Unidad Iztapalapa

División
Ciencias Biológicas
y de la Salud
Iztapalapa

Posgrado en
Biología Experimental

2nd International Symposium on Experimental and Translational Medicine

El nuevo horizonte de las neurociencias

Abstract Book

Plenary Sessions

Poster Session

- Rectoría Universidad Autónoma Metropolitana Iztapalapa
- Dirección de la División de Ciencias Biológicas y de la Salud
- Departamento de Ciencias de la Salud
- Departamento de Biología de la Reproducción
- Posgrado en Biología Experimental
- Coordinación de Posgrados CBS

Professor José Luis Gómez Olivares, Ph.D.
Director de la División de Ciencias Biológicas y de la Salud
Director of Division of Biological and Health Sciences
Universidad Autónoma Metropolitana Iztapalapa

Bienvenidos

El Simposio Internacional en Medicina Experimental y Traslacional es una actividad académica organizada por profesores de la División de Ciencias Biológicas y de la Salud de la Universidad Autónoma Metropolitana unidad Iztapalapa, que tiene como objetivo central la presentación de temas relevantes y de actualidad en la materia por parte de profesores investigadores destacados de nuestra Universidad y de instituciones nacionales e internacionales. También es una oportunidad para escuchar a egresados destacados de nuestros programas de estudios que han tenido mucho éxito en la academia y la investigación en instituciones en México y el mundo. En esta ocasión se hace un homenaje a la trayectoria académica y científica al Dr. Javier Velázquez Moctezuma por sus contribuciones al avance de las neurociencias en México, área que, en esta segunda edición del Simposio, se toma como eje central además de otros temas como el cáncer, la medicina regenerativa, las enfermedades pulmonares y el envejecimiento. Es importante destacar que también tenemos el gusto de conocer el trabajo que se realiza en nuestra Universidad y en otros centros académicos de excelencia en México y otros países.

Sean bienvenidos a este Segundo Simposio Internacional
en Medicina Experimental y Traslacional.

Welcome

The International Symposium on Experimental and Translational Medicine is an academic activity organized by professors from the Division of Biological and Health Sciences of the Universidad Autónoma Metropolitana Iztapalapa, whose main objective is to present the relevant and current topics in the subject of outstanding researchers from our university and national and international institutions. It is also an opportunity to listen to important graduates of our academic programs who have succeeded in academia and research in institutions in Mexico and the world. On this occasion, a tribute is paid to the academic and scientific career of Dr. Javier Velázquez Moctezuma for his contributions to the advancement of neuroscience in Mexico. This area in this second edition of the Symposium is taken as the central axis of this meeting, including other topics such as cancer, regenerative medicine, lung diseases, and aging. It is essential to highlight that we also have the pleasure of knowing the work carried out at our university and in other academic centers of excellence in Mexico and other countries.

Welcome to this Second International Symposium
on Experimental and Translational Medicine.

Program

8:00-8:50 — **Registration Desk**
Registration/Posters

8:50-9:10 — **Opening Ceremony**
Welcome and Opening Ceremony
- Rector General
- Rectora de Unidad
- Secretaria General
- Director de CBS

9:10-9:40 — Opening Lecture 1
New Therapeutic Options for Major Depression
Dr. Lenin Pavón Romero
Instituto Nacional de Psiquiatría | SNI-3

9:40-10:00 — **Tribute to Dr. Javier Velázquez Moctezuma**
Dra. Beatriz Gómez González
University authorities

10:00-10:15 — **Official Photograph**

10:15-10:45 — Lecture 2
Examining Patient-Specific Heterogeneity in Zero-Passage Organoids of Luminal Breast Cancer
Dr. Kevin Janes
University of Virginia

10:45-11:15 — Lecture 3
Mechanisms Associated with Pulmonary Fibrosis and Aging
Dra. Annie Pardo Cemo
Facultad de Ciencias, UNAM | SNI-Emérita

11:15-11:30 — **Coffee Break**

11:30-12:00 — Lecture 4
The Nucleolus in Pathology and Evolution
Dr. Luis Felipe Jiménez García
Facultad de Ciencias, UNAM | SNI-3

12:00-12:30 — Lecture 5
Autoimmune Psychosis: A Scientific Bridge Between Neurology and Psychiatry
Dr. Jesús Ramírez Bermúdez
Instituto Nacional de Neurología y Neurocirugía | SNI-2

12:30-13:00 — Lecture 6
Transforming Experiences: Neurobiology of Memory Updating, Implications for Alzheimer's Disease
Dr. Federico Bermúdez Rattoni
Instituto de Fisiología Celular, UNAM
Premio Nacional de Ciencias | SNI-3

13:00-13:30 Lecture 7

The Origin and Evolution of Rna Virus: the case of Covid-19

Dr. Antonio Lazcano Araujo

Facultad de Ciencias, UNAM
El Colegio Nacional | SNI-3

13:30-15:00 **Lunch/Poster session**

15:00-15:30 Lecture 8

Traumatic Brain Injury, and Post-Traumatic Epilepsy: Therapeutic Prevention Strategies

Dra. Luisa Rocha Arrieta

CINVESTAV | SNI-3

15:30-16:00 Lecture 9

An Axl Antibody Blocks Osimertinib Resistance Better than an Axl Kinase Inhibitor: Implications for Targeting Adaptive Mutability in Egfr-Mutant Lung Cancer

Dr. Arturo Simoni Nieves

The Foundation for Liver Research | London, UK

16:00-16:30 Lecture 10

Cellular Senescence Participation in Brain Neuroinflammation and Cognitive Decline: From The in Vitro to The in Vivo Model

Dra. Mina Konigsberg Fainstein

UAM-I | SNI-3

16:30-16:50 **Coffee Break/Poster session**

16:50-17:20 Lecture 11

Human Dopaminergic Neurons: Differentiation, Degeneration and Restitution

Dr. Iván Velasco

Instituto de Fisiología Celular, UNAM | SNI-3

17:20 -17:50 Lecture 12

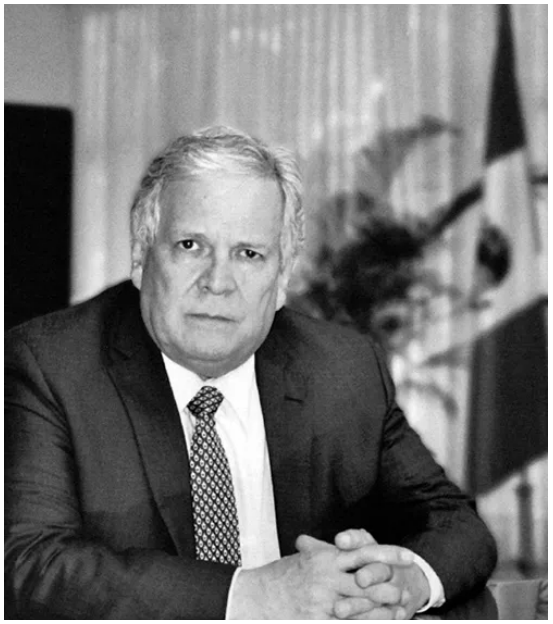
Application of Human Chemically-Derived Hepatic Progenitor Organoids (Hcdhos) in Regenerative Medicine, Hepatotoxic and Disease Modeling

Dra. Soraya Salas-Silva

Hanyang University Seoul, South Korea

17:50-18:00 **Conclusion**

Final remarks



Tribute to Dr. Javier Velázquez Moctezuma

El Dr. Javier Velázquez Moctezuma estudió la carrera de Medicina en la Universidad Nacional Autónoma de México (UNAM); posteriormente, estudió la maestría en Psicobiología en la misma institución y el doctorado en Biología de la Reproducción en la Universidad Autónoma de Tlaxcala (UATX). Fue galardonado con la Medalla Gabino Barreda por sus estudios de pregrado en la UNAM.

Es Investigador Principal del Departamento de Biología de la Reproducción de la Universidad Autónoma Metropolitana (UAM). Es experto en el campo de la Neurociencia, especialmente en fisiología del sueño, trastornos del sueño, neuroinmunoendocrinología, neurobiología del comportamiento sexual, estrés y modelos animales de depresión y ansiedad. Ha publicado más de 100 artículos en revistas internacionales; tiene más de 2300 citas de sus artículos y un índice h de 19. Ha editado 12 libros en español e inglés, publicados por universidades de alto prestigio académico. El Dr. Velázquez Moctezuma ha contribuido a la formación de recursos humanos altamente especializados; ha graduado ocho maestros y 15 doctores.

Fue jefe del Departamento de Biología de la Reproducción y Rector de la Unidad Iztapalapa de la Universidad Autónoma Metropolitana (UAM). El Dr. Velázquez Moctezuma fue el fundador y actualmente es el jefe de la Clínica de Trastornos del Sueño de la Universidad Autónoma Metropolitana. También fue fundador y principal impulsor de la Feria de las Ciencias de la Universidad Autónoma Metropolitana (UAM), con una amplia producción de programas radiales, artículos de difusión y materiales científicos para estudiantes de primaria de todas las edades.

UNIVERSIDAD AUTÓNOMA METROPOLITANA
Casa abierta al tiempo

2nd International Symposium on Experimental and Translational Medicine

Second International Symposium on Experimental and Translational Medicine

Abstract Book

Table of Contents

Second International Symposium on Experimental Medicine and Translational Medicine

Plenary Sessions

Traumatic Brain Injury: Understanding Pathophysiology, Novel Approaches, and Treatment Strategies

Félix López-Preza, Ph.D, Luisa Rocha, Ph.D.

Pharmacobiology Department. Center for Research and Advanced Studies. México.

Traumatic Brain Injury (TBI) is defined as a disruption in brain function or other evidence of brain pathology caused by external physical force, which can result in physical, psychiatric, emotional, and/or cognitive disabilities (Maas et al., 2022; Kaur & Sharma, 2017). According to the Glasgow Coma Scale (GCS), TBIs are categorized as mild, moderate, or severe. Globally, TBI is the condition with the highest prevalence and incidence when compared to diseases such as Alzheimer's or Parkinson's (Maas et al., 2022). The United States and Canada have the highest incidence rates of TBI. Conversely, in Mexico, TBI represents the fourth leading cause of death, with an incidence rate of 38.8 per 100,000 inhabitants. Presently, TBI ranks as the ninth leading cause of death, with a higher incidence in males than females, occurring predominantly among individuals aged 15 to 45 years (Leo & McCrea, 2016).
Two types of injuries have been identified following TBI: primary and secondary injuries. Primary injuries involve direct brain damage that occurs immediately after impact, resulting in hemorrhages, contusions, axonal injury (focal or diffuse), and disruption of the blood-brain barrier (BBB). These primary injuries can progress to secondary injuries, which encompass a range of cellular, physiological, and molecular processes affecting all cells in the brain. These processes include the following processes (Kaur & Sharma, 2017; Ng & Lee, 2019; Jarrahi et al., 2020):

• Excitotoxicity characterized by augmented excitatory glutamatergic neurotransmission, resulting in hyperexcitability and neuronal death.

• Oxidative stress, characterized by increased reactive oxygen species (ROS) leading to lipid peroxidation and cell damage.

• Neuroinflammation, mediated by an increase in the release of inflammatory mediators such as cytokines in the brain parenchyma.

The alterations occurring immediately after experiencing a TBI can persist for weeks, months, or even years. A severe TBI results in weight loss, sensorimotor impairment, and hippocampal hyperexcitability in the long term after the trauma. This latter effect is associated with an increase in extracellular release of glutamate and neuronal death (Santiago-Castañeda et al., 2022; Segovia-Oropeza et al., 2020). Post-TBI hyperexcitability leads to alterations in brain electrical activity, reflected in changes in the power bands of an electroencephalogram (Ianof & Anghinah, 2017), primarily in gamma waves (Ichkova et al., 2020). These changes are associated with the development of cognitive disorders (Mably & Colgin, 2018) and the emergence of pathological high-frequency oscillations, indicating the potential development of post-traumatic epilepsy (PTE) (Li et al., 2021). The neuronal hyperexcitability following TBI results from various processes, including brain damage,

disruption of the blood-brain barrier (BBB), and neuroinflammation, among others (Kharatishvili et al., 2010; Kuo et al., 2022; Gruenbaum et al., 2022; Fakhoury et al., 2020).

Clinical and preclinical evidence indicates that the short-term physical impact causes cerebral edema and neuronal death (Turtzo et al., 2013). Immediately after a TBI, endothelial cells forming the BBB are altered, leading to the infiltration of peripheral immune molecules into the CNS, microglial activation, and subsequent neuroinflammation (Sulhan et al., 2020). BBB dysfunction persists in the long term post-TBI, a condition that facilitates neuroinflammation, and vice versa, creating a vicious cycle between both alterations (Van Vliet et al., 2020).

TBI also induces long-term cerebral atrophy (Cole et al., 2018). Cerebral atrophy leads to the formation of aberrant circuits in different brain structures, potentially associated with deficits in sensorimotor function and emotional disturbances such as depression (Fakhoury et al., 2020). All of these changes can contribute to the development of neurodegenerative diseases such as Parkinson's (Gardner et al., 2018) and Alzheimer's (Ramos-Cejudo et al., 2018), as well as other conditions like PTE (Figure 1) (Verellen & Cavazos et al., 2014).

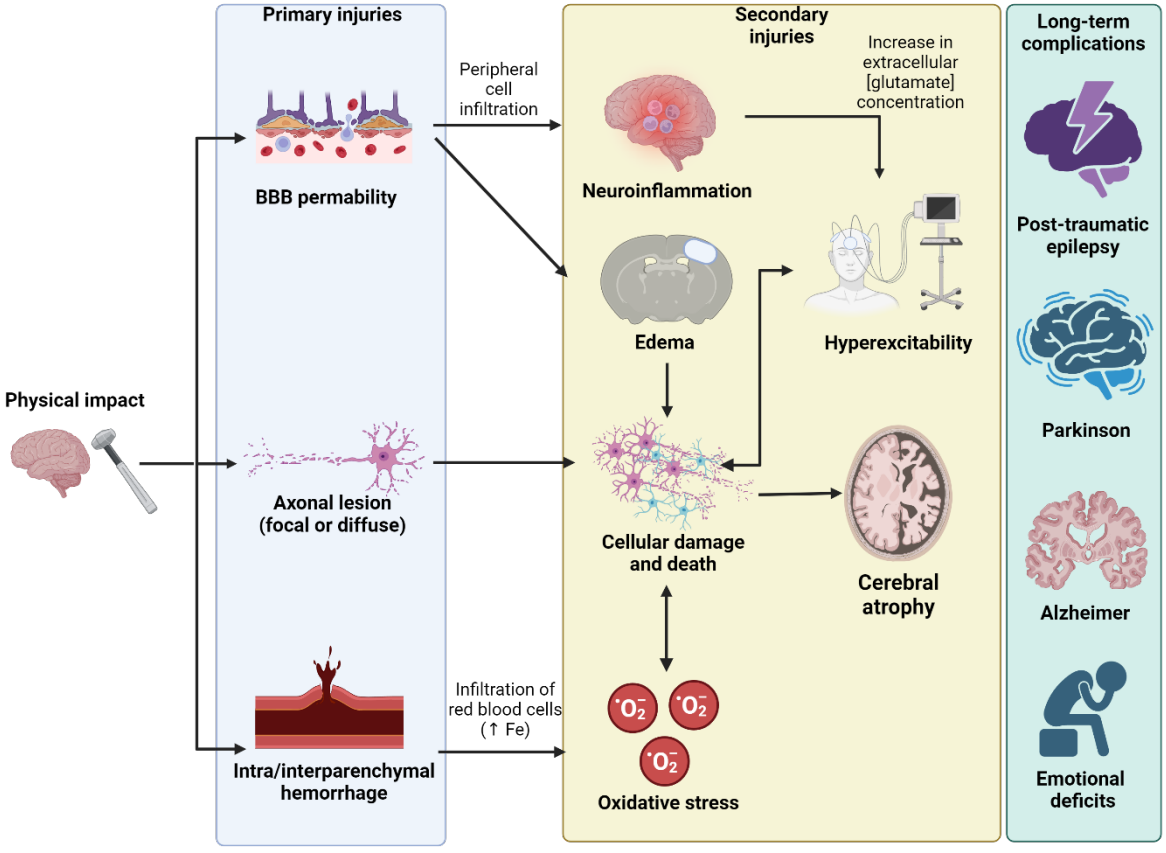


Figure 1. Physiopathology of TBI.

The treatment of TBI is a multifaceted and intricate process as consequence of various factors involved, including the severity of the injury, the specific areas of the brain affected, and the potential complications that may arise. In mild TBI, characterized by minimal structural damage or neurological deficits, conservative management strategies focusing on symptomatic relief and supportive care may be employed. This can include rest, over-the-counter pain medications for headache management, and close monitoring for any worsening symptoms. However, in cases of moderate to severe TBI, where there is significant intracranial bleeding, cerebral edema, or extensive neuronal damage, a more aggressive approach is often necessary. Hospitalization is typically required in these instances, allowing for close monitoring of vital signs, neurological status, and intracranial pressure.

Medical interventions may involve the administration of medications such as corticosteroids and osmotic agents like mannitol or hypertonic saline focused on reducing cerebral edema and inflammation. Surgical interventions may be necessary to address life-threatening complications such as intracranial hemorrhage or to alleviate elevated intracranial pressure. Surgical procedures may include craniotomy for hematoma evacuation, decompressive craniectomy to reduce intracranial pressure, or the placement of intracranial pressure monitoring devices. Moreover, rehabilitation plays a crucial role in the management of TBI, aiming to maximize functional recovery and improve quality of life for affected individuals. This comprehensive rehabilitation program may encompass physical therapy, occupational therapy, speech therapy, and cognitive rehabilitation, tailored to address the specific deficits and challenges faced by each patient. Furthermore, ongoing psychological support and counseling are often integral components of TBI rehabilitation, addressing emotional and behavioral changes that may arise as a result of the injury.

A multidisciplinary approach involving various medical specialists, including neurologists, neurosurgeons, intensivists, rehabilitation therapists, neuropsychologists, and social workers, is essential to ensure comprehensive and coordinated care for individuals with TBI (Levin et al., 2019; Maas et al., 2017; NINDS, 2020).

Despite significant advances in the understanding and treatment of TBI, there is an ongoing need to identify new molecules and therapeutic approaches to improve clinical outcomes and the quality of life of patients. One fundamental reason for seeking new molecules to treat TBI lies in the diversity of brain injuries that can result from this type of trauma. The brain is a highly complex and heterogeneous organ, and injuries associated with TBI can range from superficial contusions to diffuse axonal injuries and intracranial hemorrhages. This diversity of injuries requires equally diverse therapeutic approaches that address the different pathophysiological pathways involved in TBI, such as brain inflammation, excitotoxicity, oxidative stress, and blood-brain barrier dysfunction.

Furthermore, many current therapies for TBI focus on managing symptoms and secondary complications, such as intracranial hypertension and post-traumatic epilepsy, rather than directly addressing the underlying mechanisms of brain injury. Therefore, there is an urgent need to develop neuroprotective treatments that can prevent or mitigate primary brain damage and promote neuronal recovery and repair of damaged brain tissue.

NeuroEPO represents a novel therapeutic strategies to reduce neurodegenerative disorders. NeuroEPO is a glycoprotein derived from EPO that lacks erythropoietic activity due to its low sialic acid content (Garcia Rodríguez & Rama, 2012). NeuroEPO acts on the heterodimer of the EPO receptor and the common beta receptor, whose signaling induces the formation of anti-apoptotic, anti-inflammatory, and other protective molecules (figure 2) (Gómez, 2018). Various clinical and preclinical studies report that the use of NeuroEPO

does not induces toxic effects. Its half-life in blood is very short due to high hepatic degradation; however, intranasal administration extends its half-life in the brain for up to 12 hours (Lagarto & García, 2012).

At present, NeuroEPO is used in clinical trials for diseases such as Alzheimer's, Parkinson's, stroke, and ataxia. The results of clinical trials using NeuroEPO in Alzheimer's and Parkinson's are favorable (Pedroso et al., 2018; Bringas et al., 2021), while trials in patients with stroke or ataxia are still ongoing (International Clinical Trials Registry Platform, 2022).

Preclinical evidence indicates that NeuroEPO prevents in vitro the death of cortical cells exposed to high glutamate concentration (Garzón et al., 2018). NeuroEPO also prevents such damage in in vivo ischemia models in gerbils (Teste et al., 2012). According to these findings, it is possible to suggest that NeuroEPO could prevent post-TBI alterations.

An ongoing study is focused to determine if subchronic intranasal NeuroEPO administration prevents long-term consequences induced by severe TBI. The results obtained indicate that the subchronic intranasal NeuroEPO administration reduces the TBI-induce sensorimotor dysfunction, body weight loss, expression of ROS long-term after the trauma (Lopez-Preza et al., in process). These results support that repeated NeuroEPO administration may be a therapeutic strategy to prevent long term consequences post-TBI.

Therapeutic strategies such as NeuroEPO should be investigated to reduce the long-term consequences of TBI.

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Transformando las experiencias: Neurobiología de la actualización de la memoria

Federico Bermúdez Rattoni, Eduardo Hernández-Ortíz, Andrés Agoitia, Luis Rodríguez-Durán y Daniel Osorio-Gómez.

Departamento de Neurociencia Cognitiva, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.

Resumen

El desafío de las neurociencias es modular selectivamente a los circuitos cerebrales y evaluar el impacto de su activación e inhibición sobre la memoria y la cognición. Recientemente, el advenimiento de las técnicas optogenéticas ha abierto una vía prometedora para superar esta limitación, permitiendo una modulación espacial y temporal precisa de las neuronas y sus proyecciones en tiempo real durante la ejecución de una tarea. En nuestro laboratorio, nos enfocamos en investigar cómo la estimulación selectiva de las neuronas dopaminérgicas del área tegmental ventral (VTA) o de sus terminales en la corteza insular modula el establecimiento de memorias de reconocimiento y contextuales apetitivas. También, hemos explorado el papel de los circuitos corticales que proyectan río abajo (top-down) al VTA y la amígdala basolateral, mediante la liberación de dopamina y glutamato, en la formación y actualización de la conducta de preferencia de lugar en tiempo real. Al dilucidar estos mecanismos, nuestro objetivo es mejorar nuestra comprensión de la intrincada interacción entre la actividad de los circuitos neuronales y los procesos cognitivos, así como arrojar luz sobre aspectos fundamentales de la formación y actualización de las memorias adictivas.

Antecedentes

El aprendizaje y la memoria son procesos cruciales para la adaptación de los organismos a los cambios en su entorno, implicando modificaciones tanto morfológicas como funcionales en diversos circuitos del sistema nervioso central. La adquisición de información (aprendizaje) y su retención a lo largo del tiempo (memoria) sobre la localización de recursos como alimentos o refugios, así como la capacidad para reconocer y evitar situaciones asociadas con consecuencias negativas, son comportamientos adaptativos moldeados por la experiencia. Durante la consolidación de la memoria, la información se estabiliza mediante la síntesis de proteínas en los circuitos neuronales participantes (McGaugh, 2000; Lamprecht y LeDoux, 2004). Sin embargo, durante la evocación de la memoria, esta información previamente consolidada regresa a un estado lábil, sometiéndose a un proceso similar a la consolidación denominado reconsolidación de la memoria (Nader y Einarsson, 2010). Durante este proceso dinámico, la información almacenada puede ser modificada, lo que sugiere que la reconsolidación de la memoria implica la actualización de la información previamente consolidada (Rodríguez-Ortíz et al., 2005, Flavell y Lee, 2013). Se ha demostrado que la evocación de recuerdos es susceptible de modificarlos e incorporar nueva información, un proceso conocido como actualización de la memoria. Esta actualización puede implicar el fortalecimiento o el cambio en la información contenida en la memoria (Bermudez-Rattoni & McGaugh, 2017; Rodríguez-Ortíz et al., 2005).

En este sentido, hemos investigado la posibilidad de editar memorias contextuales reforzantes mediante el proceso de actualización de la memoria (ver Gálvez et al., 2022). Lo que sugiere que la actualización de la memoria podría ser un objetivo terapéutico para tratar recuerdos perniciosos, como los asociados con adicciones o traumas. Esta idea se respalda en estudios clínicos que han explorado la modificación de recuerdos maladaptativos mediante la manipulación de la actualización de la memoria, ya sea modificando el contexto o administrando fármacos específicos (Milton & Everitt, 2012; Pigeon et al., 2022).

Por otro lado, la adicción a sustancias de abuso se caracteriza por el consumo compulsivo de las mismas a pesar de las consecuencias negativas, y está relacionada con diversos sistemas de aprendizaje y memoria, incluidos los circuitos cerebrales de emociones y de recompensa. La corteza insular (IC) se ha considerado como un integrador multifuncional (hub) de procesos interoceptivos, sensoriomotores, cognitivos y emocionales (Bermúdez-Rattoni, 2014; Chen et al., 2016; Quabs et al., 2022; Guzmán-Ramos et al., 2022). Además, se ha relacionado a la IC anterior (aIC) con el mantenimiento de la adicción a sustancias de abuso (Fonseca et al., 2018; Naqvi et al., 2014). Estudios clínicos han demostrado que individuos con historia de tabaquismo severo que sufrieron lesiones en la aIC, como resultado de un accidente cerebrovascular, dejaron de fumar súbitamente después de la lesión cortical (Naqvi et al., 2007; Naqvi & Bechara, 2010). Además, estos sujetos no presentaron recaídas e incluso un paciente informó que "... es como si mi cuerpo hubiera olvidado las ganas de fumar" (Gray & Critchley, 2007; Naqvi et al., 2007; Naqvi & Bechara, 2010). Estos hallazgos reportados en humanos han sido corroborados en animales de experimentación, donde se demostró que la participación cortical en la supresión de la memoria adictiva, así como la recaída, no ocurre solo para la nicotina, sino también para otros agentes adictivos como la anfetamina y la cocaína (Arguello et al., 2017; Contreras et al., 2012; Cosme et al., 2015; NIDA, 2018). De manera que, se ha propuesto que la función de aIC en la adicción consiste en representar los efectos interoceptivos de las drogas y asociarlos con los estímulos exteroceptivos que conducen a su búsqueda compulsiva (Pushparaj & Le Foll, 2015).

Como se mencionó, estudios preclínicos y clínicos recientes sugieren que la adicción a las sustancias de abuso está asociada con la actividad del circuito cerebral de recompensa (Russo & Nestler, 2013). Este circuito está constituido principalmente por las neuronas dopaminérgicas del área ventral tegmental (VTA), las cuales proyectan hacia el núcleo accumbens, las cortezas perirhinal, insular y prefrontal, así como a la amígdala central y basolateral (BLA), entre otras áreas (Gray & Critchley, 2007; Naqvi & Bechara, 2009; Russo & Nestler, 2013). Varios estudios sugieren la existencia de circuitos clave, como la comunicación entre la BLA y la aIC, en la búsqueda y en el consumo de sustancias de abuso. (Stefanik & Kalivas, 2013; Sutherland et al., 2013). De hecho, se ha demostrado que la actividad glutamatérgica en la BLA regula el mantenimiento de la búsqueda de cocaína (Luo et al., 2015). Además, la aIC recibe inervación de la BLA y que tiene la capacidad de inducir cambios plásticos en esta región cortical (Escobar & Bermúdez-Rattoni, 2000; Jones et al., 1999; Rodríguez-Durán et al., 2023). Además, se ha sugerido que la comunicación recíproca entre la aIC y la BLA (Wang et al., 2018, Kayyal et al., 2019) estaría involucrada en el mantenimiento de recuerdos asociados a eventos gratificantes.

El circuito VTA-aIC en la producción y actualización de memorias apetitivas

Para este escrito, utilizaremos dos ejemplos de cómo podemos modificar circuitos a través de la inhibición y estimulación optogenética de algunos de las vías involucradas en las adicciones. Para ello, en nuestro laboratorio hemos investigado los circuitos provenientes del VTA que comunican con la aIC involucrados en el mantenimiento de una memoria contextual recompensante. Utilizamos la estimulación optogenética de las neuronas dopaminérgicas del VTA mediante la administración de un virus adenoasociado (AAV) con la secuencia del gen que codifica un canal de rodopsina sensible a luz (ChR2) en ratones TH-Cre. Esta cepa modificada genéticamente, permite la expresión específica de una enzima Cre con actividad recombinasa bajo el control transcripcional de la tirosina hidroxilasa (TH); dándole selectividad para la fotoestimulación de neuronas catecolaminérgicas e inducir una preferencia de lugar condicionada en tiempo real (rtCPP) (ver Fig- 1; Gil-Lievana et al., 2020). El rtCPP es un modelo de comportamiento utilizado para estudiar la recompensa asociada con señales contextuales (Qi et al., 2014; Tsai et al., 2009; Wang et al., 2015) y ha sido ampliamente utilizado para medir la adicción a las drogas y su recaída (Bardo & Bevins, 2000; Tzschentke, 2007). Para evaluar la participación de la aIC en el mantenimiento y la extinción de la memoria contextual recompensante, primero caracterizamos el rtCPP inducido por estimulación optogenética de las neuronas TH+ del VTA. Los resultados mostraron que la fotoestimulación del VTA indujo una modulación electrofisiológica de las respuestas neuronales de la aIC, y produjo una fuerte preferencia de lugar condicionada por el lado asociado con la estimulación optogenética (Fig. 1). Además, la fotoactivación de las neuronas TH+ del VTA indujeron una fuerte liberación de dopamina en la aIC, analizada mediante microdiálisis *in vivo*. Posteriormente y en ausencia de fotoestimulación, se observó la liberación simultánea de dopamina, glutamato y noradrenalina en la aIC durante la evocación de la memoria de preferencia (Fig. 1d, e) (Gil-Lievana et al., 2020). Estos resultados sugieren que estos tres neurotransmisores podrían estar involucrados en el mantenimiento de la memoria contextual apetitiva.

Para comprobar el papel de la actualización de memorias apetitivas en el circuito VTA-aIC, decidimos editar el mantenimiento de la memoria rtCPP, como lo realizamos en las vías catecolaminérgicas que proyectan al hipocampo dorsal (Gálvez et al., 2022). Para editar la memoria apetitiva, promovimos la extinción del rtCPP mediante la fotoinhibición del circuito glutamatérgico proveniente de la BLA, durante la primera sesión de evocación; dicha manipulación aceleró la extinción de las respuestas condicionadas de búsqueda (post-cond) y evitó el restablecimiento de rtCPP (Fig. 2, Post-cond) (Gil-Lievana et al., 2020). Este procese requiere de la actividad de los receptores NMDA a glutamato, ya que la infusión de un antagonista a dichos receptores en la aIC inmediatamente después de la primera sesión de evocación generó un efecto similar a la fotoinibición (Gil-Lievana et al., 2020).

Recientemente, se ha reportado que la estimulación optogenética de la actividad glutamatérgica de la aIC produce respuestas apetitivas (Wang et al., 2018). Los autores sugieren que el incremento en las respuestas apetitivas se debe a las proyecciones de la aIC hacia la BLA; sin embargo, las estimulaciones directas tanto de la BLA como de la aIC no provocan respuestas contextuales apetitivas a menos que estén asociadas con sustancias de abuso. Dado que la estimulación directa del VTA produce un robusto rtCPP,

proponemos que se requiere la activación de circuitos descendentes para retroalimentar la vía aIC-VTA y contribuir al mantenimiento de los condicionamientos reforzantes. A pesar de que se han descrito anatómicamente las vías descendentes de la aIC hacia el VTA (Watabe-Uchida et al., 2012) y la BLA, se desconocía su participación funcional durante la formación y el mantenimiento de las memorias recompensantes. Como se mencionó, los pacientes con lesiones en la aIC olvidaron súbitamente la "memoria adictiva", lo que sugiere que el control que ejerce la IC sobre la búsqueda y el consumo de sustancias de abuso sobre estructuras subcorticales es un proceso modificado por la experiencia. Por tanto, decidimos identificar los circuitos descendentes de la aIC que podrían estar involucrados en el mantenimiento de memorias gratificantes e incluso adictivas. En primer lugar, caracterizamos la vía aIC-VTA mediante técnicas neuroquímicas, electrofisiológicas y optogenéticas en modelos animales de adicción a las drogas, para comprender la neurobiología de los circuitos descendentes implicados en la formación y el mantenimiento de los recuerdos asociados a la adicción (ver, Hernández-Ortiz et al., 2023).

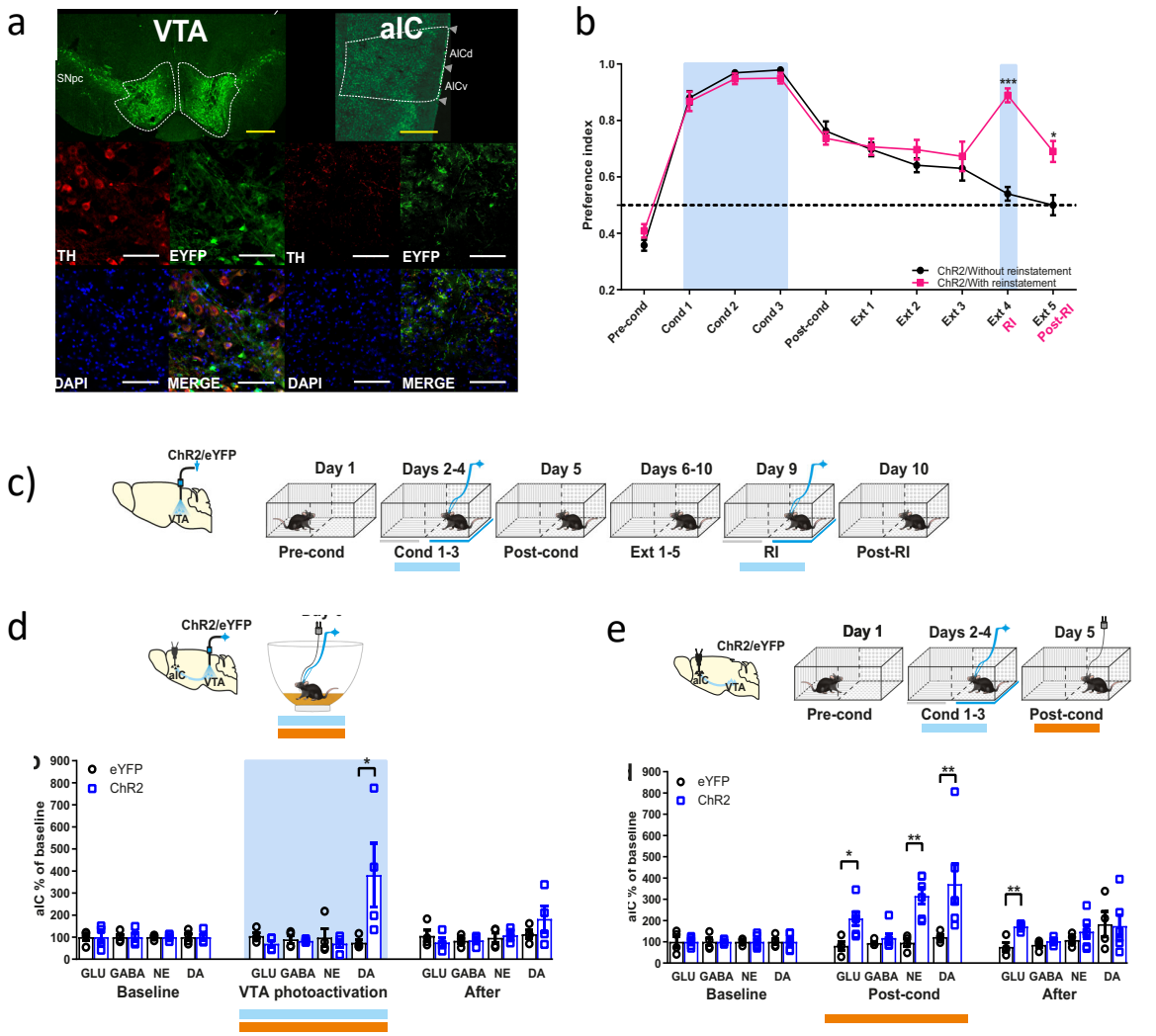

Figura 1. Fotoactivación de neuronas VTA-TH+ induce rtCPP. a) Imágenes representativas de inmunofluorescencia triple para eYFP (verde), TH (rojo) y DAPI (azul) en somas del área tegmental ventral (VTA) y axones de la corteza insular anterior (aIC) de ChR2+ que

muestran una infección viral en el VTA y fibras claras en el aIC procedente del VTA. La barra de escala amarilla representa 500 µm y la barra de escala blanca representa 100 µm. AICd, corteza insular agranular dorsal; AICv, corteza insular agranular ventral. b) Los ratones fueron condicionados durante 3 sesiones de fotoestimulación de 20 min. Tasa de extinción durante 6 sesiones de 20 min de condicionamiento de preferencia de lugar (Ext 1-5) (grupo de extinción) y restablecimiento de preferencia de lugar (Post-RI) después de 5 min de estimulación optogenética (RI) (ChR2+/Grupo de reincorporación). Las líneas discontinuas indican que no hay preferencia. c) El recuadro muestra un diagrama de inyección de virus y fotoactivación en neuronas VTA TH+. La preferencia de los compartimentos se determinó durante el pre-condicionamiento (Pre-cond). La estimulación optogenética se realizó en el lugar menos preferido. En el post-condicionamiento (Post-cond), se probó la preferencia de los compartimentos después del condicionamiento de estimulación optogenética. d) Muestra los resultados de la microdiálisis in vivo durante la fotoestimulación, en donde se librera DA. e) Resultados de la microdiálisis en el día de la evocación (post-cond), en donde se liberan la DA, NE y Glutamato (Modificado de Gil-Lievana et al., 2020).

Regulación de circuito aIC-BLA en la búsqueda exacerbada del ansia de consumo

Uno de los problemas fundamentales en el mantenimiento de las memorias adictivas es la vulnerabilidad a la reincidencia, es decir, la búsqueda desmedida del consumo de drogas que se intensifica con el tiempo de privación o abstinencia del individuo. Esta exacerbación de las conductas aprendidas y dirigidas hacia la búsqueda y obtención de sustancias de abuso depende del período de abstinencia, constituyendo el correlato conductual del fenómeno conocido como "incubación del ansia de consumo". Este fenómeno ha sido descrito con varias sustancias de abuso, como cocaína, heroína, anfetamina, alcohol, nicotina e incluso en ciertas condiciones de consumo de sacarosa (Pickens et al., 2011). Estudios farmacológicos han demostrado que la aIC participa funcionalmente en la expresión de conductas de búsqueda de consumo de sustancias de abuso (Arguello et al., 2017; Contreras et al., 2007; Venniro et al., 2017).

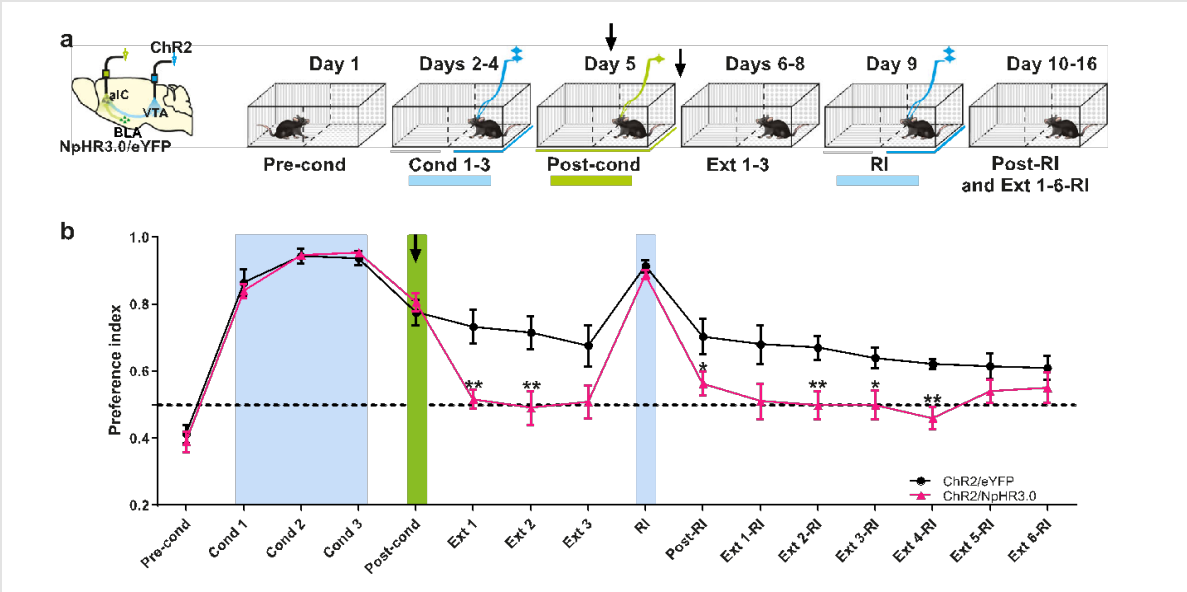

Figura 2. La fotoinhibición de la proyección BLA-aIC acelera la extinción de las respuestas reforzantes. a. El recuadro muestra un diagrama de la inyección de virus en las neuronas VTA TH+ y la fotoinhibición de las neuronas BLA. La preferencia de los compartimentos se determinó durante el pre-condicionamiento (Pre-cond). Los ratones se condicionaron durante 3 sesiones de 20 min de fotoactivación de neuronas VTA TH+ (Cond 1-3; barra azul) y las entradas de fibras aIC de BLA se fotoinhibieron (barra amarilla) durante el post-condicionamiento (Post-cond). b) La fotoinhibición de la aIC acelera la extinción (Post-cond) en el grupo NpHR3.0, pero no en el grupo eYFP. Se observa la resistencia a la recaída (RI) por fotoestimulación en el día 9 (barra azul). Los datos se muestran como media ± SEM. La flecha indica el tiempo de administración de la fotoinhibición. Las líneas discontinuas indican que no hay preferencia (Modificado de Gil-Lievana et al., 2020).

La respuesta al ansia exacerbada de consumo ante la exposición a estímulos condicionados sugiere el desarrollo de alteraciones a lo largo del período de abstinencia, incluyendo la interacción/modulación de los circuitos neuronales relacionados con la gratificación, así como en los circuitos relacionados con las memorias ansiogénicas. Por ejemplo, en la amígdala, estructura límbica, se observa un aumento de la actividad neuronal durante la exposición a estímulos condicionados después de treinta días, pero no después de solo un día de abstinencia, en un protocolo de autoadministración de cocaína. Este aumento en la actividad está mediado por señalización glutamatérgica a través de la actividad de receptores NMDA, que participa funcionalmente en la expresión exacerbada de respuestas condicionadas.

Recientemente, se ha señalado una importante proyección monosináptica glutamatérgica de la aIC a la amígdala (Venniro et al., 2017). Por tanto, decidimos evaluar si la actividad de la ínsula, así como su proyección descendente glutamatérgica a la amígdala basolateral (aIC-BLA), están mediando la expresión de la memoria adictiva, así como su participación en la incubación del ansia de consumo. Llevamos a cabo un estudio mediante condicionamiento de preferencia al lugar (CPP) inducido por anfetamina, donde se observó un aumento de preferencia de lugar con las claves contextuales asociadas a la anfetamina. También observamos un aumento en la expresión de actividad neuronal (c-Fos) en la IC de roedores sometidos a catorce días de abstinencia, en comparación con solo un día de abstinencia (Agoitia et al., 2024). Este incremento está relacionado con el aumento en la preferencia de lugar. Además, logramos, mediante técnicas de fotoinhibición optogenética, editar la memoria del ansia de consumo a través de la supresión de la actividad de la aIC y la amígdala basolateral, así como de las proyecciones glutamatérgicas a la amígdala (aIC-BLA). (Fig. 3; Agoitia et al., 2024).

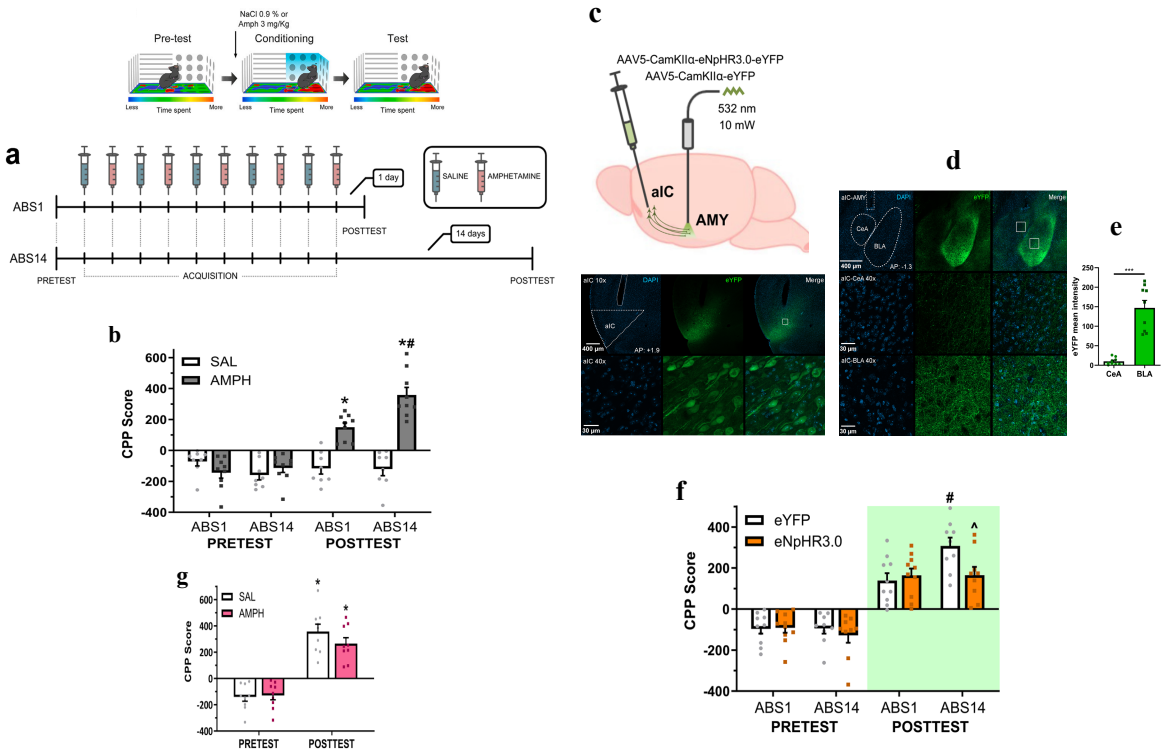

*Figura 3. a) Esquema del experimento de preferencia de lugar. SAL: solución salina isotónica, AMPH: anfetamina. b) Puntaje de preferencia, antes (pretest) y después (postest) del condicionamiento. *: p < 0.01 distinto de preferencia inicial. #: p < 0.01 distinto de puntaje POSTTEST ABS1 vs. abs14. c) Inyecciones del plasmido conteniendo la opsina NpHR3.0 o eYFP en la CI. d) Expresión de las terminales provenientes de la aIC en la amígdala basolateral. e) Cuantificación de las terminales marcadas con eYFP provenientes de la aIC en los núcleos basolateral y central de la amígdala. f) Efecto de la inhibición optogenética en la expresión de la preferencia de lugar con 1 y 14 días después del tratamiento con 5 inyecciones de anfetamina (***p < 0.01) muestran las medias (± EEM). g) Inyecciones de*

anfetamina el día 13 previo a la prueba no modificó la abstinencia exacerbada a los 14 días. (Modificado de Agoitia et al., 2024).

Los resultados publicados muestran que tanto la inhibición optogenética de la aIC como en las terminales provenientes de la aIC-BLA reducen significativamente el ansia de consumo en el día 14 de la evocación de las memorias reforzantes (Fig. 3 f). Es importante destacar que las respectivas fotoinhibiciones tanto de la aIC como de la proyección aIC-BLA solo reducen el ansia de consumo exacerbada en el día catorce, sin afectar la memoria reforzante después del condicionamiento de CPP en el primer día de evocación. Es decir, se puede editar el ansia del consumo sin afectar la memoria reforzante.

En conclusión, este trabajo demuestra cómo la actividad de varios circuitos neuronales puede controlar, mediante la inhibición/activación de los circuitos, la liberación de diferentes neurotransmisores y neuromoduladores involucrados en conductas tan complejas como las memorias placenteras e incluso ansiogénicas inducidas por la ausencia de reforzadores, como las drogas.

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Cellular Senescence Participation in brain neuroinflammation and cognitive decline: from de in vitro to the ion vivo model.

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The life expectancy in Mexico, as well as in most of the world, has increased. Data from the 2020 population census of the National Institute of Statistics and Geography (INEGI) revealed that the population aged 60 years and over in Mexico went from 9.1% in 2010 to 12.0% in 2020, while the population aged 0 to 17 years decreased from 35.4% to 30.4% in the same period (1). Despite this change in the population pyramid due to the transformative power of science and technology, there are still multiple difficulties faced by older adults in our country. For example, the loss of physical and mental capacities has increased the number of people with chronic degenerative diseases (2), hence, the last 6 to 10 years of an older adult's life are often affected by disabilities and chronic diseases, which has put a strain on Mexico's precarious health system (3).
Aging is a multifactorial and detrimental process which diminishes the functionality of most organs and systems. Among the tissues most affected by aging is the central nervous system (CNS), which suffers morphological and functional alterations in response to chronic inflammation, oxidative stress and senescent cells accumulation.
Cellular senescence, which has been recognized as one of the cellular hallmarks of aging (4), is a state of irreversible cell cycle arrest reached by cells in response to stress and damage, or due to an excess of cell divisions and telomere shortening (5). It is characterized by altered gene expression, lack of response to apoptotic or mitogenic stimuli, epigenetic alterations and the production of a secretome called Senescent Associated Secretory Phenotype (SASP) (6). The SASP includes a variety of molecules, such as proinflammatory cytokines, chemokines and metalloproteases, which alter the cellular microenvironment and generate chronic low-grade inflammation and are associated with the development of aging-related diseases (7).
Currently, there are mainly two senotherapeutic approaches to contend with the accumulation of senescent cells: senolytics, which promote their death, and senomorphics, which modify the secretory profile of these cells to reduce their negative impact. Molecules such as Quercetin and Dasatinib (D+Q) have been repositioned as senolytics and have shown promising results in animal models and pilot studies in humans (8,9). Among the

senomorphics, sulforaphane (SFN), which is extracted from cruciferous vegetables, stands out (10,11).
We have previously shown that the SASP composition depends on the stimuli used to induce senescence (12) and that astrocytes primary cultures induced to senescence with H_2O_2 modify their secretory profile towards a less inflammatory one after SFN treatment (13), supporting SFN potential to improve the cellular microenvironment in the brain. This was proven *in vivo* by treating Wistar rats of both sexes and different ages with SFN, finding a protection in cognitive performance and a decrease in inflammatory, oxidative and senescence markers, but only in middle-aged rats. SFN did not reverse the damage in old rats (14).

Among the main risk factors for developing chronic diseases, in addition to aging, is obesity (15), which increases morbidity and mortality through its contribution to the development of different chronic diseases (16), among which cognitive impairment and dementias have recently been included (17). This is mainly related to the fact that systemic inflammation generates alterations in the blood-brain barrier, oxidative stress, neuroinflammation and accumulation of senescent cells (18).
So, in the lab we developed a model to induce senescence in rat primary astrocyte cultures using sodium palmitate (PA) and have started to study the SASP in this model and how to modify it. However, a problem we face when studying the response of astrocytes to a stressor stimulus and becoming senescent, is that astrocytes can also be activated into reactive astrocytes or gliosis, which also produce pro-inflammatory molecules such as those in the SASP (19). That is why we developed an astrocyte primary culture model where we induce gliosis or senescence with different PA concentrations, in order to study the differences between the secretions of both phenotypes, since they coexist in the brains of obese older adults.

We have also studied the impact of a hypercaloric diet in middle-aged female Wistar rats. In the *in vivo* model, we found that the induced obesity increased neuroinflammation and senescence in key brain areas, such as the brain cortex and the hippocampus, leading to cognitive impairment (20). When we treated those rats with the senotherapeutics (D+Q or SFN) for two months (from age 12 to 14) we found that D+Q did not prevent cognitive decline, but SFN did prevent it, and also decreased inflammation. Interestingly, D+Q increased neuroinflammation in rats feed with standard diet, indicating potential adverse effects. Currently, we continue to evaluate the effects of both treatments on blood-brain barrier permeability and senescent cell clearance in the brain.

On the other hand, we have also started to evaluate some serum markers of brain damage in women over 60 years of age with and without obesity and cognitive impairment, in order to find a pattern that could help in the early diagnosis of these alterations.

Thus, the idea of our lab is to contribute to healthy aging in order to achieve the best quality of life in older adults.

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AXL-specific antibody better blocks osimertinib resistance than the respective kinase inhibitor: relevance to adaptive mutability.

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Non-genetic mechanisms have emerged as important drivers of cancer therapy failure, where somecancer cells can enter a reversible drug-tolerant persister state to survive treatment. A rare subset of these persister cells can paradoxically continue proliferating in the presence of drugs. However, little was known about the mechanisms enabling their proliferative capacity under drug pressure.To study this transiently resistant, proliferative persister population, researchers developed Watermelon - a high-complexity expressed barcode lentiviral library to trace each cell's clonal origin, proliferative state, and transcriptional profile. Using this system, they showed that cycling and noncycling persisters arise from distinct cell lineages with unique transcriptional programs and metabolic adaptations. Upregulation of antioxidant response genes and shifting towards fatty acid oxidation were associated with persister proliferative capacity across multiple cancer types. Inhibiting oxidative stress defenses or blocking this metabolic reprogramming reduced the fraction of cycling persister cells. Importantly, the transcriptional programs linked to cycling persisters induced minimal residual disease from patients treated with various targeted cancer therapies. Interestingly, the researchers found similarities between cycling cancer persisters and bacteria exposed to antibiotics. Like the bacterial SOS response, drug treatment of lung cancer cells with EGFR inhibitors engaged endogenous mutator pathways that promote genetic diversity and drug tolerance.
The receptor tyrosine kinase AXL emerged as a key node driving the upregulation of error-prone DNA polymerases, activating their organizer RAD18 via neddylation, and inducing MYC-mediated metabolic reprogramming that increases mutagenic purine levels and cholesterol metabolism. Different factors activate pre-existing oncogenic EGFR mutations, but EGFR-specific kinase inhibitors (KIs; e.g., osimertinib), unlike cetuximab, an anti-EGFR monoclonal antibody (mAb), can block cancer progression. Unfortunately, drug resistance emerges in osimertinib-treated patients, probably due to the induction of AXL and adaptive mutability. To inhibit AXL and resistance to osimertinib we compared two anti-AXL drugs, an antibody, mAb654, and a KI, bemcentinib. While no pair of osimertinib and an anti-AXL drug was able to prevent relapses in animal models, triplets combining osimertinib, cetuximab, and either anti-AXL drug were initially effective. However, prolonged monitoring revealed clear superiority of the mAb654-containing triplet, likely due to the inactivation of intrinsic mutators. Hence, we constructed a bi-specific antibody that engaged both AXL and EGFR. As predicted, when combined with osimertinib the bi-specific antibody persistently inhibited tumor relapses. These observations warrant clinical trials, and they are discussed considering AXL’s ability to activate endogenous mutators.
Targeting AXL pharmacologically, or using antibodies to simultaneously block AXL and EGFR, prevented the acquisition of resistance mutations and tumor relapse in patient-derived xenograft models. These findings unveiled non-genetic mechanisms of adaptive mutability that enable a subset of persister cells to proliferate under drug pressure. By exposing vulnerabilities in this rare cycling persister population, new therapeutic strategies could potentially delay or prevent disease recurrence by disabling these endogenous mutator pathways that fuel acquired resistance.

Application of human chemically-derived hepatic progenitor organoids (hCdHOs) in regenerative medicine, hepatotoxic and disease modeling.

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Background. Liver organoids, an attracting source for studying cell to cell interaction, drug screening, and disease modeling in three-dimensional systems have been demonstrated to be developed from $EpCAM^+$ cells. Our group previously reported chemically derived hepatic progenitors (hCdHs), reprogrammed from human primary hepatocytes (hPHs) with EpCAM-positive characteristic and showed bi-potent differentiation capacity into hepatocytes and biliary epithelial cells. Here we extended this culture system to generate hCdH-derived liver organoids for diverse biomedical applications.
Methods. To generate hCdHs, hPHs were cultured with reprogramming medium consisted of HGF, A83-01 and CHIR99021 for 7 days. Human liver cells isolated by a two-step collagenase perfusion method and hCdHs were cultured on Matrigel with organoid medium to generate human adult liver organoids (hALOs) and hCdHs derived liver organoids (hCdHOs).
Results. hCdHOs were morphologically undistinguished and showed high generation efficiency compared with hALOs. hCdHOs were stably expanded over the 6-month period and expressed the liver organoid-specific markers. hCdHOs cultured in hepatic differentiation medium (hCdHO_DM) showed increased expression of hepatic markers and functional capacity as CYP activity. Transcriptome analysis also demonstrated that whole gene expression patterns of hCdHO_DM were clustered with hPHs after hepatic differentiation. Upon transplantation in FRG mice models, hCdHOs effectively repopulated the injured liver. In drug-induced injury assays in vitro, hCdHOs showed a similar or higher sensitivity response than hPHs. We further developed an alcoholic liver model using hCdHO_DM under ethanol treatment which presented alcoholic liver disease associated pathophysiologic changes.
Conclusions. Our studies suggest that hCdHOs have a potential to be a novel liver organoid cell source to performing disease modeling, toxicological analysis, and their use in regenerative medicine.

Second International Symposium on Experimental Medicine and Translational Medicine

Poster Session

P-1

Design and synthesis of new molecules derived from 2-aminobenzothiazoles focused on the treatment of diabetes mellitus and its complications.

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Introduction. Diabetes mellitus (DM) is a chronic progressive metabolic disorder of carbohydrates, lipids and proteins. The prevalence of this disease continues to increase worldwide, being a constant public health problem. There are various treatments for DM, however all are accompanied by undesirable side effects for the patient, which is why it is necessary to continue searching for new alternatives. It has been shown that 2-aminobenzothiazole derivatives are molecules that have good activity on DM.
Aim. Carry out the in silico study, synthesize and evaluate in vivo compounds derived from 2-aminobenzothiazole as antidiabetic agents with activity on PPAR-y.
Methods. The molecular docking assay was carried out using Autodock 4.2 software with the objective of select from a 9 molecules family, the one or those that had the greatest affinity for the PPAR-y receptor. The synthesis route was designed as follows; initially the intermediate dimethyl (6-substituted-1,3-benzothiazol-2-yl) dithioimidocarbonate was formed, subsequently a second reaction was carried out with 2-aminophenol and o-phenylenediamine to obtain the compounds GB-18 and GB-19 respectively. Subsequently, the toxicity of both compounds was evaluated in Sprague Dawley male rats, according to the 425 OECD guideline, using 1750, 550 and 175 mg/kg doses, all of them administered intragastrically. The antidiabetic evaluation of the compounds was done in Sprague Dawley male rats with experimental DM2 induced using a high-calorie diet (caloric intake 4900 Kcal/kg) and streptozotocin. The acute and subchronic assays consisted on the administration of the compounds intragastrically at an equimolar dose with pioglitazone of 15 mg/kg. In the acute assay, the treatments were administered, and blood glucose was measured at 0, 0.5, 1, 2, 3 and 4 hours to observe the effect after the treatment. In the subchronic assay, the treatments were administered daily for 4 weeks, blood glucose values were recorded weekly and glycated hemoglobin (% HbA1c) at the beginning and in week 4, all samples were obtained by puncture of the caudal vein in the tail. of the rat.
Results-Discussion. The docking assay showed that GB-18 and 19 have the best affinity on PPAR-y with DG values of -8.4 and -8.45 respectively. The synthesis was carried out satisfactorily. The toxicity test showed that both compounds are not toxic and have an LD50 < 1750 mg/kg. Both compounds significantly reduce hyperglycemia in acute and subchronic assays. GB-18 presented the best antidiabetic activity, reaching normoglycemic values and

adequately reducing %HbA1c with values like a healthy rat, being better than the control drug pioglitazone.

Conclusion. The results suggest that GB-18 and 19 molecules that can be candidates for the development of new drugs focused on the treatment of DM2.

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P-2

Anticancer effect of dichloroacetate and curcumin in acute lymphoblastic leukemia cells.

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Background. Acute Lymphoblastic Leukemia (ALL) is the most common form of cancer and the leading cause of death in children. ALL is a disease characterized by a rapidly evolving disordered proliferation of immature lymphoid cells (lymphoblasts) that arise from stem cells in the bone marrow. Mexico has one of the highest incidence and mortality rates of ALL worldwide, in addition to a high frequency of early relapses (17%) compared to that of developed countries (4.5%). Treatments to combat ALL based on chemotherapy can be aggressive and present various side effects due to their great toxicity, which can put the patient's life at risk. It is very important to develop new therapies that are more effective and have fewer harmful side effects. In recent years, the compoundcurcumin (Cu) from the Curcuma longa plant has been shown to have anticancer effects in clinical and preclinical studies. The anticancer effects of curcumin include inhibition of angiogenesis and inhibition of tumor growth. It has also been reported that in cancer cells, the use of dichloroacetate (Di), an inhibitor of pyruvate dehydrogenase kinase (PDK), activates oxidative phosphorylation and promotes apoptosis by increasing the flow of the electron transport chain, causing depolarization of the mitochondrial membrane potential and the release of cytochrome c.
Aim. Study the effect of different drugs with anticancer properties onthe death pathway in acute lymphoblastic leukemia cells.
Methodology. In this work, the cell death pathway exhibited by ALL cells cultured in the presence of Cu and Di was determined to demonstrate the anticancer properties of both compounds. To do this, ALL cells were cultured with Cu, Di, and a mixture of both (Di + Cu); the viability percentage and the main elements of the apoptosis pathway, such as Bax, Bcl-2,and caspase 7, were determined.
Analysis of results. Di has a sensitising effect that allows it to potentiate the anticancer action of Cu on Jurkat cells. The use of Di and Cu in Jurkat cells decreases their cell viability.The anti-apoptoticprotein Bcl-2 decreases,andthe pro-apoptotic proteins Bax and Caspase-7 tend to increase in relation to treatments with Diand Cu.
Conclusion. These results show important support for understanding the intracellular biochemical events thatcause the death of Jurkat cells due to exposure to drugs with anticancer properties and provide us with a reference point for future studies concerning possible therapyalternativesfor treatingALL. This work was funded byInstituto Nacional de Pediatría2022/067 andCONAHCYT CF-2023-I-811.

P-3

Determination of antidiabetic activity and safety of the aqueous extract from the leaves of *ficus carica* linn in an experimental type 2 diabetes mouse model.

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Introduction. Type 2 diabetes has a high prevalence in Mexico and worldwide. In this context, it is essential to consider that pharmacological treatment entails both adverse effects for the patient and a high cost for both individuals and the healthcare system. Therefore, there arises a need to explore complementary and alternative approaches for this disease, such as the use of traditional Mexican medicine.
AIM: To determine the antidiabetic effect and safety of infusions made from Ficus carica Linn leaves in Balb/c mice with experimental type 2 diabetes (DM2-experimental).
Methods. The aqueous extract of F. carica (EAcFc) was standardized from an herbal infusion. This infusion was obtained from 1.5g of F. carica leaf powder, which was mixed with distilled ethanol in a 1:1 ratio. The solvent was then removed through filtration and evaporation until dryness (maintaining constant weight) using a rotary evaporator under reduced pressure at a temperature of 40°C. Subsequently, the acute oral toxicity of EAcFc was evaluated following OECD guideline for testing of chemicals 423. Female Balb/c mice weighing between 19-23 g, with n=3 for each batch, were administered a dose of 3000 mg/kg. The assessment of the antidiabetic activity of EAcFc was conducted in male Balb/c mice with experimentally induced DM2 using the Streptozotocin-Nicotinamide method. The acute and subchronic assays involved intragastric administration of EAcFc at a dose of 300 mg/kg. During the acute test, EAcFc was administered, and blood glucose was measured at 0, 1, 3, 5, and 7 hours to observe the extract's effect. In the subchronic test, EAcFc was given daily for 4 weeks, with weekly glucose measurements and glycated hemoglobin (% HbA1c) recorded in the second and fourth weeks. All samples were obtained through tail vein puncture in mice.
Results-discussion. Standardization of the extract obtained from herbal infusions resulted in an average of 0.35574 g, equivalent to 23.69865%. EAcFc has an LD50 >3000 mg/kg and falls within OECD category 5. EAcFc at a dose of 300 mg/kg, both acute test and subchronic test, demonstrated an effect compared to the DM2 control group.
Conclusion. The results suggest that EAcFc is safe for human consumption. Additionally, the equivalent of 300 mg/kg of the obtained extract prevents hyperglycemia in animals with experimental DM2.

P-4

Assessment of Novel Synthetic Steroid Drugs on Myelin of Brain. *In Vitro* Assays.

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Background. Steroids are biomolecules with a basic structure made up of cyclopentano perhydrophenanthrene. Two steroids derived from cholesterol are testosterone, as natural androgen, and progesterone, as natural antiandrogen. Antiandrogens can be prescribed to treat an array of diseases and disorders as Gender dysphoria. In men, antiandrogens are most frequently used to treat prostate cancer and hyperplasia. In fact, antiandrogens are 5α-reductase inhibitors and prevent the conversion of testosterone to Dihydrotestosterone (DHT), necessary for development of both external male sex organs. The present study has the **aim** of pharmacological evaluation of several new steroid derivatives (**seven new compouds**).
Methods. These steroids were prepared from the commercially available 16-dehydropregnenolone acetate, and evaluated by kinetic assays with 0.5, 1, 2 to 4mM NADPH as sustrate and the inhibition of 5α-reductase enzyme on myelin of brain. **Results.** The overall data of this study showed very clearly all compounds are good inhibitors for the 5alpha-reductase enzyme on myelin of brain.
Discusion. The 5 α-reductase enzyme, is present in several Central Nervous System structures of the rat, and the myelin possess a 5 α-reductase activity several times higher, and male rats possess a very similar apparent Km and the Vmax values. However; The novel steroids evaluated would be efficacious in the treatment of dihydrotestosterone related disorders.
Conclusion. Probed efficacy of these novel steroids respect spironolactone *in vitro* assays, appears to be a promising compounds for future hormonal therapy in patients.

P-5

Kinetic instability and unfolding are aggravated in human triosephosphate isomerase enzyme deficiency due to the propensity for glycation: The case of the E104D variant.

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Background. Protein glycation affects enzyme activity, disrupts normal metabolic processes, and is associated with the development of chronic degenerative diseases. The enzyme human glycolytic triose-phosphate isomerase (HsTIM, TPI1 or HsTPI) is associated with several disorders, such as enzymopathies, caused by various genetic mutations. It also possesses plurimer functions and is now recognized as a potential cancer marker. Background: Reports of HsTPI mutants have shown a significant increase in methylglyoxal (MGO) by triose accumulation.
Aim. To compare how catalysis exerts differential effects on the kinetic and structural stability of the E104D mutant enzyme reported for human deficiency by tracking over time the fluorescent argpyrimidine adduct (ARGp), which is formed through protein interaction via aminoacid Arginine-MGO residues.
Methods. Circular dichroism and fluorescence spectroscopy were explored on the -E104D variant. The loss of activity over time was analyzed, as well as the accessibility to cysteines and the propensity for catalysis-induced unfolding upon incubation with the substrate Glyceraldehyde 3-phosphate (G3P) or MGO.
Results. The -104D enzyme increased the signal of ARGp vs -WT. The adducts formed caused drastic changes in their structure. Our study sheds new light on the increased susceptibility observed in this mutant to generate MGO adducts. Because HsTPI-E104D potentiates a negative feedback loop between loss of activity by catalysis and induction to unfolding over time; this variant showed a propensity for the formation, accumulation and formation of MGO-adducts that gain access and promote their denaturation.
Conclusion. The cumulative and accelerated increase of adducts can be considered as a neglected but present factor in the development of the pathological state of this enzymatic variant, which could be partially reversed with MGO scavengers such as Arginine.
Keywords: Non-enzymatic post-translational modifications (MPT/NE); Metabolic dysfunction; Spectropolarimetry and spectrofluorometry analysis; Conformational changes.
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P-6

Unraveling the potential of human deamidated triosephosphate isomerase: A promising pharmacological target for cancer drug development.

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Background. According to the World Health Organization, more than 300,000 cases of childhood cancer are diagnosed each year worldwide, with acute lymphoblastic leukemia (ALL) being the most common cancer. Despite the availability of various treatments, their effectiveness is limited by the widespread impact of side effects. By identifying the dependence of cancer cells on glycolysis for energy production, researchers have focused on enzymes in this pathway as potential therapeutic targets. Structural abnormalities in these enzymes offer compelling elements to distinguish between normal and cancerous cells. In particular, post-translational modifications (PTMs) have been observed in the glycolytic human triosephosphate isomerase (HsTPI), potentially more pronounced in cancer cells. This highlights the possibility of examining enzyme differences both with and without MPT to identify molecular targets. Aim. To characterize the functional and structural properties of deamidation as a post-translational modification in HsTPI, and to provide evidence demonstrating its susceptibility to selective inhibition by diverse compounds.
Methods. Through molecular docking studies, the structural differences between the crystallographic structures of the HsTPIWT and HsTPIN16D (deamidated) complexes with selective inhibitors were identified. Recombinant forms of HsTPIWT and HsTPIN16D enzymes were overexpressed and purified. After purification, these enzymes were exposed to compounds, and their inhibitory effects were evaluated by enzymatic assays. Furthermore, intrinsic fluorescence techniques were used to elucidate the structural modifications induced by these compounds.
Results. In silico analyzes revealed a notable increase in the number of cavities in HsTPIN16D, predominantly located at the homodimer interface, where weak contacts are established between identical subunits. In particular, compounds such as curcumin and disulfiram exhibited higher affinity in this critical region compared to HsTPIWT. Enzymatic assays demonstrated the selective inhibitory potential of both compounds in HsTPIN16D, at concentrations ranging between 200 and 500 µM. Interestingly, these concentrations did not cause enzymatic inhibition in the WT enzyme. Furthermore, fluorescence experiments revealed distinctive alterations in the intrinsic fluorescence emission spectra upon incubation of HsTPIN16D with the compounds. In contrast to its WT counterpart, which experienced subtle structural alterations. These findings indicate important structural perturbations within the deamidated enzyme, potentially extending to the immediate environment of the fluorophores.

Conclusion. The deamidated enzyme emerges as a promising molecular target for the strategic design of pharmacologically potent molecules aimed at combatting cancer.

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P-7

Variations in the reading of genes belonging to the insulin signaling pathway in adipose tissue rehabilitated by ellagic acid treatment in rats induced by a high-fat diet.

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Introduction. Obesity is a global health problem that greatly affects the population, with Mexico being one of the countries with the highest rates of this condition, which is associated with a large number of diseases: insulin resistance, type 2 diabetes, high blood pressure, metabolic dysfunction associated with fatty liver disease (MAFLD) and also several types of cancers. The high-fat diet has been associated with disturbances in the insulin signaling pathway, particularly that related to glucose metabolism (Irs1, Irs2, Pi3k1r, Akt1, Tbc1d4, and Slc2a4). Resistin (RETN) is a cytokine that alters glucose homeostasis and its name is due to the resistance it generates to insulin, mainly in conditions of obesity and type 2 diabetes mellitus (T2DM).
Ellagic acid (EA) is a naturally occurring polyphenol found in various fruits and vegetables. Numerous studies have shown that ellagic acid has anti-inflammatory, antioxidant, and anti-apoptotic effects, which may represent a new treatment for insulin resistance.
Objective. To evaluate the gene expression of *Retn, Insr, Glut4, Akt, Pi3k* and *Tbc1d4* mRNAs and associated miRNAs in retroperitoneal and epididymal adipose tissues in an obese rat model treated with EA.
Materials and methods. 24 Male Wistar rats randomly distributed in three conditions (n=8 per group): standard diet (SD, untreated control), high-fat diet (HF, untreated obese) and high-fat diet plus EA (HF+EA) treatment. For the AE-treated group, a dose of 50 mg/kg per day was administered intragastrically for three months. Subsequently, the animals were sacrificed and adipose tissue was obtained, total RNA was isolated and purified by TriZol methodology, cDNA was synthesized by retrotranscription and the relative expression by ΔΔCT method of *Retn, Insr, Irs1, Irs2, Akt1, Pi3k, Tbc1d4* and *Slc2a4* was evaluated by qPCR. In addition, a network analysis was performed with the miRNET 2.0 network visual analitycs platform tool and identifying microRNAs and other interesting potential markers.
Results. After the intervention, we found a decrease in body weight and swelling of the treated rats compared to the untreated obese (FH) group. Quantitative analysis showed significantly that treatment with ellagic acid (HF+EA) reduced the expression of *Retn* and increased the expression *of Insr, Ins1, Ins2, Akt, Pi3k and Tbc1d4, Slc2a4* at the end of protocol.
Network analysis shows *Retn, Insr, Glut4, Akt, Pi3k, Tbc1d4, Slc2a4 and associated* microRNAs as potential markers of interest.

Conclusion. Treatment with EA has a modulatory and inhibitory effect on inflammation as well as on glucose metabolism in HF+AE rats by restauration of gene expression of glucose transport through increase Insr, Irs1, Irs2, Akt1, Pi3kr1 and Tbc1d4, Slc2a4 and decreasing Retn. Treatment with EA represents a possible alternative to insulin resistant associated with different clinical conditions.

P-8

Ellagic acid modulates the mRNA expression of FTO, NLRP3 inflammasome, TNF, and NFkB1 in adipose tissue of diet-induced obese Wistar rat.

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Introduction. Ellagic acid (EA) is a polyphenol, it can be found as precursor by the name of Ellagitannin as secondary metabolite in fruits and vegetables, such berries, nuts and wine, which can be metabolized by microbiome gut to liberate EA, it sustains anti-oxidant, anti-inflammatory, antimutagenic and antiproliferative properties.
Since ellagic acid possess anti-inflammatory properties, we can explore the ability to regulate different effects in the inflammatory response, in a variety of markers such Fat mass obesity associated FTO, Inflammasome NLRP3 (NLRP3, IkkA, IL-1β, IL-18 and Caspase1) Rela, and other pro-inflammatory genes like TNF and NFkB1.
Clinical studies in animal models confirm the potential role of EA as a therapeutical approach in the inflammatory process. FTO have been shown to affect obesity, body mass index, type 2 diabetes, cardiovascular disease, energy homeostasis, and inflammation. FTO exhibits efficient oxidative demethylation activity of abundant N6-methyladenosine (m6A). FTO knockdown with siRNA leads to an increased m6A level in mRNA, whereas FTO overexpression resulted in decreased m6A level in human cells. FTO suppresses the transcription of a distinct set of Interferon-stimulated denes (ISGs), including many know pro-inflammatory genes, and that this regulation requires its catalytic activity but is not through the actions of FTO on m6A but through the depletion of FTO that produces the activation of the transcription factor STAT3. Here we propose that EA produce a sequential inhibition of FTO transcription and expression of pro-inflammatory genes like TNF, NFkB1 Inflammasome NLRP3 and Rela.
Objectives. Evaluate the expression of FTO, NLRP3 inflammasome, TNF, and NfkB1 mRNA in qPCR
Methodology. Animal model: Wistar rats was randomly distributed into three groups: standard diet (SD), high-fat diet (HD), and high-fat diet plus ellagic acid (EA), with n=6 for each group. The EA dose of 50 mg/kg per day via intragastrical was administrated.
Retroperitoneal adipose tissue was obtained, isolated total RNA with TriZol methodology, synthesized cDNA and evaluated the expression of the mRNA of FTO, Inflammasome NLRP3, IL-1β, IL-18, TNFα, and NFkB1 in qPCR.
Results. Ellagic Acid reduces the expression of FTO, pro-inflammatory genes of inflammasome NLRP3: NLRP3, IL-1β and IL-18, Caspase1, and others: TNF and NFkB1 which expressions is reduced in group EA vs HD.
Conclusions. Ellagic Acid possess anti-inflammatory effects in obese Wistar rat adipose tissue, modulating the expression of FTO, inflammasome NLRP3 genes, TNF and NFkB1.

P-9

Identification of yeasts in children with and without Autism Spectrum Disorder.

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Background. The concept of autism has evolved from Bleuler's initial description to its current recognition as Autism Spectrum Disorder (ASD), encompassing diverse manifestations. Globally, it affects approximately 1 in every 160 children, with higher estimates in the Mexican context, about one in every 115 births, which makes early intervention difficult.
Aim. To identify the presence of yeast in individuals with and without ASD, with the purpose of understanding how this imbalance could influence the neurological symptoms of this disorder.
Results. A higher frequency of Nakaseomyces glabratus isolates was identified in children with ASD, as well as significant differences in yeast colonization between the two groups.
The findings suggest a connection between specific yeast species, especially Nakaseomyces glabratus, and ASD. This association could imply a modification in the gut microbiota, but further investigations with larger and more diverse samples are required to validate these assumptions.
Conclusion. The study raises the possibility of therapeutic interventions based on gut microbiota modification to improve symptoms associated with ASD. It highlights the importance of continuing research to better understand the relationship between gut microbiota and this disorder, evaluating its potential clinical impact.

P-10

Analysis of miRNAs and proinflammatory genes expression in semen from overweight and obese men.

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Background. Obesity promotes systemic oxidative stress is associated with proinflammatory cytokines overproduction and changes in microRNAs gene expression; Among the proinflammatory cytokines, the following stand out: interleukin-6 (IL-6) and tumor necrosis factor (TNFα). Systemic inflammation occurs in obesity as one of the damage responsible mechanisms on spermatogenesis process.
MicroRNAs (miRNAs) are non-coding single-stranded RNA sequences between 9 and 25 nucleotides length involved in the gene post-transcriptional regulation and also in DNA methylation as an epigenetic mark. Currently, the different microRNAs expression has been analyzed in overweight and obese semen samples without revealing the male reproductive system deterioration mechanism and the consequent state of subfertility or infertility. In our study we focused on measuring microRNAs and pro-inflammatory gene expression levels.
Aim. To describe overweight and obese men gene expression levels in semen, specifically from miR-146a microRNAs; miR-155-5p and miR-34a and their association with interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) mRNA expression in overweight and obese men compared with a normal weight control group.
Results. Overweight and obese men's groups have miR155-5p and IL-6 and TNFα increase gene expression compared to the control group.
Conclusion. microRNAs elevated gene expression in sperm, particularly miR-146a-5p; miR-155-5p and miR-34a-5p are related with IL-6 and TNFα under expression processes in overweight and obese men.

P-11

The effect of cadmium from smoking on the development of osteoarthritis of the knee.

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Background. The effect of tobacco use as a source of cadmium (Cd) exposure at the joint level has been little studied. It has been reported that exposure to tobacco smoke promotes oxidative stress (OS) as part of its damage mechanism and that this may be caused by some of its compounds such as Cd. *In vitro* studies by our research group have shown that Cd affects the viability of chondrocytes that produce OS.
Objective. Evaluate whether exposure to Cd by smoking increases cartilage damage through the generation of OS.
Materials and methods. Peripheral blood (PB) and synovial fluid (SF) samples from patients with OA were analyzed. Samples were stratified by smoking status, Kellgren-Lawrence score, pain and PB cotinine concentrations. Malondialdehyde (MDA), methylglyoxal (MGO), advanced protein oxidation products (APOPs) and myeloperoxidase (MPO) were assessed; the activity of antioxidant enzymes such as gamma-glutamyl transferase (GGT), glutathione-S-transferase (GST) and catalase (CAT) was determined, as well as the activity of arginase, which favors cartilage destruction. Finally, the concentration of Cd in PB was determined and correlated with oxidant and antioxidant molecules.
Results. When stratified according to smoking habits, smokers had a significant concentration of Cd (**P<0.01**). In relation to smoking, individuals <60 years of age had higher levels of MDA, APOPs and antioxidant system in OA-S. In relation to a Cd concentration **> 0.035 ng/ml**, there was a higher degree of pain and more severe OA, associated with a positive correlation with MDA, MPO, arginase and GGT (**P<0.01**) and a negative correlation with catalase (P=0.977).
Conclusion. Exposure to Cd through active smoking may be an important risk factor for the development of OA, presenting a positive activation with oxidant molecules and, on the other hand, reducing antioxidant enzymes such as catalase, influencing a greater degree of pain and OA.

P-12

Inhibition of Wnt/β-Catenin Signaling by ICRT14 Drug Depends of Post-Transcriptional Regulation by HOTAIR in Human Cervical Cancer HeLa Cells.

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Background. In Cervical cancer (CC), in addition to HPV infection, the most relevant alteration during CC initiation and progression is the aberrant activation of Wnt/β-catenin pathway. Several inhibitory drugs of this pathway are undergoing preclinical and clinical studies. Long non-coding RNAs (lncRNAs) are associated with resistance to treatments. In this regard, understanding the efficiency of drugs that block the Wnt/β-catenin pathway in CC is of relevance to eventually propose successful target therapies in patients with this disease.
Aim. To characterize the mechanism of HOTAIR regulation of Wnt/β-catenin pathway activation in the presence of drugsin CC cells.
Methods: We analyzed the levels of expression of 249 components of the Wnt/β-catenin pathway in a group of 109 CC patients. Three drugs that blocking specific elements of Wnt/β-catenin pathway (C59, NSC668036 and ICRT14) by TOP FLASH assays and qRT-PCR were tested in vitro in CC cells.
Results. 137 genes of the Wnt/β-catenin pathway were up-regulated and 112 down-regulated in CC patient's samples, demonstrating that this pathway is dysregulated. C59 was an efficient drug to inhibit Wnt/β-catenin pathway in CC cells. NSC668036, was not able to inhibit the transcriptional activity of the Wnt/β-catenin pathway. Strikingly, ICRT14 was neither able to inhibit this pathway in HeLa cells, due to HOTAIR interaction with β-catenin, maintaining the Wnt/β-catenin pathway activated.
Conclusions. These results demonstrate a mechanism by which HOTAIR evades the effect of ICRT14, a Wnt/β-catenin pathway inhibitory drug, in HeLa cell line. The emergence of these mechanisms reveals new scenarios in the design of target therapies used in cancer.

P-13

GDF11 reduces the aggressiveness of cells derived from human acute lymphoblastic leukemia.

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Background. The American Cancer Society estimates more than 60530 new cases and 23100 deaths per year due to leukemia, among which acute lymphoblastic leukemia (ALL) stands out. In Mexico, malignant neoplasms occupy first place in the child population (5-9 years). GDF11 has been shown to display antineoplastic properties.
Aim. Determine the effect of GDF11 on human acute lymphoblastic leukemia (Jurkat).
Results. The data suggest an activation of the canonical pathways (smad) after 5 minutes. No changes were found in proliferation, morphology, or viability, neither in glycolytic metabolism, but in the modulation of the oxygen consumption rate and related to the levels of reactive oxygen species, which was reflected in direct regulation of the FOXP3 factor, a marker of aggressiveness. The migration assay corroborated that GDF11 reduces the migratory capacity of Jurkat cells by a mechanism dependent on FOXP3 repression, indicating a decrease in aggressiveness. Furthermore, a clear reduction in FOXP3 expression was demonstrated in the nucleus.
Conclusion. GDF11 reduces the aggressiveness of LLA cells by compromising their metabolism.

P-14

Characterization of HGF-induced protection mechanisms in a murine model of experimental cholestasis and search for new therapeutic targets.

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Background. Intrahepatic cholestasis is a syndrome characterized by partial and total obstruction of bile flow, triggering chronic inflammation and excessive production of reactive oxygen species (ROS) in hepatic tissue. It has been reported in *in vitro* models that Hepatocyte Growth Factor (HGF) regulates the NADPH oxidase enzyme in liver cells through a dual action: activation and generation of ROS, to promote hepatoprotective signaling. Furthermore, in *in vivo* models, physiological protection against cholestatic insults to involved organs such as the liver, intestine, and kidneys has been reported, evidenced by macroscopic and microscopic reduction in damage.
Aim. Characterize the mechanisms of systemic protection induced by HGF in experimental cholestasis.
Methods. Male CD1 mice aged 8-10 weeks were randomly divided into 4 experimental groups: 1) untreated control group (NT), 2) ANIT-treated group via intragastric administration at a dose of 60 mg/kg, 3) ANIT+HGF-treated group, where HGF will be administered at a dose of 10 µg/kg intravenously 24 hours after ANIT administration, and 4) control group treated only with HGF. Mice were sacrificed at 30 h, 36 h, and 48 h post-treatment initiation for liver tissue and serum collection. The collected samples were used for biochemical assays, Western Blot, immunofluorescence, immunohistochemistry, TBARS, and H&E staining.
Results. Using α-Naphthylisothiocyanate (ANIT) as a model of intrahepatic cholestasis administered with HGF in murine subjects at different time intervals, it was observed that at 30 hours after the initiation of the experiment, the presence of HGF in a context of cholestatic injury activates the MAPK/ERK signaling pathway and favors the activation of NRF2 pathway, translocating into the nucleus to initiate the transcription of genes such as γ-GCS, GSTm, and GPx, all of which are related to antioxidant response. This was reflected in the reduction of lipoperoxidation, necrotic areas, inflammatory infiltrate, and biochemical tests of serum damage. Furthermore, it was also observed that HGF, in order to diminish this damage, promotes cell cycle regulation, thereby encouraging proliferation. At 36 and 48 hours of treatment, a decline was observed in both HGF/c-Met mediated signaling and the transcription of antioxidant genes, correlated with an attenuation of ROS-induced damage.

Conclusion. Our findings support the hypothesis of HGF as a hepatoprotector in cholestatic injury by activating the MAPK/ERK pathway, promoting antioxidant gene expression, and reducing damage. However, a decline in HGF/c-Met-mediated activity and antioxidant gene transcription is observed over time, suggesting a progressive attenuation of ROS-induced damage. **CONAHCYT: 1320.**

P-15

The consumption of a high-cholesterol diet induces acute pancreatitis in the mouse.

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Background. Acute pancreatitis (AP), one of the gastrointestinal diseases that most frequently requires hospital care, is a sudden inflammatory process of the pancreas associated with the release of hydrolytic enzymes, and inflammatory cytokines that seriously damage the organ. (Lee & Papachristou, 2019). On the other hand, cholesterol is a vital compound, essential in cell membrane formation, sex hormone production, and bile salt formation, among other functions (Yang et al., 2013). Although each lipid has specific effects on cells and tissues, excess cholesterol accumulation induces cell dysfunction. Despite its relevance, there are no studies on the impact of a high-cholesterol diet on the onset and progression of acute pancreatitis.

Aim. Determine the mechanism of damage produced at a pancreatic level by a high-cholesterol diet.

Results. Our data showed that the high cholesterol (HC) diet increased visceral fat content in the animals. Cerulein (Cn), an inductor of AP, increased pancreatic weight concerning body weight after 30 days of treatment. The HC diet alone, HC plus Cn, and Cn alone increased serum amylase and lipase activity by approximately 50% compared to the NT group, which is associated with AP. Serum total cholesterol (TC) and total triglycerides (TG) content did not present a difference; however, content in the pancreas, TC increased in Cn treatment, and TG increased in the HC diet, with no difference in HC plus Cn. Histological studies showed that the HC diet-induced fat deposition in the parenchyma and edema, cellular disruption, and inflammatory infiltrate, parameters that increase in Cn treatments, are characteristic of AP. Also, the HC diet produced oxidative stress by increasing

superoxide anion and lipid peroxidation. Finally, we analyzed the ERK survival pathway by Western blot. Results show an increase in pERK/ERK in HC and Cn groups.

Conclusion. Data show that the HC diet induces acute pancreatitis, increasing serum amylase and lipase activity and producing oxidative stress. As a result of pancreatic damage, pERK/ERK content increases as a survival mechanism. Cholesterol-enriched diets could promote pancreatic disease.

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P-16

Study of growth factor 11 (GDF11) as a possible regulator of the redox state in mouse liver damaged by acetaminophen administration.

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Background. Growth and Differentiation Factor 11 (GDF11) belong to the TGF-β superfamily, which can detract aggressiveness to hepatocarcinoma-derived cells (HCC). On the other hand, the model of drug-induced liver injury (DILI) caused by Acetaminophen (APAP) has shown that the generation of the reactive metabolite NAPQI (N-acetyl-p-benzoquinone imine) is responsible for the decrease of hepatic glutathione and the onset of toxicity, altering the cellular REDOX state and generating a necrotic cascade to hepatocytes. It is reported that the cellular REDOX state is fundamental for hepatic homeostasis due to its participation in signaling, especially in key processes such as proliferation, differentiation, apoptosis, and even survival. However, there are still no reports on the effect of GDF11 on the REDOX state in a DILI model.
Aim. Determine the effect of GDF11 on REDOX signaling in an APAP-induced DILI model.
Results. We divided 4 groups of four 12-week-old CD1 male mice (NT, APAP, GDF11, GF11+APAP). NT mice received no treatment, mice in APAP and GDF11+APAP groups were given (300 mg/kg) APAP with 12h fasting, and 12h later GDF11 (0.1 mg/kg) was administrated to the GDF11+APAPAP and GDF11 groups. Mice were sacrificed 36h after APAP injection next serum and liver were obtained. The biochemical profile was performed in serum in which AST and ALT activity was measured to determine the hepatic damage caused by APAP to see the effect of GDF11, curiously we did not observe significant differences. To verify this event, we performed a TBARS assay to that damage by reactive oxygen species (ROS) through lipoperoxidation, in which again we didn´t find significant differences, therefore, we can hypothesize that APAP and GDF11 didn´t generate hepatic damage which may be due to the exposure time of APAP in the organism. Finally, we analyze by western blot the role of the cell cycle in the DILI model induced by APAP in which we measured the proteins Cyclin A, Cyclin D1, Cdk6, Cdk4, p21, and p27 where we only found significant differences in the content of Cyclin D1 protein in GDF11 group which indicates an alteration in the G2 phase of the cycle which is the phase that precedes mitosis and whose protein regulates the transition of this step, which suggests that GDF11 promotes the cell proliferation.
Conclusion. The treatment with APAP at 36h doesn't present hepatic damage, to the other hand GDF11 doesn't promote or diminish the liver injury at the protein level GDF11 presents an increase in the content of Cyclin D1 involved in the transition from the G2 phase to mitosis, we suggest carrying out the treatment at short times to know the effect of GDF11 in a hepatic damage model.

P-17

Dietary biotin supplementation increases proliferation pathways in mice testes without affecting serum follicle-stimulating hormone levels and stem cell factor expresión.

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Background. Supplements containing pharmacological concentrations of biotin are commercially available over the counter. Classical toxicity studies have considered biotin administration as harmless, particularly because of the therapeutic potential of biotin supplementation in the treatment of diabetes, metabolic syndrome; however, recent investigations have shown that biotin supplementation modifies tissue morphology, without changes in toxicity markers, raising concerns about the consequences of morphological changes on tissues' functions and the safety of pharmacological concentrations of the vitamin. Testes are very sensitive to toxicants, and testicular histology is a reliable method to study its function. In this work, we investigated the effects of dietary biotin supplementation on testis morphology and molecular pathways and cell proliferation activated by biotin.
Aim: In this work, we investigated the effects of dietary biotin supplementation on testis morphology and molecular pathways activated by biotin.
Methods. Male BALB/cAnNHsd mice were fed a control or a biotin-supplemented diet (1.76 or 97.7 mg biotin/kg diet) for eight weeks. We evaluate ERK & AKT pathways by WB, cell cycle genes expression qRT-PCR, hormones from hypothalamic-pituitary-testicular axis by ELISA.
Results. Compared with the control group, the biotin-supplemented mice presented augmented protein abundance of the c-kit-receptor and pERK1/2Tyr204 and pAKTSer473, the active forms of ERK/AKT proliferation signaling pathways. Analysis of mRNA abundance found an increase in cyclins/cyclin-dependent kinase complexes, and cell cycle inhibitors; the mRNA expression of activators of the cell cycle, *Cyclin D3, Cdk4, Cyclin E, Cdk2, Cyclin A2*, and the transcription factor *E2F*, were increased; the mRNA expression of *p21* was decreased, and notwithstanding with the induction of cell cycle proliferating cyclins, *p16* was decreased; these effects were associated with augmented mRNA expression of *Sp1* and *Sp3* transcription factors and increased CKIT-receptor expression, proteins associated with spermatogonia proliferation. Interesting, no changes were observed in the testis expression of the stem cell factor (SCF) and in the serum levels of the follicle-stimulating hormone (FSH), the effects of biotin- supplementation on cell proliferation of testes were not elicited via the hypothalamic-pituitary-testicular axis.
Conclusion. The biotin supplementation modifies testicular tissue morphology, increased number of elongated seminiferous tubules, which presented cellular disorganization, modification in the germinal epithelium and more than three spermatogonia layers, due to increased spermatogonia proliferation, through a molecular mechanism involving increased

protein expression of c-kit-receptor, the active forms of ERK/AKT signaling proteins, and Cyclin D3, with the consequent activation of the cell cycle machinery at the G1/S boundary during cell proliferation; In accordance with its role as a cyclin dependent-kinase inhibitor, Cell proliferation was independent of serum follicle-stimulating hormone levels and the stem cell factor protein expression, indicating that the effects of biotin- supplementation on cell proliferation of testes were not elicited via the hypothalamic-pituitary-testicular axis. Therefore, the effect of biotin is *in situ*. This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT 219787 and A1-S-10101), Tonatiuh Salazar-Anzures was a doctoral student from the Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM), and received fellowship CVU/Becario 421313 from CONACyT, México.

P-18

A high-cholesterol diet induces inflammation and morphological changes in the mouse Gallbladder.

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Background. Gallbladder cancer is a highly lethal disease and is one of the most common cancers of the biliary tract. It is usually diagnosed in advanced stages and has an unfavorable prognosis. It is often discovered incidentally in tissue samples after gallbladder removal surgery due to biliary cholelithiasis. For GBC, there are currently no recognized specific biomarkers. The etiology of this disease is poorly understood, but cholelithiasis is a common sign in patients with GBC. Due to the main route of cholesterol elimination being the biliary tract and the gallbladder being the destination, we are interested in studying the impact of cholesterol overload induced by consuming a cholesterol-enriched diet in male and female mice.
Aim. To characterize the effects of consuming a high-cholesterol diet in the mouse gallbladder.
Material and methods. Eight-week-old male and female C57BL/6 mice were fed a highcholesterol (HC, 2%) diet for 30 days. After this time, the mice were euthanized, and serum, gallbladder, and liver were collected for biochemical and histological studies.
Results. Our data show that the HC diet did not induce physiological changes in mice after one month of treatment and did not induce changes in gallbladder weight at one month. Furthermore, the HC diet doesn't cause liver damage at one month of treatment. On the other hand, the histological studies addressed by H&E staining showed that the HC diet induces morphological changes in the gallbladder tissue (epithelium, lamina propria, serosa) and an inflammatory response in the gallbladder, particularly neutrophils judged by myeloperoxidase immunofluorescence (IF) and without macrophage infiltration according to F4/80 IF, at this time of the diet.
Conclusion. A high-cholesterol diet for 30 days induces inflammation of the gallbladder, particularly by neutrophil infiltration and morphological changes in the gallbladder tissue.
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P-19

Subchronic cadmium effects in the liver of a murine model

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Background. Non-alcoholic fatty liver disease (NAFLD) is an umbrella term for liver diseases that are characterized primarily by storage of excess vesicular fat because a perturbation of the homeostatic mechanism that regulate synthesis versus utilization of fat in the liver. The mechanisms for NAFLD pathogenesis are not completely understood. Epidemiological findings showed that cadmium (Cd) exposure is associated with the development of NAFLD. Cd is a widely dispersed element, that is naturally occurring in the environment, but also by anthropogenic activities. Once in the environment, Cd moves easily through the soil and is taken up into the food chain. Cd is known for its long half-life, and once it enters the body, it can cause damage to several organs, among them the liver. Studies suggest that autophagy is a way to degrade lipids besides cytosolic lipases. It has been reported in an in vitro model that Cd impairs autophagy process in hepatocytes obtained from mice with high cholesterol diet. We are interested in studying cadmium effect in the liver after a subchronic exposure.
Aim. To explore the effects of cadmium ingestion during one month in the liver.
Results. We exposed CD-1 mice of 8-12 weeks old to subchronic (4 weeks) non-toxic, relatively low concentration of Cd (10 mg/L) via drinking water and Chow diet ad libitum. We evaluated the water and food consumption during this period, that were similar between control and experimental groups. Liver macroscopical evaluation didn't present difference as well as the liver weight nor liver/body weight ratio. We evaluated parameters such as lipid peroxidation by TBARS and the content of antioxidant enzymes without finding changes in these parameters. Hepatic liver function enzymes determined in serum didn't change after 4 weeks treatment. Neither changed the content of cholesterol and HDL-c in serum. Only a reduction in the triglyceride content in serum of mice treated with Cd was found. Also, triglyceride and cholesterol content in liver were determined but we didn't find changes. We did a morphometric analysis in samples stained with H&E, showing that Cd induced an increment of pyknotic nuclei, an indicative of hepatocytes in regeneration.
Conclusion. Ingestion of drinking water with 10 mg/l of CdCl2, during 4 weeks by CD-1 mice, did not affect liver macroscopically, neither alter the redox balance, liver function test enzymes and the triglycerides and cholesterol liver content. Although no apparent damage was found, an interesting result is that Cd promotes the increment of pyknotic nuclei, that

shows regenerating hepatocytes, indicating that damage cause by Cd is being counteracted at this time and with this Cd concentration.

P-20

In Silico Screening of Drugs That Target Different Forms of E Protein for Potential Treatment of COVID-19.

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Background. Recently the E protein of SARS-CoV-2 has become a very important target in the potential treatment of COVID-19 since it is known to regulate different stages of the viral cycle. There is biochemical evidence that E protein exists in two forms, as monomer and homopentamer. An In silico screening analysis was carried out employing 5852 ligands (from Zinc databases).
Aim. To select the most promissory ligands through computational techniques which include: molecular dynamics simulations and free energy MM-PBSA calculations of the protein- ligand complexes.
Results. From our computational studies we could identify five compounds: irinotecan, alectinib,saquinavir, nilotinib and dutasteride which were best evaluated. Particularly, alectinib could inhibit the functions of the ion channel as well as avoiding the binding of other proteins involved in pro-inflammatory processes. On the other side, saquinavir, nilotinib and alectinib were also considered as a promising multitarget ligand because they seem to inhibit three targets. Additionally, saquinavir was shown to be able to bind to E protein both in its monomeric as well as pentameric forms so it could act in different steps of the viral replication cycle.
Conclusion. The following ligands: nilotinib, dutasteride, irinotecan, saquinavir and alectinib were the best evaluated. We thank CONAHCYT for Project Ciencia Frontera CF-2023-G-2024

P-21

Effect of Sulforaphane or Dasatinib + Quercetin on cognitive impairment and neuroinflammation in an experimental model of chronic obesity in middle-age female Wistar rats

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Introduction. Obesity is a worldwide health problem. In Mexico, the highest prevalence is observed at 59 years of age, particularly in women. This disease induces neuroinflammationandaccumulation of senescent cellsin the brain,processes that have been related to cognitive deficitduring aging. Senolyticsare molecules that eliminatesenescent cells (such as dasatinib+quercetin, D+Q) and senomorphics are molecules that modulate SASP (such as sulforaphane, SFN); bothhave been reported to have beneficial effects on organisms.However, the effect of these molecules on neuroinflammation and cognitive declinerelated to chronic obesityin femalesis unknown.
Objectives. To determine the effect of chronic obesity on cognitive decline. To analyze the expression of local and systemic inflammation markersandfinally, toanalyze the effect of the senotherapy in our model.
Methods. Female Wistar rats were fed with a hypercaloric diet (HD) from 21 days of birth until they reached middle age (14 months). SFN was administrated subcutaneously (0.5 mg/kg, 5 days a week) and D+Q by nasogastric tube (5 mg/kg and 50 mg/kg respectively, once a month). Both treatments were administered from 12 to 14 months of age. Memory and learning at 5, 10, and 14 months of age were evaluated using the Novel Object Recognition test (NOR) and the Barnes maze test. The expression of inflammation markers (IL-1β, IL-6, TNF-α, IFN-γ) in cerebral cortex, hippocampus, and serum wasdetermined byELISA assay.
Results. HD-fed rats showedmild cognitive impairment from 10 months on, which becameevident at 14 months of age compared to the group fed with a standard diet (SD). Senolytictreatmentdid not prevent the cognitive deficit, while SFN improved the performance in the NOR and Barnes maze. The cognitive deficit in HD rats was associated with an increase in the expression of markers of local and systemic inflammation, which werereversed with senotherapy, with sulforaphane being more effective.
Conclusion. Chronic obesity is associated with cognitive deficit and neuroinflammation in middle-aged female rats. These effects could be prevented by modulating the SASP with the senomorphic, but not by eliminating senescent cells with the senolytics. We thank Dr. María de los Ángeles Guerrero-Aguilera from UAM-I for providing the necessary animals needed for this project. This work was supported by FORDECYT-

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P-22

Participation of Cholinesterases (ChEs)in the development of Nonalcoholic Fatty Liver Disease (NFLD)

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Background. For many years themain roleof cholinesterases has been described as neuronal function; however, in the last years it has been observed that they have participation in other biological events, such as cell proliferation, inflammatory processes, metabolic disorders, and detoxification. In the case of the Acetylcholinesterase (AChE), this enzymehas been characterized as the mainhydrolyzer of Acetylcholine(ACh), which has an anti-inflammatory function and has recently been describedas an actor in the control ofhypoglycemia.Therefore, the enzymatic activity of the AChE is relevantfor the control of these processes.One the other hand BChE has been identified as a participant in energy control through satiety, hydrolyzing to the hormone ghrelin. Therefore, bothenzymesconverge in the control of nutritionand the disposition of nutrients in glycodependent tissues such as the liver, where they could play a role in the appearance and development of nonalcoholic fatty liver.
Aim. Determinatethe alteration of enzyme activity induced by a high fructose diet and changes in acetylcholine concentration.
Method. The enzyme activity was measured by Ellman´s method and was normalized by Bradford´s method.The AChconcentration was quantified by kit,assuggest the fabricant.
Results. We observed a decrease in serum BChE activity in the third month of treatment, however, from the fifth month onwards the activity increased compared to the control. In the case of liver tissue, the activity of both enzymes increases in the treated group from the fifth month onwards. In correlation with the enzymatic activity of both ChE, the concentration of ACh decreases in the liver tissue from the fifth month onwards.
Discussion. The consequence of low BChE activity is a poor degradation of ghrelin (as suggested by various authors), this means that there is an increase in ghrelin and consequently an increase in hunger. This encourages greater consumption of foods that provide immediate energy, such as carbohydrates (fructose); however, the favored metabolic pathway is de novo lipogenesis due to excess intake that occurs in the liver, which produces an increase in intracellular fat in the liver tissue. In addition to de novo lipogenesis, the consumption of a high-fructose diet has inflammatory effects on adipose tissue that results in poor internalization of fatty acids, and consequently, these fatty acids are expelled for storage in the liver, strengthening the presence of fatty liver. Furthermore, the increase in the enzymatic activity of both ChE in the liver tissue, starting from the fifth month, could contribute to the establishment of pro-inflammatory conditions due to the low concentration

of ACh caused by the high enzymatic activity. These conditions could favor an accelerated transition from a fatty liver to nonalcoholic steatohepatitis. (NASH).

Conclusion. The catalytic activity of ChEs has effects on energy control and inflammation that are key points for the development of NAFLD, therefore, the alteration of these enzymes could result in the appearance of NAFLD.

P-23

Alteration of the central nervous system produced by the effect of Bisphenol-A in chickembryos

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Background. Bisphenol A (BPA) is an industrial chemical used in the synthesis of polycarbonates, epoxy resins, and thermal paper, therefore it commonly appears in various products for daily use. Its mutagenic effect, the production of reactive oxygen species, and its ability to act as a selective estrogen receptor modulator (SERM) have been documented, therefore, it can alter biological systems in small doses. Food and water are the most important source of exposure for the human population, with an exposure dose for humans documented at 0.23mg/kg/day.
Aim. To understand the histological alterations producedin the central nervous systemby direct exposure to Bisphenol A during the embryonic development of the chicken.
Results. With the first dose, no results were obtained in terms of malformation and mortality; increasing the dose resulted in an almost exponential increase in both malformations and mortality at the same time. A double dose on day two of development was toxic and consequently caused significant mortality. The most frequent malformations were "disorganized embryos" (40%) along with those in the CNS; embryos exhibited anencephaly (25%), exencephaly (20%), and alteration of the brain vesicles (15%). Under microscopic observation, significant dysmorphology with suggestive apoptotic bodies of the somatic mesoderm was observed.
Conclusion. Bisphenol A (BPA) increases the frequency of morphological alterations that occur spontaneously in the species, with alterations in the neural tube being one of the most frequent; anencephaly, exencephaly, and alteration of brain vesicles predominate. At the histological level, there were no significant changes in the spinal column with a dose of 0.46 mg/kg, but neural tube malformation was observed in disorganized embryos.

P-24

Effects of naringenin on diabetes kidney disease.

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Background. Chronic hyperglycemia activates metabolic pathways promoting oxidative stress, endothelial dysfunction, hypertension,and inflammationinstead leading tokidney damage. Naringenin is a flavanone found in fruits from the citrus genusthat has shownanti-diabetic, antioxidant, and anti-inflammatory activities. The aim was to evaluate the effect of naringenin on diabetic kidney disease.
Methods. Male Wistarrats (280-300 g) were as signed to control, diabetes (streptozotocin 55 mg/kg i.p.), and diabetes + naringenin (50 mg/kg/day gavage). All the groupsBody weight, blood glucose, leptin, blood pressure, anddiuresis were analyzed. Kidney tissue samples were obtained at 30 days of follow-up under anesthesia. As renal damage markers creatinine, BUN, and proteinuria were determined. Renal hypertrophy index and oxidation of lipids and proteinsin the kidney were assessed.
Results. The diabetic animals showed, hyperglycemia, polydipsia, polyphagia, polyuria and body weight loss compared to the control group. In diabetic the blood pressure increased from day 15 until the final of the study. Diabetes induced increase in BUN, and creatinine in serum and urine, which was associated to an increased creatinine clearance, and proteinuria. Alterations in renal function observed in the group with diabetes were related to renal hypertrophy and increase in the lipid and protein oxidation in kidney. After 30 days of treatment with naringenin, hyperglycemia, polydipsia, polyuria, and polyphagia were decreased. The decrement in polyphagia was related to increase in plasma leptin levels. Naringenin reduced hypertension, hypertrophy andattenuates the lipid and protein oxidation in the kidney, improving renal function during diabetes.
Conclusion. Naringenin through the attenuation of hyperglycemia, hypertension,and oxidative stres sattenuates kidney damage diabetes-induced. Further studies are required to understand the antihypertensive and antioxidant mechanisms.

P-25

Differences in redox state of primary rat cerebral cortex astrocytes induced to cellular senescence or gliosis with palmitate.

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Background. Obesity is a global health problema related to brain deterioration and cognitive decline. To study the mechanisms underlying this phenomena, here we used sodium palmitate (PA) to mimicthepathological neuroinflammatoryand oxidative environment caused by obesity. Astrocyte s are the most abundant cells of the Central Nervous System (CNS) and show an adaptive plasticity that defines the functional maintenance of CNS. As a response to astressful stimuli, astrocytes can enter in a state of cellular senescence (SC) or be activated to gliosis. Both phenotypes play an important role in the establishmentof neuroinflammation and neurodegenerative diseases associated with aging.
Aim. Our aim was to comparedthe redox state of these two phenotypes to understand their differential participation in the neuroinflammation caused by obesity.
Methodology. Primaryastrocytes were isolated from Wistar rats brain cortex and treated for 24 h with different concentrations of PA to induce gliosis (40 μM) orsenescence (200 μM). Markers of senescence (γH2AX, Lamin B1, p16 and SA-β-Gal) or gliosis (C3, S100A10 and GFAP) were evaluated to confirm the phenotypes; GHS and GSSG concentrations were determined by HPLC to assess the redox status.
Results. PA treatment significantly modified celular proliferation, which decreased in senescence and increased in gliosis. The SA-β-Gal and safranin assay showed that 200 μM PA treatment increased the number of senescent cells, but not the control or the 40 μM treated cells. The redox state was more significantly more oxidized in both PA treated astrocytes when comparted to control cells, but was even more oxidized in senescent than in gliotic cells.
Conclusion. We confirmed the induction of the differentastrocytes states depending on the PA concentration used. Both phenotypes present differences in the redox state between them and compared to the control, with senescent astrocytes presenting a more oxidized state. We thanks to Dr. Ángeles Guerrero-Aguilera. This Project is financed by -FORDECYT-PRONACES/263957/2020. LTM and AGKE are CONAHCyT scholarship holders.

P-26

Electroencephalographic profile of Salvia amarissima Ortega and amarisolide A in the absence and presence of PTZ-induced seizures in mice.

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Background. Epilepsy is one of the most severe and most prevalentbrain disorders in the world, affecting more tan 70 million people. This is characterized by a long-lasting predisposition to generate recurrent, spontaneous, and unpredictable epileptic seizures. Although several antiepileptic drugs are available to control different types of epilepsies, they are not enougheffective and safe to completely control seizures. Thus, othertherapeutic alternatives such asthe use of natural products of plant originare required. Salvia amarissima Ortega is a plant used in traditional medicine to treat CNS conditions. Despite its depressant properties in anxiety and fibromyalgia, there is no scientific evidence for its ability to control seizure activity.
Aim. To investigate the effects of the S. amarissimaaqueous extract (SAAE) and its metabolite amarisolide A (AMA) on the electrocorticographic (ECoG) activityalone and using thepentylenetetrazole(PTZ)-induced tonic-clonic seizures, and by evaluating the involvement of the opioid, GABAA, and 5HT1Aserotonin receptors as a possible mechanism of action.
Methods. For this purpose, SW mice were implanted for ECoG recording organized into 16 groups (n=8) receiving an intraperitoneal acute dose of the treatments. The ECoG profiles were previously and concurrently analyzed to the PTZ (85 mg/kg, i.p.)-induced seizure behavior after thirty min of the administration of several doses of the SAAE (1, 10, 30, or100 mg/kg, i.p.) and two doses of AMA (0.5 or1 mg/kg, i.p.). A dosage of AMA (1 mg/kg,i.p.) was selected to explore a possible mechanism of action using antagonists of inhibitory receptors such as picrotoxin (1 mg/kg, i.p.) for GABAAreceptor or WAY100635(1 mg/kg, i.p.)for 5-HT1Aserotonin receptor. Results: Significant changes in the frequency bands and the spectral power were observed after the treatment alone. Additionally, SAAE and AMA produced significant and dose-dependent anticonvulsant effects by reducing the incidence and severity of seizures and increasing latency or survival. Both antagonists partially prevented the effects of AMA in the severity score of seizures and survival during the tonic-clonic seizures.
Conclusions. In conclusion, our preclinical data support that S. amarissima possesses anticonvulsant properties, in part due to the presence of amarisolide A, mediated by different inhibitory mechanisms of action. Our scientific evidence suggests that this Salviaspecies

and amarisolide A are potential neuroprotective alternatives to control seizures in epilepsy therapy. Acknowledgments: To the project NC123280.0 and CONACYT 256448.

P-27

Antinociceptive effects of Raphanus sativusL. cv. Sango sprouts involve the opioid and 5-HT1A serotoninreceptors, cAMP/cGMP pathways

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Background. Raphanus sativus L. cv. Sango, commonly known as red radish, is widely consumed around the world as a vegetable, but its benefit in pain relief is not sufficiently investigated.
Aim. This study aimed to evaluate the anti-nociceptive effects of R. sativusand a possible mechanism of action.
Methods. An aqueous extract of R. sativus sprouts (AERSS) was investigated by parenteral (10, 30, and 100 mg kg−1, i.p.) and enteral (500 mg kg−1, p.o.) administration in the neurogenic and inflammatory phases of the formalin test, where gastric damage was also evaluated as a possible adverse effect. Ketorolac (5 mg kg−1, i.p.) was used as the reference drug. Endogenous opioid and 5-HT1A serotonin receptors, as well as the cAMP/NO-cGMP pathways, were explored in the study of a possible mechanism of action by using their corresponding antagonists: naloxone, 1 mg kg−1, i.p., WAY100635, 1 mg kg−1, i.p., and enzymatic activators or inhibitors, respectively.
Results. A significant and dose-dependent antinociceptive activity was observed with the AERSS resembling the antinociceptive effect of the reference drug, with an equivalent significant response with a dose of 500 mg kg−1, p.o. without causing gastric damage. The participation of the endogenous opioid and 5-HT1A serotonin receptors at central and peripheral levels was also observed, with a differential participation of cAMP/NO-cGMP.
Conclusion. Our preclinical evidence supports the benefits of consuming Raphanus sativus cv. Sango sprouts for pain relief.
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P-28

Raman spectroscopy: Towards a possible implementation in the clinical diagnostic field

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Background. While molecular spectroscopy has been known for its great versatility in different fields for a long time, Raman spectroscopy has historically seen limited utilization within the biomedical sciences.Raman spectroscopy'smain strength isdiscerning between different chemical bonds and translating said particularities into wavenumbers, allowing for the display of a spectral profile that represents the analyte.In this presentation, we describe the principle of using Raman spectroscopy as a histopathological diagnostic tool, employing triple negative cancer cells from both murine and human cell cultures to provide the foundationforintegratingthis instrument in the medical field.
Aim. To describe how Raman spectroscopy and artificial intelligence could be included as supporting techniques in the medical field for diagnosing different pathologies, using breast cancer as our main focus.
Methods. We obtained Raman spectra from three cellular lines: 4T1, found in murine hosts, MCF7, and MDA, both found in human hosts. We used RPBI as their culture medium, with 10% bovine fetal serum added toreplicate physiological conditions. The acquisition of Raman spectra was made by a Raman micro-spectrometer; allowingus to obtain single spectrums from a specific cell point, spectrum lines that cross cell regions, and spectrum maps from an entire single cell. We applied multivariate statistics and machine learning tools to analyze the large amount of data obtained.
Results. TheRaman signals we obtainedcan be used to distinguish betweeninside the cells, the cell boundaries, and the cellular matrix. We have insights that it can be possible to differentiate intracellular components, like proteins. The implementation of the multivariate analysis makes possible these findings since we are analyzing hundreds of Raman spectra and optical images.
Conclusion. We can conclude that Raman spectroscopy not only can give us specific molecular information but also be combined with artificial intelligence to increase their capabilities.

P-29

Interferon-stimulated gene 15 and ISGylation are upregulated in glioblastoma.

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Background. Interferon-stimulated gene 15 (ISG15) encodes for a 15 kDa protein consisting of two ubiquitin-like domains. ISG15post translationally modifies proteins bycovalent binding to lysine residues on its target proteins through a catalytic process that is denominated ISGylation. ISG15 can be found in non-conjugated form (free ISG15), which is secreted to act as a cytokine-like protein in some cellular contexts. Protein ISGylation is associated with modulation of protein stability and protein-protein interactions. The expression of ISG15 in some types of cancer is deregulated; however, in glioblastoma, a highly aggressive brain tumor, ISG15/ISGylation levels have not been thoroughly studied.
Objective. Determine ISG15/ISGylation levels in glioblastoma cells.
Methodology. Glioblastoma-derived cell linesand glioblastoma tissue samples were analyzed. The detection of ISG15/ISGylation in glioblastoma cells with or without treatment with IFN-γ was analyzed using immunoprecipitation and western blotting assays.To detect ISG15 protein in glioblastoma tissue, an immunohistochemical assay was performed on tissue microarrays. Finally, the determination of gene expression in glioblastoma-derived cell lines was performed in silico using data from databases such as EMBL-EBI, UALCAN.
Results-Discussion. ISG15 protein levels are significantly higher in glioblastoma than healthy brain tissues and up-regulated in response to IFN-γ stimulus. Furthermore, higher ISG15 expression is associated with shorter survival time in glioblastoma patients, suggesting that ISG15 and ISGylation may play a central role in glioblastoma progression.
Conclusion. IFN-γ enhances ISG15/ISGylation levels in glioblastoma cells. Increased levels of ISG15 (mRNA and protein)/ISGylation may be a biomarker in glioblastoma.

P-30

Renoprotective effect of the Empagliflozin/Metformin treatment in an experimental model of Metabolic Syndrome.

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Background. Metabolic syndrome (Mets) is a set of risk factors (RF) including obesity, dyslipidemia, insulin resistance, hypertension, hyperglycemia, and others, which contribute to the development of diabetes and cardiovascular diseases. Recent studies have reported that these RF independently, as well as when occurring together, increase the risk of renal damage, with greater risk observed as more risk factors coexist. Metformin and sodium-glucose cotransporter 2 inhibitors (SGLT2i) are drugs used for the treatment of diabetes, due to their effects on several RF, these drugs could serve asan alternative therapy for protecting the kidney from damage caused by Mets.
Aim. To assess the effects of empagliflozin/metforminco-therapyon renal dysfunction in an experimental model of metabolic syndrome.
Methods. Male Wistar rats were used to induce Metswith a Paygen and sweetened drink diet. At thirty days, the animals were randomly assigned to Control (C), Mets (MS), Empagliflozin (12.5 mg/kg), Metformin (850 mg/kg), and Empagliflozin/Metformin (12.5/850 mg/kg). We evaluated biochemical parameters [glucose, triglycerides, total cholesterol, HDL colesterol (HDL-c), creatinine, BUN], blood pressure, biomarkers of kidney damage, and theactivity of mitochondrial complexes, and antioxidant enzymesin the kidney cortex.
Results. Before the administration of treatments, body weight, fasting blood glucose, blood pressure, total cholesterol, and triglycerides were increased in theMS group. Additionally, HDL-c was decreased in thisgroup. Creatinine and proteinuria were observed in the MS groups without treatment, suggesting deterioration of kidney function. Thirty days of treatment demonstrated that co-therapy is more effective in reducing weight, lipid profile, systolic pressure, and fasting blood glucose compared to stand-alone therapy. Regarding renal function, decreases in creatinine, proteinuria, and BUN levels, as well as in blood pressure were observed with the co-therapy and were not different from the independent treatments. Co-therapy also reduces oxidative stress by improving glutathione peroxidase, glutathione reductase, and superoxide dismutase activities, and possibly by enhancing the activities of mitochondrial complexes I, and II.

Conclusion. Our results demonstrate that the empagliflozin/metformin combination is effective in reducing RFand improving renal dysfunction caused by Mets. Renal protection was achieved throughanti-dyslipidemic, antihypertensive, and antioxidant activities. CONAHCYT: 815405.

P-31

Effects on Glucose Levels After Running Long-distance Races.

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Introduction. Glucose stands as the body's primary fuel source, crucial for maintaining functional muscle levels during high-intensity physical activity. Its regulation hinges upon various factors including exercise intensity, frequency, duration, and insulin levels. Glycemic variability, reflecting fluctuations in blood glucose levels throughout the day, holds significant implications for an athlete's performance and overall health. This study aims to discern the interstitial glucose concentration and glycemic variability in ultra-marathon runners.
Methods. This cross-sectional study examines the interstitial glucose concentration and glycemic variability among endurance runners during and after an ultra-marathon race.
Results. Analysis reveals a notable difference in average glucose levels between the race and post-race periods (114.9 ± 17.8 vs. 94.7 ± 18.53 mg/dL; p=0.001), accompanied by a mean glycemic variability of 16.2 ± 2.7%. Age and gender exhibit no discernible association with these variables. Remarkably, some runners fail to adhere to the recommended monitoring frequency (at least every 8 hours) for interstitial glucose levels during and post-race. Additionally, examination of continuous glucose monitoring metadata identifies nine participants experiencing low glucose events post-run (levels equal to or below 69 mg/dL).
Discussion. Energy deficits, commonplace among ultra-marathon participants, are linked to suboptimal exercise recovery and sustained fatigue. Hence, maintaining adequate post-race nutrition is pivotal for efficient performance and mitigating adverse health effects.
Conclusions: Glucose levels and nutrition play pivotal roles in optimizing training, facilitating recovery, and fostering metabolic adaptation. This study underscores their significance, emphasizing the importance of post-race nutrition for ultra-marathon runners. The study adhered to a significance level of $p \leq 0.05$.

P-32

Dihydrosanginarine, metabolite partially responsible for the anticonvulsant activity of Bocconia arborea S. Watson.

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Background. Epilepsy is a multicausal chronic disease characterized by spontaneous and recurrent seizures, which affects 50 million people worldwide. Despite the availability of many antiepileptic drugs, any of them are efficacious enough to completely control epileptic seizures. The use of medicinal plants or their metabolites represent an option in the search for new drugs. In traditional Mexican medicine, the Bocconia arborea S. Watson (Papaveraceae) tree (B. arborea) is used for the treatment of skin, kidney, and infectious diseases, as well as antidiabetic. Furthermore, it has been reported that extracts of different polarities of B. arborea cause central nervous system (CNS) depressant effects such as anxiolytics and analgesics involving the opioid and GABAA receptors. The above information suggests that this species could possess anticonvulsant activity. Aim: To evaluate the anticonvulsant activity of extracts of different polarity of B. arborea and one of its secondary metabolites called dihydrosanguinarine (DHS) on tonic-clonic seizures induced with pentylenetetrazole (PTZ) and maximum electroshock tests in mice.
Methods. The study consisted of two experiments. In the first, thirty-six male Swiss Webster mice were implanted for electroencephalographic recording and divided into six groups: vehicle, diazepam (1 mg/kg), hexane (HEX), dichloromethane (DCM), methanol (MeOH), and the metabolite DHS (100 mg/kg). The treatments were intraperitoneally administered with an acute dose 30 min before the convulsant PTZ (85 mg/kg). For the second experiment, sixty-nine mice were used, which were subjected to the maximum electroshock test 30 min after the administration of each treatment.
Results. Animals treated with the HEX extract presented a decrease in the incidence ($p < 0.005$) and an increase in the latency ($p < 0.005$) of tonic-clonic seizures induced with PTZ. In addition, a lower rate mortality ($p<0.005$) was observed. These results were similar to those obtained with the reference drug diazepam. Animals treated with the MeOH extract and DHS also showed significant responses, although to a lesser extent than HEX ($p<0.05$ for both). In the second experiment, animals treated with diazepam, the extracts (HEX and DCM) or DHS presented a protective effect on tonic-clonic seizures induced with the maximum electroshock test, as well as a shorter duration of seizures ($p<0.0001$).
Conclusion. Our study provides evidence that constituents of HEX and MeOH extracts (low and high polarity, respectively) are involved in the anticonvulsant properties of B. arborea, where the DHS metabolite was partially responsible, and its effect could be mediated by the modulation of the GABAergic system. Acknowledgments: To the projects CONACYT 226454 and 256448.

P-33

Pharmacological evaluation of the CNS depressant activity of an aqueous extract of Rosa centifolia L. on the behavior and ECoG in mice.

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Background. Currently, anxiety disorders have had an exponential increase due to the SARSCOV-2 pandemic condition. Unfortunately, anxiety treatment often includes medications that are not always effective and can produce unwanted effects such as dependence, lethargy, tachycardia, and even exacerbate anxiety symptoms, among others. Ethnomedical knowledge referscentral nervous system (CNS) depressant properties as anxiolytic with the use of the infusion of Rosa centifolia L. However, there is no scientific evidence that validates its potential therapeuticon the CNS affections.
Aim. To investigate the anxiolytic-like effects of the R. centifolia aqueous extract (RcAE) in the behavior andon the electrocorticographic (ECoG) activityin mice.
Methods. In this study, male SW mice (25-30 g) were used, grouped into at least 6 individuals, administered intraperitoneally with different doses (10, 30 and 100 mg/kg) of the RcAE and compared to the vehicle and the anxiolytic drug diazepam, as reference (DZP,1 mg/kg). Thirty minutes after the treatments, the open-field, the hole-board, and the plus-mazetests were started to evaluate the ambulatory activity and the exploratory behaviorof mice, as well as the to observe changes on the ECoG in mice.
Results and discusión. Mice receiving the extract showed significant reduction in anon-dose ordose-dependent manner depending on the parameter analyzed in the plus-maze assay at doses of 10 and 30 mg/kg, i.p. resembling the effects of DZP (1 mg/kg, i.p.) in the behavioral response and in the neuronal activity observed in the ECoG analysis. The dose of 100 mg/kg caused the greatest decrease in the examination of the ambulatory and exploratory activities in mice referring a sedative response. In the ECoG, the effects of the extract were different to that observed with the clinical drug DZP.
Conclusion. The aqueous extract of R. centifolia produces CNS depressant effect as anxiolytic and/or sedative reinforcing the usefulness of this plant in traditional medicine as an alternative for the therapy of anxiety. Acknowledgments:To the project NC123280.0 and CONACYT256448.

P-34

Antitumor activity of Incomptine A in murine breast cancer modelby downregulation of Hexokinase II.

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Background. Breast cancer is the most common and lethal cancer in women worldwide. Glycolytic metabolism is a distinctive characteristic of cancer cells where there is greater expression and activity of enzymes such as Hexokinase II (HKII); which is involved in the inhibition of apoptosis of cancer cells. We have previously reported that Incomptine A (IA), a sesquiterpene lactone found in leaves of Decachaeta incompta(DC), exhibit anticancer effects against human breast cancer cells.
Aim. In this study we decided to evaluate the in vivo effect of IAand propose a mechanism of action that involves HKII and the induction of apoptosis.
Methods. 4T1 murine breast cancer cells were inoculated subcutaneously into the abdominal mammary gland area at a concentration of 1×10^5 cells/mouse in 100 µL, after 7 days of inoculation; animals with palpable tumor were selected and the antitumor activity of IA at different doses was evaluated. Tumors from treated mice were compared with untreated mice. To evaluate the effect of IA on proteins, western blot studies were performed.
Results. We demonstrated that IA-treatment decreases the tumor growth in female BALB/c mice inoculated with 4T1 breast cancer cells. Additionally, the expression of HKII was downregulated while the expression of caspase 3 increased in IA-treated tumors. These results suggest that the induction of apoptosis in breast cancer cells is associated with the decrease in HKII given by IA.
Conclusion. IA possesses anti-tumor effects against breast cancer cells by downregulation of HKII and could potentially be used as a new anticancer agent for the treatment of breast cancer.

P-35

Antidepressant-and anxiolytic-like activities of the Psilocybecubensismushroom in experimental models in mice.

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Background. Major depressive disorder (MDD) occupies the first place of disability worldwide. It has been exacerbated by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic condition alone or in comorbidity with anxiety disorders, among others mental ailments. Despite alternatives like Psilocybemushrooms for mental health there is almost nothing about basic research to evidence their CNS benefits.
Aim. To evaluate the antidepressant-and anxiolytic-like effects of P. cubensis mushroomin experimental murine models.
Methods. First the acute toxicity (lethal dose fifty, LD50) was determined to know the safety doses to be explored in the study. Then, the anxiolytic-like effects were assessed using the open-field and plus-maze tests to evaluate the ambulatory,exploratory, and rearing behaviors, as well as the antidepressant responses in the forced swimming test. Each assay was evaluated after thirty min of administration of a dosage of 1000 mg/kg, p.o., of the whole P. cubensismushroom or the polar aqueous (AQ) or methanolic (MeOH) extractions (1, 10, and/or 100 mg/kg, intraperitoneal, i.p.) in comparison to the reference drugs buspirone (4 mg/kg, i.p.), fluoxetine and/or imipramine (10 mg/kg, s.c. and i.p., respectively).
Results and discusión. The LD50was calculated to be higher of 2000 mg/kg, i.p. Whereas, significant and dose-dependent responseswere produced in the antidepressant-like effects in the presence ofthe whole P. cubensismushroom after parenteral administration of the AQ or MeOH extractions resembling the effects of the p.o.administration of mushroom and reference drugs. The antidepressant behavioral responses were associated with its anxiolytic-like effects in the open-field assay.
Conclusion. Our results provide preclinical evidence of the antidepressant-and anxiolytic-like effects of the P. cubensismushroom after enteral or parenteral administration. This studyreinforcesthebenefits of the P. cubensismushroom in mental health and therapy for anxiety and depression. Acknowledgments. To the project NC123280.0 and CONACYT 256448.

P-36

In silico pharmacological study of thalidomide analogues with application in liver cancer.

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Background. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. It is a disease with multiple etiologies that causes alterations in hepatocytes, causing their multiplication to be accelerated and uncontrolled. In Mexico, HCC accounts for 80-85% of malignant liver cancer tumors, with a mortality rate of 4.7 per 100,000 inhabitants in 2020. The transcription factor SALL4 plays a critical role in the carcinogénesis and proliferation of cancer cells. Thalidomide has been shown to have inhibitory activity on SALL4 and is a promising pharmacophore for the design of new compounds with lower toxicity, as well as greater efficacy and therapeutic safety.
Aim. To identify in siliconew inhibitors of the SALL4 protein analogous to thalidomide.
Methods. A library of 500 molecules was formed and obtained from PubChem. Anticancer activity was evaluated on the Way2Drug server. Molecular properties, acute and chronic toxicity parameters were determined inthe Osiris Poperty Explorer and ProTox II platforms, respectively; as well as the pharmacokinetic and pharmaceutical properties in SwissADME. Finally, the molecular dockingof the molecules under study at the ligand binding site (SUL) of the SALL4 protein (PDB 7BQV) was performed with the Molegro Virtual Docker software.
Results. 3 thalidomide-like molecules were obtained with two main structural modifications, an aromatic ring at position 6 and the removal of the carbonyl at position 2 of the phthalimide nucleus. The prediction of T-235, 243 and 302 as antineoplastic drugs is 32.2%, 29.3% and 51.3% and as liver cancer agents is 24.7%, 24.6% and 21.3%, respectively; compared to 25.9% and 18.8% for thalidomide. The 3 analogues comply with the suggestions of Lipinski and Veber. They also have no chronic or acute toxicity, with the exception of molecule 243, which can be irritating. The OECD safety level for all three molecules is IV, while thalidomide has a classification of III. The pharmacokinetic properties are good aqueous solubility, high intestinal permeability, and absence of promiscuity by CYP450. Its pharmaceutical characteristics are adequate with high similarity to drugs, they do not present PAINS and phthalimide as structural brenks. The affinity energies in SALL4 of thalidomide, T-235, 243 and 302 are -7.82, -9.58, -9.49 and -9.58 Kcal/mol, respectively. They also interact with the amino acids tryptophan 400, 380, and 386 in SUL, which are essential in inhibiting SALL4.
Conclusion. It is suggested that the elimination of the ketone group and the addition of a third ring to the structure decreases the toxic effects and provides better stability to the molecules, presenting a greater affinity for SALL4 SUL and with it, a potential application as therapeutic agents in liver cancer with oral administration.

P-37

Benzothiazoles with therapeutic potential in type 3 diabetes: in silico study.

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Background. Type 2 diabetes mellitus (DM2) is a chronic disease whose main indicator is insulin resistance and Alzheimer's disease (AD) is neurodegenerative disease characterized by the progressive decline of memory. In the last decades, both DM2 and AD are constantly increasing; the relationship of these two diseases has been termed type 3 diabetes (DM3). The accumulation of amyloid beta peptide (Aβ) and tau protein(p-tau) are the main biomarkers in the pathophysiology of AD. Aβ and hyperphosphorylated tau may also have roles in pancreatic β-cell dysfunction and reduced insulin sensitivity and glucose uptake, also overactivation of glycogen synthase kinase-3 (GSK-3) has been linked to glucose intolerance and memory impairment in model animals, and is thought to be the link between DM2 and AD. As well as the peroxisome proliferator-activated receptor gamma (PPARγ), it is a positive regulatory potential by activating genes and signaling mechanisms that respond to insulin.

Aim. To evaluatein silicobenzothiazole derivatives in therapeutic targets of DM3: Aβ, p-tau, GSK3 and PPARγ.

Methods. The validation of docking was performed with the co-crystallized ligand of β-amyloid, p-tau, GSK3 and PPARγ proteins from PDB. The docking study of the 2-aminobenzothiazole derivatives was carried out with the Molegro Virtual Docker software, Discovery Studio was used for interaction analysis and with PlayMolecule Kdeep the affinity energies were obtained.

Results. The binding free energy values (ΔG, kcal/mol) of the docking between the therapeutic targets and the molecules under study are shown in the following table.

	IT-1	IT-2	GB-1	GB-2	GB-3	GB-18	GB-19	GB-24	GB-25
PPAR-γ	-6.09	-6.52	-5.66	-5.87	-6.77	-7.47	-8.18	-8.16	-8.24
GSK3-β	-6.13	-7.03	-5.65	-5.98	-6.75	-6.24	-6.4	-7.63	-7.96
β-amiloide	-4.98	-5.31	-5.26	-5.00	-5.66	-5.93	-6.01	-6.3	-6.43
p-tau	-4.61	-5.74	-4.99	-5.41	-5.4	-6.35	-6.34	-6.1	-6.83

The molecules derived from 2-aminobenzothiazole that exhibited the best affinity energies compared to the reference compounds and the most favorable interactions with amino acid residues at the catalytic or ligand-binding sites are GB-25 and GB-19 on PPARγ, GB-25 and GB-24 with GSK3-β and the β-amyloid peptide, while on p-tau they are GB-25 and GB-18.

Conclusion. Based on the above results, the GB-25 molecule may have a potential therapeutic action on the pharmacological targets of DM3. SIPMIULTI 20242297; SIP 202400559; SIP 20240101; SIP 20240211; CONAHCYT-CVU1346988.

P-38

Challenging memory loss: the combined treatment of physical exercise and gdf11 as a defense against alzheimer's disease

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Background. Alzheimer's Disease (AD) is a progressive and irreversible neurodegenerative pathology, which leads to a loss of mental abilities (memory, thinking, and language). AD is the leading cause of dementia, affecting 10% of the elderly population. Although its causes have not been fully elucidated, the most accepted hypothesis is that neurodegeneration occurs due to the accumulation in the brain of two proteins: beta-amyloid protein (Aβ) and tau protein, and currently there are no treatments to cure this pathology or stop its progression. Thus, the need arises to implement different treatments like the one in this research, which is relevant as it is one of the first studies aiming to investigate the combined effect of the properties of physical exercise (which several reports mention improve memory and cognitive functions, reduce Aβ and tau protein levels in the brain, as well as increase antioxidant enzymes and reduce oxidative damage) with the properties of GDF11, which reports indicate improve cerebral vascularization, enhancing neurogenesis, besides decreasing Aβ vascular and brain tissue levels. Therefore, the investigation of this combined treatment is of interest, as it will provide information about its application in triple transgenic mice (3xTG-AD), an AD model.
Aim. To evaluate the therapeutic potential of the combination of physical exercise and GDF-11 treatment in AD, by applying it in the triple transgenic mouse model 3xTg-AD.
Methods and results. The training and conditioning of triple transgenic animals were performed according to a low to moderate intensity exercise routine, wherein the speed was gradually increased from 5cm/s to 25cm/s every 5 minutes over a period of half an hour ona rodent treadmill, conducted five days per week. Since the beginning of the project, accumulating 10 months with physical exercise at this time. The GDF11 treatment (Peprotech 120-11, 0.1 mg/kg) was administered IP; the treatment started at 7 months of age of the transgenic mice and lasted for four weeks, administering three times per week. Behavioral tests of Novel Object Recognition (NOR) and Morris Water Maze (WM) were conducted. As a result of the NOR test, there is a significant difference ($p<0.05$) when applying the ANOVA test between the control group and the treatments, with the physical exercise group showing the highest recognition percentage. Regarding the WM test, it can be evidenced that there is greater spatial recognition evaluated by the trajectory in EF, GDF11, and combined treatment animals compared to the control group. Food consumption and animal weight were evaluated to investigate if these influenced the observed effect;

however, they did not present significant differences ($p<0.05$) when applying the ANOVA test.

Conclusion. The results suggest that treatment with physical exercise and GDF11 could improve memory and learning in 3xTg-AD mice; however, it is necessary to continue with the project phases to evaluate its effectiveness.

P-39

Polymorphic frequencies in genes *SLC22A1, SLC22A2, SLC22A3, ABCB1*, and *CYP2C9* among DMT2 Mexican-Mestizo patients and their effect on HbA1c levelsand metformin's plasmatic concentration.

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Introduction. Diabetes Mellitus Type 2 (DMT2) is a metabolic disease characterised by an insufficient activity of endogenous insulin. In Mexico, first line treatment is metformin monotherapy, whilst the prevalent combined therapy is metformin+glibenclamide. Some SNPs have been associated with responses to these drugs, for instance: *SLC22A1* (rs12208357, rs2282143, rs594709, rs622342, rs628031, rs683369 and rs72552763), *SLC22A2* (rs316019), *SLC22A3* (rs2076828 and rs8187725), *ABCB1* (rs1128503, rs1045642, and rs2032582), and *CYP2C9* (*2, *3 and rs1934969). Metformin transit is enabled by Organic Cation Transporters (OCT 1, 2, and 3), expressed by *SLC22A* 1, 2 y 3 respectively, whilst it has been found that *ABCB1* expresses an extrusion protein which also transports metformin, whereas *CYP2C9* metabolises glibenclamide.

Objective. Determining the frequency and effect of SNPs in *SLC22A1* (rs12208357, rs2282143, rs594709, rs622342, rs628031, rs683369 and rs72552763), *SLC22A2* (rs316019), *SLC22A3* (rs2076828 and rs8187725), *ABCB1* (rs1128503, rs1045642, and rs2032582), and *CYP2C9* (*2, *3, and rs1934969) over HbA1c% and metformin plasmatic concentration (ng/dL) among DMT2 Mexican-Mestizo patients.
Methods. Out of a 104 patient sample from HRAEI, we conducted a descriptive analysis where genotypic frequencies of each SNP were determined and the Hardy-Weinberg equilibrium was calculated. We probed HbA1c levels and metformin plasmatic concentration (ng/dL) through an inference analysis by every chosen SNP. Normalcy tests were performed, followed by either Kruskal-Wallis or Mann-Whitney's U test, depending on group numbers for every genotype. A $p<0.05$ value was considered statistically significant.
Results-discussion. Every determined SNP frequency reported Hardy-Weinberg equilibrium except for *SLC22A3* rs2076828. Only *ABCB1* rs2032582 reported significant differences in metformin plasmatic concentration (ng/dL) across genotypes. T/A in rs2032582 reported a greater metformin plasmatic concentration [1348.77 (1196.95-

2286.01 ng/dL), $p<0.05$]. Even though T/A and G/A in rs2032582 reported the highest HbA1c levels, no statistical difference was found across genotypes.

Conclusion. T/A in rs2032582 affects metformin plasmatic concentration and it might influence its therapeutic response, likewise glibenclamide response or their combination amongst DMT2 Mexican-Mestizo patients. An analysis of a stratified sample, divided according to treatment, would be necessary to confirm these results.

P-40

Study of the IFN-gamma Signaling Effect on Protease Profile in Glioblastoma Cells.

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Background. Glioblastoma, a highly aggressive primary brain tumor, poses a significant clinical challenge due to its highly aggressive nature and fatal prognosis, with an average life expectancy of only15 months for affected patients. This cancer is characterized by a complex inflammatory microenvironment that favors its development and progression. Within this inflammatory context, various cytokines are found, among which interferon-gamma stands out. IFN-γ, a key proinflammatory cytokine, modulates the immune response. Upon binding to its receptor complex, it triggers a signaling cascade culminating in the activation of the transcription factor STAT-1. The latter regulates the expression of various genes, including genes encoding proteases. Specifically, these proteases are involved in critical biological processes such as cell migration, which play a fundamental role in tumor progression.
Aim. Determine the effect of the IFN-gamma signaling pathway on the protease profile involved in glioblastoma progression.
Methods. The effect of IFN-gamma on protease expression will be analyzed using bioinformatics and experimental analyses, including protein microarray.
Results. Our results demonstrate that IFN-gamma signaling can modulate the levels of some proteases in glioblastoma cells.
Conclusion. IFN-gamma may promote pro-tumorigenic actions by modulating the expression levels of some proteases in glioblastoma cells.

P-41

Hepatoprotective effect of Empagliflozin/Metformin co-treatment in Metabolic Syndrome.

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Background: Metabolic syndrome (MS) is a group of risk factors (RF) that favours fat deposits in the liver, due to non-alcoholic factors, and could leads to steatohepatitis, fibrosis, and cirrhosis. These alterations belong to a non-alcoholic fatty liver disease (NAFLD), a comorbidity closely related to MS. Treatment of NAFLD is based on the control of RF through changes in lifestyle and drugs, but yet has no specific approved pharmacotherapy, hence the need for pharmacological strategies. Sodium-glucose cotransporter type 2 inhibitors (SGLT2i) and metformin have shown beneficial effects on obesity and insulin resistance, RF associated with the pathogenesis of NAFLD.
Aim: To evaluate the effect of Empagliflozin/Metformin co-treatment in NAFLD.
Methodology: MS was induced in male Wistar rats (200-220g) with a type Paygen diet. Once established MS, rats were randomly assigned to MS untreated, MS + Empagliflozin/Metformin (12.5/850 mg/day gavage), Empagliflozin, and Metformin. We assess body weight, blood pressure (BP), fasting blood glucose, glucose tolerance test, and lipid profile as MS markers. Also were analysed aspartate aminotransferase (AST), alanine transaminase (ALT), antioxidant stress and mitochondrial activity as indicators of liver dysfunction.
Results: The Paygen diet increase blood pressure, fasting glucose and impaired the GTT and lipid profile. Also, the liver transaminases were increased. The results demonstrated establishment of MS and liver damage. The co-therapy showed improvement in BP, fasting glucose, GTT, and lipid profile compared to the MS group. The liver changes in AST, ALT, antioxidant enzymes activity and mitochondrial complexes I and II MS-induced were prevented with the co-therapy. The empagliflozin/metformin co-therapy did not restore the value of the parameters assessed respect to the control group but were statistically different when compared with MS-untreated.
Conclusion: The Empagliflozin/Metformin co-therapy was better than each one treatment for the control of risk factors of MS, and therefore offers an hepatoprotective role, suggesting that may delay the progression to NAFLD and could be considered as a therapeutic option in NAFLD induced by MS. **CONAHCYT**: 815405, and 815062, 1235288,1281406, 1281430.

P-42

Title Identification and validation of microRNA as biomarkers for Rheumatoid Arthritis in Mexican families.

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Background. Rheumatoid arthritis (RA) is a chronic autoimmune condition that affects the Mexican population and is most common in women aged between 30 to 50 years old. The RA etiology is multifactorial and genetic factors play a significant ro. MicroRNAs are small molecules that regulate at post-transcriptional expression of specific genes. Several studies have shown that microRNAs are altered during the progression of RA and could affect first-degree relatives of patients with RA.
Objective. To validate the miR-939, miR-4695, Mirlet7a-1 and miR-125A identified by the Affymetrix GeneChip miRNA 3.0 Array in RA patients and their firts-degree relatives.
Materials and Methods. The differential expression of peripheral blood miRNAs was analyzed using the GeneChip™ miRNA 3.0 Array (Affymetrix, USA, Cat. No.902413). The study included 3 groups: patients diagnosed with RA according to the ACR/EULAR classification criteria (Group 1), healthy first-degree relatives of patients with RA (Group 2) and healthy subjects who do not have a family history of rheumatic diseases (Group 3). Validation will be achieved by selecting two miRNAs that were both overexpressed and dysregulated. Primer design was conducted using the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) for subsequent analysis through Polymerase Chain Reaction (PCR).
Results: The GeneChip™ miRNA 3.0 Array analysis demonstrated that 11 miRNA were upregulated and 86 were downregulated in the RA patients and their first-degree relatives compared to the control group. In order to validate the miRNA, the primers defined as short sequences of acid molecules were chosen and designed. To design the primer, it was necessary the use of computational methods, detecting possible primer dimer, the amplification of alternative products, hairpin formation, extreme melting temperatures, and genotype-specific variations in the target sequence. Finally, a computational sequence analysis tools were used to select the best primer pairs from the available candidates.
Conclusion. The identification and validation of miRNA as new biomarkers involved in the RA patients and their first-degree relatives may contribute to the identification of early RA.

P-43

The drug combination doxorubicin, metformin, sodium oxamate, inhibit cell proliferation of bone sarcoma cells cultured in 2D and 3D Models.

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Background. Sarcomas are a rare and heterogeneous group of tumors with high rates for distant metastases and mortality. They are grouped according to the site of origin into soft tissue sarcoma (STS) and bone tissue sarcoma (BTS) which represent 80% and 20% of total cases, respectively. Osteosarcoma is a bone tumor of mesenchymal origin that represents the most frequent type of bone cancer in pediatric patients and the third most frequent in adults. The therapeutic aims for osteosarcoma are to reduce recurrence and minimize morbidity and mortality. Despite previous efforts to introduce new therapeutic approaches, the standard treatment for these types of tumors has remained unchanged for nearly 40 years. First-line treatments are currently led by anthracyclines as doxorubicin, alkylating agents, and taxanes. However, these compounds are not histologically driven or specific to any biomolecular pathways, therefore inducing high toxicity levels and low response rates. Recently, it was demonstrated an antineoplastic effect of the drug combination doxorubicin, metformin and sodium oxamate through apoptosis and autophagy induction as well as cell proliferation inhibition in breast, colon and cervix cancer. However, the mechanisms affected by this therapy to inhibit cell proliferation and its effects on tumors of mesenchymal origin remains unclear. The protein β-catenin have been stablished as a central regulator of cancer cell proliferation. When the protein is translocated to the nucleus, it activates the expression of gene targets as cyclin D1, c-myc and snail to promote cell cycle progression.
Aim. To evaluate the Wnt/β-Catenin proliferation pathway under the effect of the drug combination doxorubicin, metformin and sodium oxamate in an osteosarcoma cell line.
Results. In this work we demonstrated that β-catenin signaling pathway is activated in an osteosarcoma cell line. Constitutive nuclear location and transcriptional activity of β-catenin was observed. Moreover, under doxorubicin, metformin and sodium oxamate combined treatment β-catenin activity decrease and cell proliferation is diminished. In addition to these findings we stablish a organotypic 3D model, where the IC50 was lower compared to the one observed in monolayer culture.
Conclusion. These findings support the addition of metformin and sodium oxamate to doxorubicin as an alternative treatment for osteosarcoma.

P-44

Lipidic profile comparison of type 2 diabetic patients undergoing rosuvastatin (10mg) and atorvastatin (40mg) treatment.

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Introduction. Type 2 Diabetes (T2D) is one of the most frequent diseases in the world. One of its comorbidities is dyslipidemia. Among T2D patients, dyslipidemia is a modifiable risk factor in cardiovascular diseases [Khadka B et. al.; 2023]. In 2024, The National Institute for Statistics and Geography (INEGI) of Mexico reported that between January and June 2023, heart ailments were the main national cause of death, followed by diabetes. In its guideline Standards of Care in Diabetes – 2024, the American Diabetes Association (ADA) recommends the use of statins according to cardiovascular risk in T2D patients, where atorvastatin (40-80 mg) is considered a high intensity scheme and rosuvastatin (5 – 10 mg) is a moderate intensity scheme.
Objective. Performing a meta-analysis to compare benefits in total cholesterol (TC), LDLcholesterol (LDL-C), and HDL cholesterol (HDL-C) levels when prescribing atorvastatin (40 mg) and rosuvastatin (10 mg) to T2D patients.
Methods. A research was conducted in PubMed around Mesh terms: Rosuvastatin calcium, atorvastatin and diabetes mellitus using boolean operator AND. Overall, 65 articles were found, out of which 33 were left out because the present study considered only clinical trials; 13 were not open access articles; 4 were not based on T2D patients; 7 did not match atorvastatin and rosuvastatin dosage; 2 did not measure the subrogated biomarkers of interest; 3 did not match the treatment of interest; 1 was not available online. These criteria yielded 2 viable articles for their analysis using Cochrane RevMan software online version.
Results-discussion. The analysis revealed a median difference in total cholesterol reduction [9.33 (IC 95%: 2.77-15.89) p = 0.005], LDL-C [6.19 (IC 95%: 0.53-11.85) p = 0.03], and an HDL-C increase [-1.12 (IC 95%: -1.61, -0.64) p <0.00001] in favour of rosuvastatin (10 mg) over atorvastatin (40 mg).
Conclusion. When compared against atorvastatin (40 mg), rosuvastatin (10 mg) displays a greater quantitative benefit over the lipidic profile of T2D patients. PAPIIT: IN205121.

P-45

Analysis of IL-1β as a prognostic and diagnostic marker in cervical cancer.

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Introduction. Cervical cancer (CC) is the most common gynecological cancer and because most cases are diagnosed in late stages, it is necessary to search for more effective biomarkers for the disease. The cellular and molecular components of the immune response are key in the development of CC. Interleukin-1 beta (IL-1β) is a pro-inflammatory cytokine produced primarily by monocytes in the blood and by macrophages in tissues. Although it is known that, during cervical carcinogenesis, IL-1β mediates host-tumor microenvironment interactions, promoting cell proliferation and tumor angiogenesis, its role as a prognostic and diagnostic biomarker in CC has not been analyzed.
Aim. To bioinformatically analyze the prognostic and diagnostic potential of IL-1β in CC.
Methods. Differential expression of genes from healthy and CC tissues was obtained from the Gene Expression Omnibus repository (GEO) using GSE67522 and GSE63678 database, and the common genes were used in a functional enrichment analysis of pathways and biological processes using Data Base for Annotation, Visualization and Integrated Discovered (DAVID) v6.8. The Gene Set Enrichment Analysis (GSEA) was performed in WEB based Gene Set Analysis Toolkit software, a functional enrichment analysis web tool. IL-1β expression between healthy and tumor tissues was analyzed using Gene Expression Profiling Interactive Analysis (GEPIA) database, and comparative analysis with its copy number was performed in cBio Cancer Genomic Portal (cBioPortal). The prognostic and diagnostic potential was analyzed using Kaplan-Meier Plotter (KM-plotter) and easyROC: a web-tool for ROC curve analysis (ver. 1.3.1), respectively. To diagnostic analysis, the Area Under the Curver (AUC), p-value, cutoff, sensitivity and specificity were calculated from GSE67522 and GSE63678 datasets. To determine the significant differences, one-way ANOVA test and Mann Whitney test were used. A FDR<0.05 and a p value <0.05 were considered as significant.
Results-discussion. A total of 180 genes showed differential expression in both databases. In addition to the cell cycle and related pathways in cancer, the NOD-type receptor signaling pathway presented significant enrichment ($p<0.001$). Eight genes participate in this pathway, including IL-1β, so their expression level in CC compared to normal tissues was analyzed.IL1β presented a significantly higher expression level in patients with CC compared to healthy patients ($p<0.05$). The analysis of overall survival (OS) based on TCGA data showed that women with CC and overexpression of IL-1β had significantly lower

survival compared to women with underexpression of the cytokine ($p<0.001$). Finally, ROC curve analysis showed that IL-1β expression identified a sensitivity of 0.95, and a specificity of 0.68.

Conclusion. The results obtained demonstrated that the expression of IL-1β could be useful in the diagnosis of CC.

P-46

A novel 3D cell culture of triple negative breast cancer cell lines emulates molecular characteristics of tumoral tissues.

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Background. Triple negative breast cancer (TNBC) is the type of breast cancer with the highest mortality in women worldwide. Its clinical classification arises from the lack of hormone and HER-2 receptors; however, this type of cancer has heterogeneous molecular characteristics that require further study. It is known that the development and progression of TNBC involved epigenetic and genetic alterations, some of which are modulated by lncRNAs. Several applications of cell culture allowed the development of cancer research knowledge. The three-dimensional cancer cell cultures recreate several features of the tumor microenvironment which will allow deep studies in complex cancers. In this work, we established a novel organotypic culture of TNBC cell lines. This model is based on the hanging-drop methods with substantial modifications.
Aim. To determine the expression of long non-coding RNAs in a novel 3D cell culture of triple negative breast cancer and their association with tumoral characteristics.
Results. The organotypic structures formed were characterized by two fluorescence microscopy technologies and the image analysis was made using Imaris v2.0 software. The structures of cancer cells formed in 3D showed a tumor-like morphology in accordance with the biology of the cell line. Subsequently, total RNA of the same cell line cultured in traditional monolayers two-dimensional (2D) cell culture and 3D conditions was purified with TRIzol reagent and the genomic-wide profile of long noncoding RNAs (lncRNAs) was determined by microarrays technology. Data showed that 2,712 lncRNAs were overexpressed and 281 were downregulated in 3D cultures versus 2D conditions. Bioinformatic analysis identify HOTAIR, CCDC26 and NBAT1, among others, which have been stablished as main regulators of cell proliferation, invasion, and metastasis in cancer. In addition, this lncRNAs profile is more like that the observed in tumoral TNCB tissues from patients.
Conclusion. These results support the idea that organotypic 3D structures may emulates the morphological and genetic characteristics of tumors and could allow further studies of potential therapeutic targets which remain as important clinical challenges in breast cancer therapy.
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P-47

The role of miR-126 in susceptibility to Rheumatoid arthritis, first-degree relatives' study.

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Background. Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that affects the joints and other organs, causing disability. It has a global prevalence of 1 %, while in Mexico, it is about 1.6 %, mainly affecting women. The pathological mechanisms are not clear but genetics plays an important role. Currently microRNAs have been proposed as biomarkers in the pathogenesis of RA, miRNAs are short RNA molecules (19 to 25 nucleotides) involved in post-transcriptional regulation and their deregulation can lead to the progression of various diseases. Considering the genetic background, it is proposed that the first-degree relatives are individuals at very high risk of developing RA.
Aim. To determine if differential expression of miR-126 plays a key role in the identification of subjects susceptible to develop RA particularly the first-degree relatives.
Methods. Clinical, anthropometric and demographic data were collected from the three groups (RA, relatives and controls). The total RNA was derived from the peripheral blood and the miRNA expression was analyzed by RT-qPCR in the PCR cycler Rotor-Gene Q 5plex HRM system using the RT SYBR Green qPCR Mastermix kit. The primers were pre designed for miRNA-126 and the RPL27 was used as the reference gene. The significance level was set at $P < 0.05$.
Results. The final population of 31 participants (14 of the study group, 10 patients with RA and 7 controls) was analyzed, made up of 29 women (93.55%) and 2 men (6.45%). The center of the country represented 83.87% of the total population analyzed, with Mexico City having the highest presence (67.74%). The demographic, clinical, and laboratory variables between groups do not show significant differences and do not represent a bias in the analysis of miRNA expression. The expression of miR-126 in peripheral blood was analyzed by real-time RT-PCR and did not show a significant difference ($P = 0.5256$) when compared the three study groups.
Conclusion. The study population belongs to the central zone of the country, with a greater establishment in the CDMX. No significant differences were found in demographic, clinical and laboratory data, as well as in miR-126 expression when the three study groups were compared.

P-48

Environmental Factors in Patients with Gout: Stress and depression.

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Background. Gout is a type of arthritis caused by the buildup of monosodium urate crystals in joints and soft tissues. The condition is usually triggered by high levels of uric acid in the body and can be influenced by genetics and diet. However, other factors such as stress, and depression can also play a role in triggering or worsening Gout. While studies have been conducted on these factors in other autoimmune rheumatic diseases, there has been limited research on their impact on Gout.
Aim. The objective of this study is to analyze the impact of depression, stress, and overall health on our population of Gout patients.
Methods. This analytical cross-sectional study included patients with Gout compared with healthy individuals matched by age and sex. All participants signed the informed consent letter (INR protocol 97/2023). Participants were asked to complete three validated questionnaires, Beck Depression Inventory-II a 21-item version, Goldberg's 12-item General Health Questionnaire, and Stress Scale. The study used the t-student test to compare the data obtained from both groups. The significance level was set at $P < 0.05$.
Results. The study included 50 male gout patients with an average of 14.96 (±13.04) years of evolution treated at the INRLGII and 43 healthy subjects. Patients met the classification criteria of the ACR/EULAR. In patients with Gout, 36% presented depression vs. controls 11%, the health questionnaire reported psychological distress in 52% vs. 37% in controls and the stress questionnaire showed 82% vs. 74% in controls. The BDI shows a significant difference ($p=0.005$) between patients and controls ($\mu=11.76\pm9.7$ and $\mu=6.70\pm6.4$, respectively). The GHQ-12 shows a tendency ($p= 0.067$) to be higher in the Gout group compared to the control group without reaching statistically significant values. The PSS questionnaire shows that the two groups studied are moderately stressed (patients: $\mu=20.36\pm7.16$, controls $\mu=18.88\pm6.81$), without significant difference ($p=0.314$).
Conclusions. This study suggests that patients with Gout experience greater levels of depression than individuals without the condition. Additionally, patients with Gout experience higher levels of psychological distress than the general population, and stress levels do not differ significantly between the two groups. New studies are required with a larger sample size of Gout patients and healthy people to determine if the depression, psychological distress, and stress may affect treatment adherence, development of metabolic syndrome, and other comorbidities, leading to a decrease in patients' quality of life.

P-49

Hypoxic microenvironment regulates CYP2S1 expression in hepatocellular carcinoma cell lines.

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Background. Tumoral hypoxia is a hallmark of solid tumors, including hepatocelular carcinoma (HCC). Hypoxia activates several signaling pathways that lead to the expression of proteins that support tumoral proliferation, metastasis, and drug-metabolizing enzymes such as cytochromes p450, more specifically, CYP1A1, CYP3A6 and CYP24A1. However, the effect of hypoxia on CYP2S1 expression in HCC is unclear. We previously demonstrated that hypoxia increases the CYP2S1 expression levels in HepG2 cells. However, this effect has not been reported in other representative HCC cell lines. Moreover, has been reported that methylation is another mechanism that regulate de CYP2S1 expression. Therefore, using an *in vitro* model with liver cancer cell lines (HepG2, Hep3B and SNU423), we evaluated the effects of hypoxia and methylation on CYP2S1 expression.
Aim. To evaluate the effect of hypoxia and methylation on CYP2S1 expression in HCC cell lines.
Methods. The HCC cell lines were cultured in normoxia and hypoxia condition 1% O2 (0, 6, 12, 18 and 24h). The levels of mRNA of CYP2S1 were evaluated by RT-qPCR using the 2 -ΔΔCT method. The protein levels were evaluated by western blot assy. To evaluate the rol of methyltransferase DNMT1 in CYP2S1 expression, the cell lines were treated with 5μM of 5-AZA (a potent inhibitor of DNMT1) in nomoxia and hypoxia condition 1% O2 (24h). The levels of CYP2S1 were evaluated by western blot assay.
Results. Our data showed that hypoxia increase the mRNA and protein levels of CYP2S1 in HepG2, Hep3B ($p<0.05$); however, hypoxia decrease the protein levels of CYP2S1 in SNU423 cells ($p<0.05$). Moreover, our data showed that the treatment with 5-AZA promotes the expression of the CYP2S1 in SNU423 cells under normoxia and hypoxia condition ($p<0.05$), but not in Hep3B and HepG2 cell line.
Conclusion. Our data suggest that hypoxia differentially regulates CYP2S1 expression. Furthermore, our results also suggest that methylation is another important mechanism for the regulation of CYP2S1 expression.
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P-50

Progesterone treatment restores blood-brain barrier function and improves long term memory and learning in a rat model of chronic cerebral hypoperfusion.

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Background. Cerebral blood flow supplies oxygen and nutrients to support normal brain function. Aging is associated with chronic cerebral hypoperfusion (CCH). These blood flow age-related alterations might be attenuated by progesterone (P4), a neurosteroid which has been proven to exert pleiotropic neuroprotective effects in several models of brain injury. The **aim** of this study was to evaluate the effects of P4 on the blood-brain barrier (BBB) in the short term and on spatial learning and memory in the long term in rats subjected to CCH.
Methods. Male Sprague-Dawley rats (12-14 months old) were randomly distributed in the following groups: CCH+vehicle; CCH+P4 (8 mg/kg/day) and sham procedure as a control. At seven and fourteen days after CCH, the function of the BBB was evaluated through permeability assays by systemic administration of a cocktail containing Evans blue and Na-fluorescein tracers. In addition, the expression of BBB tight junction proteins and inflammation factors was evaluated by western blot. At one hundred and eighty days later, memory and learning were evaluated using the Barnes maze and the novel object recognition test.
Results. CCH induced BBB dysfunction, decreased tight junction protein expression, increases inflammation factors and impairment in memory and learning. Treatment with P4 improved the BBB function, restoring the expression of the tight junction proteins, decreased inflammation factors, and preserves learning and memory.
Conclusion. These results suggest that, in old male rats with disrupted blow-flow, P4 plays an important role in restoring BBB function, which may contribute to the neuroprotective effects that have been previously reported.

P-51

Nodular kinases in sarcoma: A bioinformatic approach for Osteosarcoma, Liposarcoma, Sinovial Sarcoma and Leiomyosarcoma.

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Background. Sarcomas is a heterogeneous group of neoplasms derived from mesenchymal cells, which in turn originate from the mesodermal germ layer (Ferrari et al., 2016). There are approximately 50 different histological varieties of sarcomas, for study purposes they are defined in two large groups: soft tissue sarcoma (STS) and bone tissue sarcoma (BS), which represent 80% and 20% of tumors respectively (Nakata et al., 2021). For the past 40 years cytotoxic agents leading by anthracyclines, alkylating agents and taxanes have remained as standard treatment for sarcomas. However, it has been known that a problem in the treatment of sarcomas is the resistance to conventional treatments (Lin et al., 2020). Protein phosphorylation is driven by kinases and the dysregulation of this process plays a fundamental role in cell proliferation, migration, and differentiation during cancer progression (Ferguson & Gray, 2018). Recently, several clinical trials using Tyrosine Kinase Inhibitors (TKI) or antibodies to block the kinase activity of receptors have been carried out for sarcomas (Pollack et al., 2018). However, few TKIs such as imatinib, pazopanib, or olaratumab are approved by the FDA for clinical use in sarcoma patients (Pollack et al., 2018). Therefore, the analysis of the axis phosphorylation/dephosphorylation through the establishment of nodular enzymes that drive it in sarcomas may be key for the improvement of new therapeutic schemes.
Methods: We used gene expression databases and bioinformatic tools to establish the overexpressed (OE) kinases in the four most frequent types of sarcomas: osteosarcoma, synovial sarcoma, liposarcoma, and leiomyosarcoma comparing with expression in healthy tissues. Then, the OE kinases shared in all analyzed sarcomas were determined. Enrichment assays were performed and finally the clinical implication of nodular kinases was stablished by kapplan-meier test.
Results: BUB1 kinase was established as nodular for the four types of sarcomas included. Interestingly, BUB1 expression is regulated by transcription factors, RNA binding protein and ncRNAs (microRNAs and long noncoding RNAs), all of them involved in signaling pathways that leads sarcoma progression through proliferation and invasion. Signaling pathways that regulate malignancy and progression of STS and BS were the most represented. Finally, high levels of the kinase correlate with poor overall survival (OS) in patients of all analyzed sarcomas.
Conclusion: All this evidence allows us to propose BUB1 such as a nodular kinase in the progression of osteosarcoma, liposarcoma, leiomyosarcoma, and synovial sarcoma. Its inhibition using target therapy could provide a pharmacologic strategy in sarcoma treatment.

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P-52

SARS-CoV-2 spike protein modifies angiotensin-converting enzyme 2 activity in hypothalamus, striatum and cortex of brain of hypertensive rats.

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Introduction. Angiotensin-converting enzyme 2 (ACE2) is an important enzyme that plays a crucial role in regulating the renin-angiotensin system. The ECA2 hydrolyzes angiotensin II (Ang II) to Ang-(1–7), which then interacts with the Mas receptor to counteract the effects of Ang II. ACE2 is also the receptor for the coronavirus 2 (SARS-CoV-2) that causes severe acute respiratory syndrome (SARS). SARS-CoV-2 contains on the outer surface of the viral particle a spike (S) protein, which interacts with the ACE2. In vitro, the spike protein increased recombinant human ACE2 activity against des-Arg9-bradykinin analog, while cleavage of angiotensin II analog is minimally affected by the binding of spike protein.
Objective. To investigate whether SARS-CoV-2 spike protein would affect the enzymatic activity of angiotensin-converting enzyme 2 (ACE2) in six brain regions: hypothalamus, amygdala, hippocampus, striatum, cortex, and brain stem of Ang II-induced hypertension rats.
Methods. Male Wistar rats (350–360 g) were divided into two groups (n = 10 each): 1) control (sham) and 2) Ang II-induced hypertensive rats (435 ng/ kg / min of Ang II) was infused through osmotic minipumps (Alzet 2002; Alza, Palo Alto, CA) for 14 days. To test the effect of the viral spike protein binding on the ACE2 activity, we measured ACE2 activity, with and without preincubation with SARS-CoV-2 spike protein, in the presence of Mca-APK (DnP) substrate at λex 320 nm/λem 420 nm at 28oC in a kinetic mode with readings recorded every 5 minutes for 30 minutes (Synergy®HTX multimode). Enzyme-Linked Immunosorbent Assay (ELISA) for ACE2 determination.
Results. In the hypothalamus of hypertensive rats, the ACE2 activity decreased compared to the control group but increased in the presence of the spike protein. In the striatum of hypertensive rats, the ACE2 activity did not change compared to the control group but increased in the presence of the spike protein. In the cortex of hypertensive rats, the ACE2 activity decreased compared to the control group but increased in the presence of the spike protein. ACE2 protein decreased in the hypothalamus, hippocampus, cortex, and brain stem and was not modified in the amygdala and striatum of the brain of hypertensive rats.
Conclusion. Spike protein modifies angiotensin-converting enzyme 2 activity in hypothalamus, striatum and cortex of brain of hypertensive rats, this mechanism can contribute to the COVID-19 pathogenesis.

P-53

Angiotensin-converting enzyme 2 activity and expression in brain of diabetic rats.

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Introduction. Angiotensin-converting enzyme 2 (ACE2) is an important enzyme that plays a crucial role in regulating the renin-angiotensin system. The ECA2 hydrolyzes angiotensin II (Ang II) to Ang-(1–7), which then interacts with the Mas receptor to counteract the effects of Ang II. Ang-(1–7)-mediated responses include, decreased sympathetic excitation leading to vasodilatation and lowering blood pressure (BP), reduced cardiac contractility, anti-inflammatory and anti-fibrosis effects, reduced physiological response to stress and anxiety.
Objective. To investigate ACE2 activity and expression in six brain regions: hypothalamus, amygdala, hippocampus, striatum, cortex, and brain stem of diabetic rats.
Methods. Male Wistar rats (300–350 g) were divided into two groups (n = 10 each): 1) control (injected with citrate buffer) and 2) streptozotocin (STZ)-induced diabetic rats (STZ, 65 mg/kg body weight). We measured ACE2 activity in the presence of Mca-APK (DnP) substrate at λex 320 nm/λem 420 nm at 28oC in a kinetic mode with readings recorded every 5 minutes for 30 minutes (Synergy®HTX multimode). Enzyme-Linked Immunosorbent Assay (ELISA) for ACE2 determination.
Results. In the hypothalamus of diabetic rats, the ACE2 activity did not change compared to the control group. In the amygdala, hippocampus, and cortex of diabetic rats, the ACE2 activity decreased compared to the control group. In the striatum and brain stem of diabetic rats, the ACE2 activity increased compared to the control. ACE2 protein decreased in the hypothalamus, hippocampus, striatum, cortex, while that brain stem was not modified in the amygdala of the brain of diabetic rats.
Conclusion. There is no correlation between the activity and the expression at the protein level of ACE2 in the six brain regions: hypothalamus, amygdala, hippocampus, striatum, cortex, and brain stem of diabetic rats. These results suggest that there are different regulatory mechanisms in each region.

P-54

Prevalence of Non-Alcoholic Fatty Liver Disease in Patients with Asthma from April 2022 - December 2022.

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Introduction. Asthma, characterized by chronic inflammation of the airways, stands apart from chronic obstructive pulmonary disease (COPD) due to its reversible nature. Key cellular players in asthma pathology include mastocytes, eosinophils, and T lymphocytes, contributing to recurrent symptoms like coughing, wheezing, and breathing difficulties. Conversely, non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver conditions, ranging from fat accumulation to fibrosis and cirrhosis. Obesity, marked by increased body fat content, correlates with the onset and inadequate management of asthma. NAFLD is intricately linked with obesity and can progress due to concurrent chronic inflammation. Various cytokines, notably TNF-α, IL-1β, and IL-6, contribute to asthma pathology by inciting inflammatory responses. TGF-β, associated with fibrosis, tends to be elevated in patients with severe asthma. In essence, obesity and NAFLD may exacerbate asthma by provoking systemic inflammation. Effective management of obesity and NAFLD holds significant promise for enhancing asthma control in affected individuals. The intricate interplay between obesity, NAFLD, and asthma underscores the importance of addressing systemic inflammation for comprehensive management.
Objective. To determine the prevalence of non-alcoholic fatty liver disease using Fibroscan® in patients diagnosed with asthma.
Methods. Observational, cross-sectional, and retrospective study in patients diagnosed with asthma, who will attend Medical Sur Hospital from April 2022 - December 2022 for Fibroscan® transient elastography, meeting inclusion criteria.
Results. In our study, we analyzed data from thirty asthma patients with a mean age of 27±14.31 years. The distribution of patients was approximately 63.3% women and 36.7% men. Furthermore, patients were classified based on the behavior of their disease, with 40% classified as controlled, 36% as poorly controlled, and 4% as partially controlled. Discussion: The analysis disease insights are crucial for understanding the patient population under investigation and interpreting the subsequent findings regarding the prevalence of NAFLD among individuals diagnosed with asthma.
Conclusion. The prevalence of NAFLD among patients diagnosed with asthma at Medical Sur Hospital from April 2022 to December 2022 was determined to be 63.33% (95% CI 46.09% - 80.58%). This finding underscores the significant burden of NAFLD in individuals with asthma, highlighting the potential comorbidities and health challenges associated with both conditions. Understanding the high prevalence of NAFLD among asthma patients is crucial for informing healthcare providers about the need for comprehensive management

strategies that address both conditions concurrently. Such insights can ultimately lead to improved patient care and better outcomes for individuals living with asthma and NAFLD.

P-55

Effect of empagliflozin treatment on magnesemia in metabolic syndrome.

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Background. Magnesium (Mg2+) is an essential ion that plays a key role in all energy-dependent transport systems, maintenance of vascular tone, and secretion and action of insulin, thus depletion has a negative impact on glucose homeostasis and insulin sensitivity as well as on the evolution of complications such as diabetes, arterial atherosclerosis, and nephropathy. Metabolic syndrome (MS) is a group of risk factors (RF) that has been related to hypomagnesemia. In contrast, it has been reported that a good metabolic control is associated with a reduction in urinary Mg wasting and normomagnesemia. Sodium-glucose cotransporter type 2 inhibitors (SGLT2i) have shown beneficial effects on RF, but its effects on magnesium homeostasis are unknown.
Aim. To study the effect of empagliflozin on magnesium homeostasis in metabolic syndrome.
Methodology. MS was induced in male Wistar rats (200-220g) with a type Paygen diet. Once established and validated MS, rats were randomly assigned to MS untreated, Empagliflozin (12.5 mg/kg/day). We validated the establishment of metabolic syndrome through body weight, blood pressure (BP), fasting blood glucose, glucose tolerance test (GTT), and lipid profile measurements. Also were analysed serum magnesium, diuresis and magnesiuria.
Results. Previous to empagliflozin treatment, the results showed an increase in blood pressure, fasting glucose, and impaired GTT, as well as an altered lipid profile, demonstrating the establishment of metabolic syndrome. Additionally, in MS group there was an increase in serum magnesium, magnesiuria and diuresis compared to the control group, indicated alterations in the body status of magnesium during MS. Empagliflozin treatment was successful in controlling blood pressure and improving fasting blood glucose, GTT, and lipid profile. The serum levels of magnesium were restored with the treatment of empagliflozin, and no significant changes were found in magnesiuria and diuresis between the treated group when compared with the untreated group. However, the urinary excretion of magnesium was higher in the MS group of 60 days compared with 30 days.
Conclusion. The treatment with empagliflozin controls the risk factors and restores magnesemia in metabolic syndrome, suggesting improvement in renal magnesium transporters function. Therefore, empagliflozin could delay the progression to diabetes and cardiovascular disease induced by MS.
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P-56

Synthesis of new benzothiazoles and docking studies on aldose reductase.

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Background. Aldose reductase 2 (ALR2) is the initial enzyme in the polyol pathway that converts glucose to sorbitol. Sorbitol is oxidized into fructose by the action of sorbitol dehydrogenase. This pathway is a mechanism of cellular detoxification under normoglycemic conditions. However, in hyperglycemia, it can cause diabetes complications such as neuropathies or microvascular complications. In this sense, benzothiazole is a privileged structure that may provide greater specificity on ALR2 than on ALR1, the main problem of ALR inhibitors currently used in the clinic.
Aim. To synthesize benzothiazole derivatives and evaluate their activity on ALR2 through molecular docking studies.
Methods. Two guanidine-benzothiazoles were synthesized from the reaction of methylamine and ammonia with the intermediate dimethyl benzo [d] thiazol-2-yl-carbono-dithioimidate, the products were characterized with nuclear magnetic resonance spectroscopy (1H and 13C-NMR) and their melting point was determined. Molecular docking studies were performed with Molegro Virtual Docker; the crystal of ALR2 (PBD 3G5E) was optimized, as well as ligands at a semi-empirical PM3 level with Spartan 08. The affinity energy was obtained with PlayMolecule Kdeep, and the analysis of the interactions was performed with Discovery Studio.
Results. The synthesis of IT-1 and IT-2 was based on accessible methods, with yields of 68% and 74%, respectively. Qualitative NMR results showed the efficiency of synthesis with minimal impurity (<5%). The melting point for IT-1 was 159-160 and for IT-2 72 0C. In addition, epalrestat has a binding energy (ΔG) of -8.89 Kcal/mol and interacts with the Val47, Trp111, Phe122, Trp219, Ala299 and Leu300 residues of the ALR2 catalytic site. Whereas IT-1 has a ΔG of -6.13 Kcal/mol and binds to Trp111, Phe122, Trp219, and Leu300. IT-2 is a ΔG of -6.75 Kcal/mol and binds to Val47, Trp111, Phe122, and Leu300. In this sense, a more negative affinity energy as that of epalrestat suggests a more favorable binding with ALR2. However, the ΔG values of IT-1 and IT-2, as well as their interactions at the catalytic site of this enzyme, position them as promising inhibitors of ALR2.
Conclusion. According to the results, IT-1 and IT-2 are stable molecules and energetically favorable to interact with ALR2. In addition, the accessibility of their synthesis offers a comprehensive and promising proposal for obtaining new ALR2 inhibitors with utility in the complications of hyperglycemia.
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P-57

Development of a tissue-engineered extraluminal tracheal splint with mesenchymal stem cells for the treatment of acquired tracheomalacia in an ovine animal model.

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Introduction. Acquired tracheomalacia (ATM) is characterized by loss of structural strength of tracheal framework, resulting in airway collapse during respiration. In patients undergoing prolonged invasive mechanical ventilation near half will suffer tracheal lesions. Nowadays, it has been described that 20% of patients who underwent mechanical intubation due to COVID19 complications will develop airway lesions leading to loss of tracheal cartilage strength. Treatment includes external splinting with rib grafts, prosthetic materials and tracheal resection. Extraluminal splinting for airway collapse aims to recover strength, reduce rejection and subsequent surgeries. It has not been described the development of extraluminal splints using mesenchymal stem cells cultured onto decellularized biomatrices. Failure in the use of prosthetic materials has made reconsidering natural origin scaffolds and tissue engineering as a suitable alternative.
Objective. To develop a tissue-engineered extraluminal tracheal splint (TE-ETS) with mesenchymal stem cells to restore adequate airway patency in an ovine model with surgically induced tracheomalacia.
Methods and Results. In this prospective pilot study, tracheal rings were partially resected to induce airway collapse in sixteen Suffolk sheep (Ovis aries). TE-ETS was developed with autologous mesenchymal-derived chondrocytes, allogenic decellularized tracheal segments and implanted above debilitated tracheal rings. Animals were followed up 8, 12, 16 weeks and 1 year postinsertion. Flexible tracheoscopies were performed at each stage. After sacrifice, histopathological study of the trachea and splint were performed. Our result showed that cell viability of oMSC was 95.7% and cell proliferation was 7.5 % at early stages of cell culture. Mesenchymal immunophenotype was CD44+(92.53%) and CD45- (2.7%). Chondrogenic differentiation induced formation of cartilaginous nodules positive for type II collagen and glycosaminglycans. DNA absence in decellularized heterologous tracheal segments was confirmed with DAPI staining, preserving malleability. Chondrocytes derived from oMSC were biocompatible with fibrin hydrogels (94.7%) confirming suitability for cell immobilization onto tracheal splints. The therapeutic extraluminal splint prevented airway collapse for 16 weeks and up to one year postinsertion. Tracheoscopies revealed a non-collapsing airway during inspiration. Histopathological analyses showed the organization of mesenchymal-derived chondrocytes in lacunae, the proliferation of blood vessels, and recovery of epithelial tissue subjacent to the splint. Splints without autologous cells did not prevent airway collapse. Airway diameter was similar to healthy animals without ATM and it was visible a slight stenosis. The tissue-engineered extraluminal splint favored tissue mechanical strength and recovery of the malacic lesion.

Conclusion. It is possible to treat acquired tracheomalacia with TE-ETS without further surgical removal since it undergoes physiological degradation. This study supports the development of tissue-engineered tracheal substitutes for airway disease. CONACYT FOSISS SALUD-2014-01-234406, SALUD-2015-01-262404.

P-58

Pilot study on the involvement of long non-coding RNAs in the development of heart failure in patients with ST-segment elevation myocardial infarction.

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Background. Cardiovascular diseases are the leading cause of death in Mexico, accounting for 4% of all health spending in the country. Acute myocardial infarction is myocardial necrosis caused by acute obstruction of a coronary artery. After an infarction, the myocardium undergoes several functional changes that will have repercussions on the ventricular parietal architecture, generating left ventricular remodeling, which is of great relevance because it is associated with the development of heart failure. Post-infarction heart failure is a public health problem, the pathogenic pathways involved in the development of this serious complication are not well identified, currently under study are long non-coding RNAs, molecules with a length of more than 200 nucleotides that epigenetically regulate gene expression, so they have multiple functions within the cell, by their functional characteristics, these molecules may be involved in the development of post-infarction heart failure.
Aim. To evaluate the participation of lncRNAs in the development of postinfarction heart failure in patients with acute myocardial infarction with ST-segment elevation in the anterior face.
Results. We have currently recruited 32 patients of which 93.8% are men and 6.2% women, with a mean population age of 56 years, presenting Diabetes mellitus 46.9%, Systemic Arterial Hypertension 56.2%, Dyslipidemia 21.9% and Smoking 78.1% being classified in patients with reduced LVEF 20%, slightly reduced 8% and preserved 4%, as well as being subjected to thrombolysis 46.4% and angioplasty 68%.
Conclusion. Within the Coronary Care Unit at the Instituto Nacional de Cardiología Ignacio Chávez, we will continue to invite patients who meet the inclusion criteria and decide to participate in the study in order to reach our sample of 130 patients.

P-59

Bioinformatic analysis of the expression of the PLK4 gene in cervical cancer.

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Background. Cervical cancer (CC) is one of the most common gynecologic malignancies worldwide. Although rapid improvements have been made regarding its prevention and treatment, little is known about disease pathogenesis and the clinical relevance of reliable biomarkers. Polo-like kinase 4 gene, also known PLK4, plays a crucial role in regulating cell cycle and division. Overexpression of PLK4 has been identified in different types of cancer, but there are few studies of the protein in cervical cancer.
Objective. In this study, we comprehensively explored the expression levels and prognostic significance of PLK4 in cervical cancer using bioinformatics. We further explored the PLK4 mutation characteristics and its potential mediated signaling pathways in cervical cancer.
Methods. Oncomine (http://www.oncomine.org/) and GEPIA (http://gepia.cancer-pku.cn/detail.php) were used to explore the expression levels and prognostic values of PLK4 in cervical cancer. UALCAN (http://manualcan.path.uab.edu.index.html) was used to measure the relationships between differentially expressed PLK4 and clinical parameters in cervical squamous cell carcinoma (CESC). The gene mutation characteristics of PLK4 in cervical cancer were explored by GSCA (http://bioinfo.life.hust.edu.cn/GSCA/#/mutation). STRING (https://string-db.org/) was used for nodes and connections between other genes with which PLK4 is related were obtained. The graph of the enrichment pathways in which the gene has been detected was obtained using the Shiny Go page (http://bioinformatics.sdstate.edu/go/).
Result. Using the UALCAN platform we obtained a difference in the expression of PLK4 between normal tissue and cervical cancer tumors, being much greater in the tumors, with a significance P = 0.001-12. In the survival analysis, using the GEPIA 2 tool, we found that there is no statistical significance between the probability of survival of patients with high PLK4 expression. (OS: p=0.77, HR= 1.1). On the other hand, a correlation was observed between high expression and a lower probability of survival in disease-free patients (DFS: p=0.037, HR=2.7). Using STRING, 9 genes with which PLK4 has a co-expression relationship were identified. Performing an enrichment analysis with these genes in SHINY GO, the cellular pathways in which they participate were found, the main ones being centriole replication, centriole assembly, spindle replication, and centrosome duplication.
Conclusion. PLK4 is overexpressed in cervical cancer tumors. There is a statistically significant relationship between gene expression and the probability of disease-free survival (DFS) of patients. PLK4 may be a potential biomarker for early diagnosis and targeted therapy of cervical cancer.

P-60

In silico studies of prodrugs derived from adenosine as possible antagonists of the human immunodeficiency virus type 1 (HIV-1).

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Introduction. In the context of antivirals, organophosphorus compounds derived from adenosine inhibit the synthesis of DNA or RNA of viruses. These antivirals are administered in the form of prodrugs, these are inactive drug precursors. Once administered, they undergo metabolic biotransformation (chemical or enzymatic) to release the drug, maximizing the therapeutic effect.
Objectives. Design and model organophosphate compounds derived from adenosine as possible antagonistic prodrugs to Human Immunodeficiency Virus type 1.
Methods. In silico prediction of the biological activity of organophosphate ligands, control (+/-) in PASSOnline. Predict druglikeness analysis by entering the chemical structure of the ligands and controls in ADMETLab 2.0. Predict ADMET analysis, compare and evaluate pharmacokinetic and pharmacodynamic properties in ADMETLab. Prediction of molecular target, performing bibliographic review, screening therapeutic objectives and corroborating on PASSOnline platform.
Results.

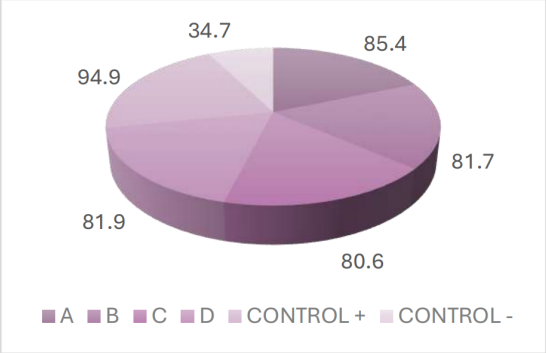

Figure 1. *Prediction of antiviral activity of organophosphorus ligands (A-D) on the PASSOnline platform; Probability of being activated (Pa) or being inactivated (Pi).*

Table 1. Values obtained from ligands B, C and positive control obeying Lipinski's Rules.

Molecule	B	C	+
PM	403.13	499.13	287.08
Acceptor of H	12	12	9
Donor of H	4	4	4
Rotatable Links	8	8	5
Log S	-1.787	-3.028	-2.318
Log P	-1.438	0.874	-1.245
Log D	-0.868	-1.059	-0.558
Density	1.149	1.094	1.164
Accepted	Yes	Yes	Yes

Conclusion. ligands B and C present better pharmacological prediction, favoring their chances of non-rejection in the preclinical and clinical stages of drug development.

P-61

CDKN3 and NUSAP1 expression in high-grade cervical intraepithelial neoplasm and squamous cell carcinoma.

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Background. Cervical cancer (CC) is one of the common gynecological tumors that seriously harm women's health, so it is particularly important to accurately explore the underlying mechanism of its occurrence and clinical prognosis. More than 90% of CC cases are caused by high-risk human papillomavirus (HR-HPV) infection; the persistence of infection with high-risk genotypes, such as HPV 16 and 18, has a high association with CC. Currently, much research has been done to identify new biomarkers that can be used to improve the detection of CC at the earliest possible stage. Some of them participate in the regulation of the cell cycle such as CDKN3 a phosphatase that regulates the cell cycle and NUSAP1 participates in spindle assembly and chromosome segregation.
Aim. The study aims to explore the clinical significance of CDKN3 and NUSAP1 through in silico analysis and their validation by immunohistochemistry (IHC) in preinvasive lesions and CC and thus provide a new research direction to find potential biomarkers of cervical cancer.
Methods. The expression of CDKN3 and NUSAP1 was initially evaluated using a public database (UALCAN). The protein expression level was analyzed by immunohistochemistry, also in other types of cancer associated with HPV. To explore the functional annotation and pathway enrichment analysis, we used the online analysis tool in ShinyGO, to determine the enriched GO terms and KEGG, and a survival analysis was performed.
Results. Our in-silico results demonstrate that there is a high expression of the CDKN3 and NUSAP1 genes in cervical cancer tissues compared to normal tissues. KM survival analysis consistently demonstrated that patients with high levels of CDKN3 or NUSAP1 in CC had poor overall survival. When the IHC analysis is performed, both proteins are overexpressed, CDKN3 staining predominated in the cytoplasm, and NUSAP1 highly expressed nuclear staining was observed, however, the expression of CDKN3 is stronger in high-grade lesions and tumors. NUSAP1 is expressed in low- and high-grade lesions similarly and the tumors are stronger. This result was compared with the report protein atlas.
Conclusion. Available online databases and immunohistochemistry confirm that CDKN3 and NUSAP1 are highly expressed in cervical cancer tissues. These genes may be potential biomarkers for screening, helping diagnostic of lesion NIC before the development of CC, for patients. Furthermore, they can provide novel ideas for targeted therapeutic strategies in the future.

P-62

Identification of the association of the DNMT1 protein with the LncRNAs: MalaT1, UCA1 and HOTAIR in cervical cancer.

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Background. Cancer is defined as the sum of a series of alterations in cellular processes that lead to the acquisition of distinctive characteristics by healthy cells to transition to states of neoplastic growth and their ability to form a malignant tumor. According to the World Health Organization, cancer was the leading cause of death worldwide in 2020. In 2020, cervical cancer ranked fourth in terms of incidence and mortality worldwide; In Mexico, cervical cancer is the second most diagnosed type of cancer and the second cause of death in women. Although the role of HPV is important for the development of CC, 75% of cases of infection by this virus are transient, so it can be deduced that this etiological factor is necessary to develop the disease, but not decisive since it genetic changes are required. and additional epigenetics for its progression. An example of this is non-mutational epigenetic reprogramming, within this reprogramming we can find DNA methylation, this process can be regulated by long non-coding RNAs that are a class of RNA molecules more than 200 nt in length similar to a messenger RNA, which are not translated into proteins. Some of these LncRNAs have been detected abnormally expressed in specific types of cancer and regulate a large number of biological processes that can lead the cell to a tumor phenotype, which is why the importance of studying these molecules is emphasized.
Aim. To analyze if there is an association between the LncRNA: MalaT1, UCA1 and HOTAIR with the DNMT1 protein in cervical cancer.
Results. As a result of a bioinformatic analysis, the LncRNAs: HOTAIR, MALAT1 and UCA1 were selected as candidates to identify their possible association with the DNMT1 protein. The analysis was carried out on a set of data downloaded from the TCGA database where the expression of these LncRNAs was compared in patients with cervical cancer in which no significant differences were found between cancerous tissue and tissue free of dysplasia. results that we verified by expression analysis with dysplasia-free tissue samples versus cervical cancer tissue samples where only LncRNA MALAT1 showed a significant difference. As a result of the immunoprecipitation of the DNMT1 protein in HeLa and HaCaT cell lines, it was obtained that HOTAIR, MALAT1 and UCA1 had a greater association in the tumor cell line compared to the non-tumor cell line. From the RIP assay performed with patient biopsies, it was found that for MALAT1, in at least 8 of the 10 samples there was a strong association of the protein with LncRNA, while for the LncRNAs UCA1 and HOTAIR in all samples this association was seen. Results that indicate that the interaction between LncRNA and protein does not depend on their expression and emphasizes the importance of these interactions even if the expression of the molecules is not modified.
Conclusion. There is a relationship between the LncRNAs: HOTAIR, MALAT1 and UCA1 with the DNMT1 protein in cervical cancer, which tells us what it is and that this interaction could interact in cellular processes such as DNA methylation.

P-63

Investigating the role of EVP1 in Candida albicans virulence.

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Introduction. Fungal pathogens are a rising human health threat that annually cause 150 million infections and over one million deaths worldwide. Among these pathogens, Candida albicans is the most frequently isolated fungal pathogen from medical devices and the most prevalent in fungal nosocomial infections, causing an annual cost of ~1.4 billion dollars in the United States of America alone. In addition, C. albicans infections are becoming more prevalent as immunocompromised individuals and the elderly population increase. Given the importance of this pathogen, it is imperative that we study how C. albicans causes damage to its host. Extracellular vesicles (EVs) are naturally released particles associated with virulence traits such as drug resistance, biofilm formation, and yeast-to-hyphae differentiation in C. albicans infections. Recently, it has been reported that the protein Evp1, is present in the plasma membrane of EVs secreted by C. albicans.
Objective. Currently, the molecular function and importance of this protein in C. albicans infection remains uncharacterized. Through this research, we aimed to elucidate the role of Evp1 in C. albicans virulence.
Methods. We phenotypically characterized an EVP1 knock-out mutant (evp1Δ) obtained from Noble (2010) and reconstituted the mutant by integrating EVP1 into the NEUT-5L locus of C. albicans. We also used our CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) systems to create repression and overexpression EVP1 mutants, respectively. We designed several sgRNAs targeting -100 to -350 bp upstream of the start codon (ATG) and transcriptional start site of EVP1, using the Eukaryotic Pathogen CRISPR gRNA Design Tool (EuPaGDT) to create 4 CRISPRi and 2 CRISPRa mutants. Real-time quantitative PCR was conducted to measure EVP1 expression in the CRISPRi and CRISPRa mutants. The mutants were subject to a Caenorhabditis elegans killing assay, growth curves and a biofilm assay to profile virulence.
Results-discussion. We observed overexpression in only one of the two CRISPRa mutants achieving 5-fold more expression than the wild-type. In the case of the CRISPRi mutants, repression was observed in the four mutants ranging from ~4 to 2-fold. Interestingly, the CRISPRa mutant with the highest overexpression and the CRISPRi mutant with the highest repression had sgRNAs that targeted the same region in the promoter located -289 upstream of the ATG. From the C. elegans killing assay, we observed a decreased virulence of evp1Δ with a worm survival of 60 % compared to the wild-type with 45%, after 72 hours. This result suggests that EVP1 could play an important role in C. albicans virulence. Also, restored virulence was achieved after EVP1 reconstitution, and no increase in virulence was observed by the overexpressing CRISPRa mutant.
Conclusion. Together, this study yields new insights about the role of EVP1 in C. albicans virulence, contributing to the research of C. albicans infections and virulence mechanisms. Future directions should include the characterization of EVP1 virulence role through filamentation assays.

P-64

Association of microRNA-1 y microRNA-133b plasma expression with fibromyalgia clinical features.

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Background. Fibromyalgia is characterized by chronic widespread pain, fatigue, sleep disturbances, and functional symptoms [Sarzi-Puttini P, et al. Nature Reviews Rheumatology 2020;16(11):645–60]. The pathogenesis of FM is not completely known although a combination of genetic and environmental factors. Both miRNA-1 and miRNA 133-b are highly expressed in skeletal muscle involved in myogenesis, regeneration, hypertrophy, and muscular dystrophy. In addition, miRNA-1 is involved in learning and memory process [McCarthy JJ, et al. Physiol Genomics 2009;39(3):219–26].
Aim. This study aimed to explore the association of miRNA-1 and miRNA-133b plasma levels with fibromyalgia clinical features.
Methods. We invited adult women suffering from fibromyalgia without metabolic or autoimmune comorbidities and age, sex, and body mass index (<35) healthy women. All individuals filled following questionnaires: Revised Fibromyalgia Impact (FIQ-R), Widespread Pain Index (WPI), Severity Symptom Score (SSS), Poly-symptomatic Distress Scale (PDS), Dysautonomia (COMPASS-31), neuropathic pain (LANSS), small fiber neuropathy (SPSS), anxiety (GAD-7), depression (PHQ9), and quality of life (EuroQol). Blood sampling was obtained outside the patients' menstrual period and verified they had at least 5 half-lives without drugs used for the pharmacological treatment of fibromyalgia (TxFM). Both miRNAs were detected with primer-specific cDNA synthesis and TaqMan probes from Applied Biosystems followed by qPCR. Plasma microRNA abundance was expressed as 50-target miRNA Cq values and by means of 2-(Cq target-Cq miR-39) formula. Results. We studied 46 adult women suffering from fibromyalgia without metabolic or autoimmune comorbiditiesand 26 age, sex, and body mass index (<35) matched healthy women. In the cohort of patients with fibromyalgia, COMPASS 31 correlated with miR-133b (Rho: -0.335, p=0.026), general condition domain (Rho: -0.381, p=0.011) and sensitivity domain (Rho = -0.377, p=0.012). of the FIQR. We found downregulated statistical significance in the expression levels of miR-133b in plasma of female fibromyalgia patients compared to controls (p<0.0001), while the expression levels of miR-1 were not significantly different (p=0.611).
Conclusion. The association between miR-133b expression levels and fibromyalgia clinical parameters suggests that this microRNA could play a role in dysautonomic symptoms and sensitivity in fibromyalgia patients. More studies are necessary to confirm these findings.

P-65

Determination of the glycosylation profile of a monoclonal antibody produced by partially synchronized hybridoma cells.

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Background. Nowadays, monoclonal antibodies (MAbs) are the most successful biotechnological pharmaceutical therapeutics globally (1) They are structurally complex glycoproteins, with a heterogeneous glycosylation profile, which is a critical quality attribute because it directly impacts the biological activity of the molecule (2). It is necessary to investigate the factors that lead to such heterogeneity in order to generate homogeneous MAbs with more specific effector responses. Monoclonal antibodies (MAbs) are currently the most successful biotechnological pharmaceutical therapeutics worldwide. These glycoproteins are structurally complex and have a heterogeneous glycosylation profile, which is a critical quality attribute. The glycosylation profile directly affects the biological activity of the molecule, making it essential to investigate the factors that contribute to this heterogeneity. The goal is to develop homogeneous MAbs that have more specific effector responses.
Aim. In the present work, the N-glycosylation profile of a MAb produced by hybridoma cells cultured partially synchronized through the different phases of the cell cycle (CS) was determined, and compared with the profile produced during the conventional non-synchronous culture (CNS).
Materials and Methods. A murine hybridoma cell line (BCF2) that produces an IgG1 MAb was used. The cultures were carried out in suspension, volume of 50 mL, 37 oC, 5% CO2, 120 RPM, in a CD Hybridoma Gibco® culture medium supplemented with 8 mM L-Glutamine. Cell synchronization was performed by countercurrent centrifugal elutriation (3) and the cell cycle determination was performed by quantification of the cellular DNA by flow cytometry. The mAb was purified by protein A affinity chromatography and the N-glycosylation analysis was performed by mean of hydrophilic interaction liquid chromatography (4).
Results. Our results revealed that under both culture conditions the glycosylation profile was highly heterogeneous. A total of 23 oligosaccharide structures were identified, comprising both high-mannose structures and complex structures. The percentage of complex structures representative of MAbs (A2G0, A2G1 and A2G2), as a whole, was the majority in both cases. However, the CS, compared to the CNS, showed an increase in the percentage of high mannose structures, accompanied by a decrease in the percentage of complex structures.
Conclusion. The CS culture versus the traditional CNS did not reduce the heterogeneity of MAb glycosylation. In the CS, an increase in the percentage of high mannose structures was determined, indicative of an incomplete processing of the Mab oligosaccharide.

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P-66

Petiveria alliacea L. extracts inhibit amyloid beta aggregation in an in vitro model

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Background. Alzheimer's disease (AD) is an irreversible progressive neurodegenerative disorder characterized by cognitive dysfunctions including memory loss. Pathologically, AD is characterized by the formation of amyloid beta peptide (Aβ) plaques. The Aβ peptide is mainly formed by fragments of 1 to 40 or 1 to 42 amino acids derived from abnormal proteolytic processes of the amyloid precursor protein (APP). APP-cleaved peptides occur as soluble oligomers and are potentially neurotoxic. Although the causes of neuronal death mechanisms in AD remain unknown, there is evidence suggesting that Aβ (1-42)-induced oxidative stress plays an important role in the AD onset brain. Several strategies to target the production and elimination of Aβ have failed. Immunotherapies against Aβ induce encephalomyelitis and possible microbleeds, and the inhibition of secretases interrupt important metabolic processes. Therefore, it is important to study new strategies to reduce the side effects of current therapies. Petiveria alliacea, a tropical plant that belongs to the Petiveriaceae family has shown a capacity to improve cognitive impairments and inhibit acetylcholinesterase, suggesting that this plant is a candidate to obtain molecules for developing therapies to treat AD.
Aim. To evaluate the effect of Petiveria alliacea on the aggregation of the Aβ peptide in an in vitro model.
Material and methods. P. alliacea leaves were collected in Catemaco, Veracruz, México. Plant material was extracted with methanol in a solid-liquid extraction system. The extract was filtered, and the solvent was removed under reduced pressure. The methanol extract was then fractionated with different organic solvents of increasing polarity (hexane, ethyl acetate, and methanol). On the other hand, the Aβ peptide aggregation was detected by thioflavin-T (ThT) fluorescence assay. Samples with Aβ in the presence of hexane, ethyl acetate, and methanol extracts from P. alliacea were incubated at different concentrations (1000, 500, 250, 125, 50, 25, 10 µg/mL) during 24 h polymerization.
Results. The fluorescence signal of the Aβ aggregates showed significantly lower aggregation of Aβ in the presence of different concentrations of methanol and hexane extracts, compared to the control without plant extract. Furthermore, the highest Aβ aggregation inhibition was shown by hexane extract to each evaluated concentration while the highest Aβ aggregation inhibition was observed at 125 µg/mL of the methanol extract. The inhibition activity could be due to the secondary metabolites like terpenes and flavonoids present in the extracts. The tested concentrations of ethyl acetate extract had no activity on Aβ aggregates.
Conclusion. Petiveria alliacea inhibits the aggregation of Aβ, and its activity is concentration dependent. Hexane and methanol extracts have secondary metabolites that could be considered promising potential therapies for AD.

P-67

The effect of the cholesterol overload on the transcriptional regulation of romo1 gene in the hepatocellular carcinoma.

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Background. The relationship between cholesterol overload and poor prognosis in HCC is a growing area of interest. While cholesterol is essential for vital functions, its elevated presence in the Western diet and its accumulation in the liver have been associated with increased incidence and progression of HCC. Overexpression of Romo1, a protein that regulates reactive oxygen species, has been linked to increased oxidative stress in HCC. Studies in mice have demonstrated that a high-cholesterol diet and exposure to HCC-inducing agents lead to greater hepatic damage and tumor growth. These findings suggest that cholesterol may influence the development and aggressiveness of HCC by altering cellular functions and inducing stress in liver cells.
Aim. To assess the impact of cholesterol overload on transcriptional regulation of the romo1 gene in an HCC model.
Methods. A Huh-7 cell line of human HCC cells from ATCC was obtained. The cells were seeded at 48,000 cells/cm^2 in petri dishes using Williams E medium for Huh-7, supplemented with 10% (v/v) fetal bovine serum (FBS). Cells were incubated at 37°C with 5% CO2 and 95% air at saturation humidity, and monolayer adhesion was verified after 4 hours. Once attached, the cells were washed with phosphatebuffered saline (PBS), and the medium was replaced with a serum-free growth medium. The cells were then treated with cholesterol-cyclodextrin (Cx) and cyclodextrin (Cdx) complex for short durations (NT, 0 h, 1 h, 1.5 h, and 3 h). Trizol was used for RNA extraction to perform RT-qPCR to measure the gene expression of romo1. The same method was used for protein extraction and Western Blot at longer durations (NT, 0 h, 12 h, 24 h, and 48 h), with n=3 for both experiments.
Results. It was found that cholesterol increased the levels of romo1 mRNA transcriptionally at 1.5 h and 3 h post-treatment compared to the untreated cells. Cholesterol can affect various pathways that activate gene expression because this molecule is found in the cellular and mitochondrial membranes. Cholesterol can bind with other proteins or disturb the mitochondrial redox state, which affects gene expression. At the protein level of ROMO1, the levels were also affected. We observed that protein levels decreased at 48 h, possibly due to translational regulation.
Conclusion. Cholesterol induces the activation of romo1 gene transcription and can also affect the protein content. We suggest that cholesterol activates certain transcription factors that bind to the romo1 promoter and may also modify protein levels.

P-68

Analysis of the mechanism of radiosensitization of resveratrol in cervical cancer cell lines.

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Background. Cervical cancer (CC) is the second most common type of cancer in the world that occurs in the female population. Most cases of CC are diagnosed in advanced stages, which is why radiotherapy is the most used therapeutic modality. The mechanism of action of ionizing radiation (IR) is to induce cell damage directly or indirectly by generating DNA double-strand breaks through the formation of free radicals. In response to this stimulus, the cell is able to activate DNA damage repair pathways by homologous recombination (HR) and non-homologous recombination (NHEJ) through the activation of the proteins Rad51, Ku70 and Ku80. New ones are currently being sought. agents with radiosensitizing properties that can be directed towards these molecular targets for cancer treatment. One compound we have set out to analyze is resveratrol (RSV), a natural polyphenol and phytoalexin, found mainly in peanuts, grapes and wines. It has been seen that RSV decreases the expression of genes and proteins involved in the repair of DNA damage in MCF7 breast cancer cells, and is also capable of reducing the viability of prostate cancer cells. It has been reported that RSV is capable of inducing DNA damage in DT40 cells.
Objective. To analyze the mechanism through which RSV acts as a radiosensitizer in cervical cancer cell lines through the inhibition of DNA damage repair pathways by homologous (HR) and non-homologous recombination (NHEJ), as well as to evaluate the effect of RSV on the induction of DNA damage.
Methodology. To this end, SiHa, C33a and HeLa DC cells were treated for 48 hours with RSV with their respective IC20 and IC50 and subsequently subjected to ionizing radiation (IR) with their corresponding LD20 and LD50 in order to evaluate the effect of the treatments alone and in combination on the levels of the KU70, KU80, Rad51 and γH2AX proteins. The Western Blot technique was also performed to analyze the levels of the KU70, KU80, Rad51 and γH2AX proteins.
Results. The results obtained so far indicate that the treatment of RSV IC50 in combination with IR DL50 is capable of significantly reducing the levels of the KU70 protein in the HeLa cell line. RSV can decrease the levels of KU70 and KU80 proteins in the HeLa, SiHa and C33a cell lines in the treatments combined with the treatment of RSV with their respective IC20 in combination with the IR DL20 and more evidently in the treatment of the RSV IC50 in combination with the RI DL50. Regarding the RAD51 protein. The results suggest that treatment with their respective RSV IC50 and also the combination of RSV IC50 plus RI LD50 is capable of reducing protein levels in the 3 cell lines. These preliminary results show

that the IC50 of RSV and the combination of RSV IC50 plus RI DL50 is capable of damaging DNA by increasingγH2AX in the three cervical cancer cell lines.

Conclusion. These results suggest that RSV inhibits proteins involved in the repair pathway by Homologous and Non-Homologous Recombination of Cervical Cancer cells. CONAHCYT: 1080728.

P-69

Blockade of the Lipid Phenotype in Prostate Cancer as Metabolic Therapy.

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Background. Prostate cancer is the second most frequent malignancy in males, is the fifth leading cause of cancer -related death in both developed and developing countries, and accounts for a significant proportion of the health burden worldwide. Even with recent advances in therapy, the disease still represents a significant morbidity and mortality issue. In this sense, has been proposed a new strategy of therapeutic repurposing of low-cost and widely available drugs could reduce global mortality from this disease. The malignant metabolic phenotype is becoming increasingly important due to its therapeutic implications. Prostate cancer is particularly lipidic; it exhibits increased activity in the pathways for synthesizing fatty acids, cholesterol, and fatty acid oxidation (FAO). Pantoprazole and orlistat are two inhibits of the enzyme fatty acid synthase (FASN) therefore, blocking the novo synthesis of fatty acids. Our previous studies demonstrated antitumor effects of orlistat in combination with lonidamine and 6-diazo-5-oxo-L-norleucine in several cancer cell lines. In the present study was analyze which of these two inhibitors is more
effective in prostate cancer.
Objective. To analyze the effect Pantoprazole and orlistat in prostate cancer.
Methodology. In the present study PC3, DU145 (negative AR), 22RV1 and LNCap (positive AR) prostate cancer cells were treated with pantoprazole and orlistat as well as in combination with bicalutamide (22RV1, LNCap) and docetaxel (PC3, DU145) for 10 days were compared regarding viability inhibition (violet crystal), pharmacological interaction and measuring cell metabolic activity (MTT).
Results. Our results shows that orlistat and pantoprazole significantly decreased the viability of the four cell lines of prostate cancer. Also analyzed the effect of bicalutamide in 22RV1 and LNCap cancer cell lines positive to AR (androgen receptor) and docetaxel in PC3 and DU145 negative to AR. Our results showed that orlistat and pantoprazole significantly decreased the viability of the four cell lines of prostate cancer. on the other hand, analyzed the effect of bicalutamide in the 22RV1 and LNCap cell lines positive to AR (androgen receptor) and of docetaxel in PC3 and DU145 cells lines negative to AR. our results demonstrated an effect with bicalutamide and docetaxel viability inhibition however this effect was more evident later combination with orlistat and pantoprazole. The analyses of the pharmacological interaction also demonstrated an effect synergic in LNCap, 22RV1 in PC3 cell lines with both combinations. Finally, the measuring cell metabolic activity mediated by MTT assay demonstrated a significantly decreased with the combination pantoprazole-bicalutamide and pantoprazole-docetaxel in the LNCap and PC3 prostate cancer cell lines, respectively.
Conclusions. These results suggest that the inhibitors of the novo synthesis of fatty acids

orlistat and pantoprazole decreased the viability of prostate cancer cells lines independently of AR suggest that more studies are needed to elucidate the mechanism responsible of this effect.

P-70

PaSTe. Blockade of the Lipid Phenotype in Prostate Cancer as Metabolic Therapy.

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Background. Prostate cancer is the second most frequent malignancy in males, is the fifth leading cause of cancer -related death in both developed and developing countries, and accounts for a significant proportion of the health burden worldwide. Even with recent advances in therapy, the disease still represents a significant morbidity and mortality issue. In this sense, has been proposed a new strategy of therapeutic repurposing of low-cost and widely available drugs could reduce global mortality from this disease. The malignant metabolic phenotype is becoming increasingly important due to its therapeutic implications. Cancer generally is characterized by hyperactivation of glycolysis, glutaminolysis, and fatty acid synthesis. However, prostate cancer is particularly lipidic; it exhibits increased activity in the pathways for synthesizing fatty acids, cholesterol, and fatty acid oxidation (FAO). Pantoprazole and simvastatin inhibit the enzymes fatty acid synthase (FASN) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), therefore, blocking the synthesis of fatty acids and cholesterol, respectively. In contrast, trimetazidine inhibits the enzyme 3-b-Ketoacyl-CoA thiolase (3-KAT), an enzyme that catalyzes the oxidation of fatty acids (FAO). It is known that the pharmacological or genetic depletion of any of these enzymes has antitumor effects in prostatic cancer.
Objective. To evaluate PaSTe (Pantoprazole, Simvastatin, Trimetazidine) effect as a metabolic therapy in prostate cancer.
Methodology. In the present study PC3, DU145 (negative AR), 22RV1 and LNCap (positive AR) prostate cancer cells were treated individually with pantoprazole, simvastatin, trimetazidine and in combination (PaSTe) as well as bicalutamide-PaSTe (22RV1, LNCap), docetaxel-PaSTe (PC3, DU145) for 10 days to analyze the effect on viability inhibition (violet crystal).
Results. Our results shows that the combination PaSTe significatively decreased the viability levels in the four prostate cancer cell lines later of 10 days the treatment.
Conclusions. We conclude that this regimen deserves to be preclinically evaluated because of its clinical potential for the treatment of prostate cancer. However, are needed more studies to elucidate the mechanism responsible of this effect.

P-71

Chronic sleep restriction promotes neurotoxic astrocytes and blood-brain barrier dysfunction.

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Background. Astroglia maintain the blood-brain barrier (BBB) by the release of diverse soluble mediators. During inflammatory environment, the BBB can be altered and astroglia may polarize, increasing C3 protein (A1/neurotoxic phenotype marker), β-galactosidase or p21 (senescence markers) and decreasing S100a10 protein (A2/neuroprotector phenotype marker). Sleep restriction promotes local inflammatory environment and increases the astrogliosis marker GFAP expression. Additionally, it has been reported that sleep one night of sleep deprivation promotes increased levels of senescent markers in serum of aged people.
Aim. To evaluate the changes in astroglia phenotypes and their impact on BBB alteration and senescent process during sleep restriction.
Results. Male C57BL/6 were sleep restricted 20h daily with 4h sleep opportunity during 3, 5 or 10 days using the multiple platform technique, then the cerebral cortex and hippocampus were dissected. BBB permeability assays were performed using Evans blue and sodium-fluorescein tracers. Changes in the expression of GFAP, C3, β-galactosidase, p21 and tight junction proteins were evaluated by western blot, and TNF-α and IL-6 by ELISA, in the cerebral cortex and hippocampus. We found that sleep restriction increased BBB permeability and C3 protein (A1 phenotype). After 10 days of sleep restriction increased levels of senescent markers and pro-inflammatory cytokines were found, while decreased levels of S100a10 protein (A2 phenotype), were found.
Conclusion. These results suggest that sleep restriction alters astroglia physiology beginning at the 5th day of sleep restriction. A1 astrocytes may promote the local increase of pro-inflammatory mediators leading and senescent process and BBB dysfunction.

P-72

α-amyrin and lupeol, triterpenes from *Hibiscus sabdariffa* L. with anti-inflammatory effect and activity.

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Background. Inflammation is a protective response of the body's tissues to irritation or injury, where macrophages play an important role, producing key cytokines. Although they are crucial for the resolution of damage, chronic inflammation favors the development of metabolic diseases such as obesity, type 2 diabetes and cardiovascular diseases. Existing anti-inflammatory medications that are useful, however, their long-term use leads to unwanted side effects. This has motivated the search for new compounds with safer and more effective anti-inflammatory properties. In traditional Mexican medicine, *Hibiscus sabdariffa* L. is reported to be used to treat obesity and diabetes. Studies have identified in this plant the triterpenes α-amyrin and lupeol, dual agonists of PPARδ/γ, which may be related to anti-inflammatory effects. Possible anti-inflammatory effect and activity of these triterpenes are evaluated with *in vivo* and *in vitro* studies.
Aim. Evaluate anti-inflammatory effects of these triterpenes in *in vivo* and *in vitro* studies.
Methodology. Mice of the CD-1 strain were used to evaluate the anti-inflammatory effect, through topical administration of TPA in the ear, triterpenes were administrated to 10 mM. Macrophages from the RAW264.7 line were treated with LPS (1μg/mL) to induce an inflammatory response. Subsequently, the cells were treated with the compounds α-amyrin [10μM] and lupeol [10 μM] for 24 hours. Secreted cytokines were quantified in medium culture by the ELISA method. Cytokines expressions were analyzed by real-time RT-PCR.
Results. α-amyrin and lupeol reduce ear edema caused by TPA; α-amyrin generates 68% inhibition and lupeol 85% compared to the CT. Both compounds even have a greater anti-inflammatory effect than positive CT, Indomethacin. On the other hand, these triterpenes reduce the expression of TNF-α and IL-6, in addition to increasing IL-10 compared to the CT, generating an effect like positive CT, Celecoxib. Likewise, both compounds decrease the secretion of proinflammatory cytokines in cultured RAW 264.7 macrophages.
Conclusion. α-amyrin and lupeol are compounds with anti-inflammatory potential, modulate the expression and secretion of pro- and anti-inflammatory cytokines. It is important to continue with the elucidation of the mechanism of action of these triterpenes, with the aim of proposing them for the development of new drugs.
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P-73

Aspergillus terreus AtfB (bZIP) differently regulates the lovastatin production in submerged and solid-state fermentation.

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Introduction. Coronary heart disease is the first cause of death worldwide, and lovastatin and its semisynthetic derivative, simvastatin, are mainly used to treat this condition. The drug lovastatin is produced by Aspergillus terreus, a filamentous fungus capable of producing several secondary metabolites of interest by fermentation in liquid medium (SmF) and solid medium (SSF). Recently the interest in the production of SSF has increased, due to its high yields and other advantages related to the physiology of the solid medium compared to SmF. Our working group identified the main stimuli that trigger the physiology of the solid medium, which are direct contact with air and contact with support stimulus. Regarding direct contact with air, our group found that reactive oxygen species (ROS), related to aerobic respiration, play a key role in the production of secondary metabolites with a positive upregulation. Currently, we found a group of transcription factors associated with the defense against oxidative stress, which has a double function acting as regulators as well in the production of lovastatin by A. terreus, among them is AtfB.
Objective. This work aimed to study the role of the AtfB transcription factor in the regulation of lovastatin biosynthesis in solid-state and submerged fermentation in A. terreus.
Methods. We phenotypically characterized atfB knocked-down mutants obtained with a constructed RNAi plasmid and transformed in A. terreus. Oxidative and osmotic stress response assays were performed with H2O2 and NaCl respectively. Lovastatin was quantified with HPLC. ROS were quantified in mycelium using a fluorogenic probe H2DCF-DA which can be oxidated. H2O2 addition in cultures was used as exogenous ROS. Quantification of atfB, lovE, lovF and sod1 expression was measured with RT-qPCR using actin gene as housekeeping.
Result-discussion. AtatfB knocked-down mutants of A. terreus showed increased sensitivity to oxidative and osmotic stress in conidia. Moreover, transformants showed decreased lovastatin production in SSF, while increased production in SmF but ROS levels were decreased as well in both
fermentation systems during idiophase. When ROS of mutants were increased with exogenous ROS to equal levels to parental strain, the differences in lovastatin production originally found were conserved in SmF while in SSF, there seems to be an exacerbation effect of a prevailing ROS regulation. AtAtfBregulates lovastatin biosynthesis in A. terreus, but has different functions in SmF and SSF, acting as a negative regulator in SmF at transcriptional level due to a higher expression of lovE and lovF in SiatfB and as an indirect positive regulator in SSF through ROS.

Conclusion. AtAtfB is part of the oxidative and osmotic stress defense of A. terreus and differentially regulates lovastatin biosynthesis, in SSF acts as an indirect positive regulator mainly through ROS and a negative direct regulator at transcriptional level in SmF. At the same time, it is part of a complex network of transcriptional factors associated with stress defense. These findings will contribute to unraveling the molecular basis of the physiology of solid medium, and probably to design other genetic improvement strategies for producing strains in SmF or SSF.

P-74

Chronic sleep restriction induces β-amyloid accumulation and changes in proteins expressed in blood-brain barrier.

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Sleep restriction (SR) is commonly observed in modern societies. In humans and rodents, lack of sleep is associated with cognitive impairment. Decreased sleep time has been observed to increase concentrations of the β-amyloid peptide in the brain of both humans and rodents. However, the exact mechanism that favors the accumulation of β-amyloid in the CNS is not yet known. It has been observed that in total sleep deprivation there is a decrease in low-density lipoprotein receptor-related protein 1 (LRP1), which is expressed mainly in the endothelial cells of the blood-brain barrier; however, little is known about the possible changes in the expression of this protein in sleep restriction.
The aim of this study is to determine the effects of SR on LRP1 expression in the hippocampus and cortex of rats. To do this, we subjected male Wistar rats to an SR protocol using the modified multiple platform method. Rats were placed on 7 cm platforms for ten days (20 hours of sleep loss per day). Every day. Immediately after daily RS, the animals were allowed to rest in their own home cages (4 hours of sleeping opportunity per day). On day 10, declarative memory was assessed using the novel object recognition test. The hippocampus and cortex were then dissected for Western blotting to determine the expression of β-amyloid peptide and LRP1 protein. We observed an increases of β-amyloid peptide and worse performance in the behavioral test and a decrease in LRP1 protein expression.

P-75

Effect of sulforaphane on primary astrocytes of rat cerebral cortex induced cellular senescence with palmitate.

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Background. High fat intake leads to obesity, linked to cognitive decline and neurodegenerative diseases. Using palmitic acid, a primary fatty acid in high-fat diets, we simulated brain lipotoxicity impacting glial cells like astrocytes. The involvement of astrocytes in the neuropathology of these diseases is attributed to their fundamental role in the proper functioning of the Central Nervous System (CNS), as well as in their ability to respond to damage or stressors leading to a cellular state known as senescence, characterized by morphological and functional changes that play an important role in neurodegeneration. On the other hand, sulforaphane (SFN) is a phytochemical present in cruciferous vegetables that is an important inducer of antioxidant response and regulator of redox homeostatic imbalance, normally present in senescent cells.
Aim. Our objective was to evaluate the protective effects of sulforaphane on senescent astrocytes caused by palmitate and related to neurodegeneration.
Methodology. Primary astrocytes were isolated from Winstar rat cerebral cortex and treated for 24 h with different concentrations of sulforaphane (1 uM, 2 uM, 4 uM, 8 uM, 16 uM, and 32 uM). Cell viability was measured with trypan blue, while HPLC evaluated the concentration of GHS and GSSG to determine redox status. The concentration with the best response was chosen and used to pre-treat the cells for 24 h and treated with palmitate to induce senescence (200 uM for 24 h). The redox status was re-evaluated.
Results. Treatment with high concentrations of SFN (32 uM) caused significant cell death compared to lower concentrations. An intermediate concentration of SFN (4 uM) was chosen, which significantly changed the redox state of senescent astrocytes.
Conclusion. We confirm that sulforaphane can be an important antioxidant and senescent protector at low concentrations and cytotoxic at high concentrations.

P-76

Changes on cholinesterases activity in the liver of CD1 mice in a model of liver injury induced by acetaminophen.

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Background. Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) are two enzymes responsible for the hydrolysis of acetylcholine (Ach), which has been involved in cell proliferation processes and as an anti-inflammatory agent. Both enzymes are found in the liver, the organ responsible for the detoxification of xenobiotic agents, such as acetaminophen or Paracetamol, which is the most used and self-medicated analgesic and antipyretic by people to treat some type of mild or moderate pain, however , it has been reported that an overdose of Paracetamol can cause severe liver damage resulting in acute liver failure (AFL). It has been reported in models of liver injury due to hepatectomy that ACh has an important participation in the regeneration of this tissue, therefore, it is suggested that the hydrolytic activity of cholinesterases must be decreased.
Aim. To evaluate if there are modifications in the enzymatic activity of cholinesterases when liver injury occurs due to a xenobiotic agent.
Method: An overdose of acetaminophen was administered to 3-month-old male mice and the enzymatic activity of cholinesterases was measured at 24 and 48 hours after administration. The enzyme activity of AChE and BChE was measured spectrophotometrically by Ellman´s method and was normalized by Bradford´s method. The concentration of Acetylcholine was quantified by kit, as suggested by the manufacturer.
Results. In the liver tissue we observed that after 24 hours of treatment there were no changes in the enzymatic activity in exposed group, after 48 hours of treatment the activity of both enzymes increased significantly in exposed group. However, the concentration of ACh after 48 hours of treatment is not altered in any group. In the case of serum, no changes.
Discussion. Liver injury induced by acetaminophen overdose occurs 24 hours after administration (as suggested by various authors), however, during this time the enzymatic activity of cholinesterases remains unchanged, due to this we suggest that the early response in the event of damage, it is the appearance of an exacerbated inflammatory process, therefore must be an anti-inflammatory system that regulates this phenomenon, such as ACh, which during this process we hypothesize is elevated, because it does not come solely from liver tissue but also from the vagus nerve; 48 hours after administration after the treatment, the concentration of ACh must decrease and is at a level similar to the physiological level, so for this to happen the activity of the cholinesterase enzymes is increased at this time.
Conclusion. Hepatic cholinesterase enzymes act in a late phase of response to damage, that is, they are not the main inflammatory control mechanism.

P-77

Changes in the *in-situ* expression of Cx43 protein in the ventricular myocardium of mice exposed to gold nanoparticles smaller than 15 nm.

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Background. In recent years, the use of gold nanoparticles (NPAu) has become popular in different industries. NPAu stands out for its potential biomedical applications for the diagnosis and treatment of several degenerative diseases. However, a debate persists about the possible toxic effects of NPAu. In the heart, cellular and histological alterations have been reported. Beyond these investigations, the effect of NPAu on the integrity of the gap junctions of the intercalated disc, which are crucial for the correct contraction of the ventricular myocardium, has not been addressed.
Aim. To evaluate the effect of NPAu of 5, 10, and 15 nanometers (nm) on the structure of the gap junctions of the intercalated discs of the ventricular myocardium.
Materials and methods. 12 male mice of the CD-1 strain, between 8 to 10 weeks of age, were used. The animals were randomly divided to form three experimental groups (“NPAu-5”, “NPAu-10” and “NPAu-15”) and a control group. The experimental groups were subjected to seven doses of 30 µL, intraperitoneally, of NPAu of 5, 10, and 15 nm, respectively. The vehicle was administered to the control group using the same route and dose. After euthanasia, we obtained blood from the inferior vena cava for clinical studies, dissected the hearts, obtained photographs, and then processed them for paraffin embedding. The histological sections were stained using the hematoxylin-eosin technique, and indirect immunofluorescence was performed to detect the gap junction protein connexin 43 (Cx43).
Results. No mortality was recorded in any of the study groups. Blood studies revealed significant changes in AST, ALT, HDL, T-cho, and TG tests, suggesting heart and liver damage and changes in cholesterol and triglyceride metabolism. Morphometric analysis of surface anatomy relationships did not show any changes. Immunofluorescence analysis revealed that there were no global changes in Cx43 protein expression. However, different subcellular reorganizations of the Cx43 protein were observed in the experimental groups: lateralizations, perinuclear accumulation, and different degrees of dispersion in the intercalated disc. These rearrangements were reflected in a significant reduction in the number of intercalated discs with gap junctions in the hearts of mice in all experimental groups.
Conclusions. Intraperitoneal administration of 5, 10, and 15 nm NPAu causes structural changes in the gap junctions of the intercalated disc in the ventricular myocardium.

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