PANEL COMMUNICATIONS
Monday, December 4, 2023

1.
EXPLORACIÓN FARMACOLÓGICA DEL ANTAGONISMO 5-HT6R COMO MODULADOR DE LA VÍA MTOR EN LA AUTOFAGIA NEURONAL. Pharmacological exploration of 5-ht6r antagonism as a modulator of the mtor pathway in neuronal autophagy.

Autophagy is a physiological process of cellular degradation and recycling highly conserved in eukaryotic cells, by which cells degrade damaged organelles, macromolecular aggregates, and pathogens, through the lysosomal pathway to maintain energetic, metabolic and proteostatic homeostasis. This process is regulated by complex interactions between stimuli (such as starvation, hypoxia, ROS, etc) and multiple proteins, with mTOR being a master regulator of the overall process. Recent research has shown that 5-HT6R has a non-canonical pathway (Gs-independent) coupled to the mTOR pathway through which it compromises cognition by increased mTOR activity associated with abnormal activity of this receptor in neurodevelopmental models. Therefore, the development of 5-HT6R antagonists could have an impact on cognition by inducing autophagy through the mTOR pathway. Aim. To evaluate the induction of mTOR-dependent autophagy by 5-HT6R antagonists with N-arylsulfonylindole/indazole structure in the SH-SY5Y neuroblastoma cell line. Methods. The subcloned neuroblastoma cell line SH-SY5Y was used. MTT assay was performed to determine the cytotoxicity of the compounds. 5-HT6R levels were determined by Western Blot. Subsequently, autophagy induction was analyzed by immunofluorescence (GFP-LC3) and mTOR-dependent autophagy protein modifications were also determined by Western Blot. Results and Conclusions. Our results show that the N-arylsulfonylindole/indazole derived compounds induce autophagy through the mTOR pathway. SH-SY5Y cells treated with the N-arylsulfonylindole/indazole derived compounds show a cell survival of over 90% at different concentrations. Compounds PUC-10 and PUC-111 exhibit an increase in the number of cells with autophagosomes as well as an increase in the number of autophagosomes per cell in SH-SY5Y cells and show a similar tendency in the decrease of mTOR and ULK1 phosphorylation as well as an increase of BECN1 phosphorylation.

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2.
MICROFIBRAS CARGADAS CON NANOPARTÍCULAS DE PLATA: UNA SOLUCIÓN PROMETEDORA PARA LA PREVENCIÓN Y TRATAMIENTO DE LA PERIIMPLANTITIS. Silver Nanoparticle-Loaded Microfibers: A Promising Solution for Peri-Implantitis. Prevention and Treatment.

Peri-implantitis is an inflammation that affects the tissues around dental implants, potentially leading to loss of the implant itself. In this study, advanced microfibers were developed with the ability to combat peri-implantitis through the controlled release of silver nanoparticles (AgNPs). Objective: The main objective of This study aimed to evaluate the efficacy of polyhydroxybutyrate (PHB) microfibers loaded with AgNPs against various bacterial strains. Materials and Methods: Initially, AgNPs were synthesized via a chemical reduction process in N,N-dimethylformamide (DMF). These nanoparticles were subsequently incorporated into a PHB solution, and from this combination, microfibers were fabricated by electrospinning. Three concentrations of AgNPs loaded on the fibers were evaluated (PHB- AgNPs 1X, PHB- AgNPs 5X, and PHB- AgNPs 10X). To evaluate the antimicrobial activity of the microfibers, tests were performed using different bacterial strains, including Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans, and Candida albicans. The antimicrobial activity of the membranes was evaluated based on the planktonic growth of the microorganisms exposed to the membranes during 24 h at 37°C, the kinetic growth was monitored each 30 min as optical density at 600 nm. Results: The PHB-microfibers loaded with AgNPs showed marked antibacterial activity against all strains evaluated at all

concentrations tested. However, only the highest concentration of AgNPs inhibited planktonic growth of C. albicans.
Conclusions: The results obtained in this study indicate that microfibers have potential in the prevention and treatment of peri-implantitis, due to their potent antibacterial activity and their ability to prevent the proliferation of planktonic bacteria that could colonize different Areas of the implant and form a biofilm.

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3.
ASOCIACIÓN ENTRE POLIMORFISMOS GENÉTICOS DE OPRM1 Y TLR4 CON LA RESPUESTA AL DOLOR ONCOLÓGICO EN PACIENTES CON CÁNCER DE COLORRECTAL AVANZADO TRATADOS CON FÁRMACOS OPIOIDES. Association between genetic polymorphisms of OPRM1 and TLR4 with oncologic pain response in patients with advanced colorectal cancer treated with opioid drugs.

Moderate to severe pain is present in 80% of patients with advanced stage cancer and weak to potent opioids are the first line. In colorectal cancer (CRC), oxaliplatin-induced peripheral neuropathy (OIPN) is one of the most frequent adverse effects. Its etiology is associated with TLR4 (Toll-like receptor 4) and the concomitant use of opioids is considered a risk factor. The genes of interest are OPRM1 (mu opioid receptor) and TLR4, they present single nucleotide polymorphisms (SNP) of interest (rs1799971 and rs510769; rs4986790 and rs4986791, respectively), which decrease the responsiveness of the receptor. General objective. Determine the influence of polymorphic variants of OPRM1 (rs1799971 and rs510769) and TLR4 (rs4986790 and rs4986791) on pain relief and degree of OIPN, respectively. In patients with advanced stage CRC under opioid treatment. Methodology. Multicenter, ethically approved case-control study. DNA samples are from tumor and non-tumor tissue. SNP analysis is performed by qPCR associated with TaqMan® probes. The Visual Analog Scale (VAS) and the opioid posology change are used to evaluate pain response, while the degree of severity and frequency of occurrence are analyzed for OIPN. An association analysis is performed with univariate logistic regression and χ^2 test in R program. Results. The rs1799971 and rs510769 (OPRM1) polymorphisms were determined to be in Hardy-Weinberg Equilibrium (n=102), with a χ^2 value of 0.25 and 0.52, respectively. It was observed that patients carrying the mutated allele for rs1799971 obtained higher VAS with standard opioid doses when compared to patients not carrying the SNP ($p<0.05$). Conclusions. The genotype carrying the mutated allele for rs1799971 (OPRM1) is significantly associated with lower pain relief when using opioids as analgesic treatment in patients with advanced CRC. Key words: Opioids; oncologic pain; pharmacodynamic; oxaliplatin-induced peripheral neuropathy; pharmacogenetics; single nucleotide polymorphism; OPRM1 polymorphism; TLR4 polymorphism.

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4.
DETERMINACIÓN DE CAMBIOS EN LA EXPRESIÓN DE LA PROTEÍNA BDNF EN UN MODELO DE RATÓN CON SOBREEXPRESIÓN DEL TRANSPORTADOR EAAT3. DETERMINATION OF CHANGES IN THE EXPRESSION OF THE BDNF PROTEIN IN A MOUSE MODEL WITH OVEREXPRESSION OF THE EAAT3 TRANSPORTER.

The pathophysiology of depression is associated with dysregulation of glutamate system and clearance mechanisms in brain regions mediating cognitive-emotional behaviors. Mice with forebrain EAAT3 overexpression (CaMKIIα-promoter driven, EAAT3glo/CMKII mice) may be linked to a resilient phenotype to chronic stress. Furthermore, EAAT3glo/CMKII mice could be have alterations in BDNF expression, a relevant factor in the depression. Aims: To evaluate the consequences of unpredictable chronic mild stress (UCMS) on the expression of BDNF in EAAT3glo (control group, EAAT3 non-overexpressing) and EAAT3glo/CMKII mice. Methods: EAAT3glo and EAAT3glo/CMKII mice were tested in anxiety and depressive-like behaviors in baseline and UCMS conditions. BDNF protein levels were measured by Western Blot. Results: EAAT3glo/CMKII mice subjected to UCMS exhibited a lower depression score compared to stressed EAAT3glo mice, indicating that the UCMS paradigm did not induce depressive-like behaviors in EAAT3-overexpressing mice. Furthermore, significant differences were found in the mRNA levels of BDNF in the hippocampus of EAAT3glo/CMKII mice in baseline conditions. The ratio of mature BDNF to preproBDNF significantly decreased in the medial prefrontal cortex of EAAT3-overexpressing mice. Furthermore, the BDNF/preproBDNF ratio and mature BDNF levels increased in the CA1 region of the hippocampus in EAAT3glo/CMKII mice. Nonetheless, these changes were not observed in chronic stress conditions. Summary and Conclusions: Differences between brain regions in mature BDNF levels and its precursors may contribute to resilience to chronic stress. Altogether, our results suggest that forebrain EAAT3 overexpression may be linked to a resilient phenotype to chronic stress, emphasizing the significance of this model for translational studies on mechanisms and biomarkers of stress resilience.

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5.
ADSORCIÓN COOPERATIVA DE ANTICUERPOS MONOCLONALES EN NANOPARTÍCULAS DE ORO: UN NUEVO ENFOQUE PARA MEJORAR LA EFICACIA TERAPÉUTICA. Cooperative Adsorption of Monoclonal Antibodies on Gold Nanoparticles: A Novel Approach for Enhanced Therapeutic Efficacy.

Monoclonal antibodies (mAbs) represent vital pharmaceutical agents in the 21st century, celebrated for their precision and safety. Nevertheless, they encounter challenges, such as limited tissue penetration and stability, which may restrict their therapeutic efficacy. In this context, nanomedicine emerges as a promising avenue to enhance various pharmacological parameters, encompassing passive targeting, tumor accumulation, and overall stability. Aim: This study aimed to evaluate the therapeutic potential of gold nanospheres functionalized with innovative mAbs as a strategy to improve existing therapies.

Methods: Our approach involved the development of a nanosystem using a "cooperative adsorption" technique to form a protein corona on gold nanoparticles. Different nanospheres were functionalized with anti-MICA and anti-sST2. The nanosystems were characterized using Z-potential, hydrodynamic diameter, UV-vis spectrophotometry, and electron microscopy (STEM). The antigen recognition capability of the nanosystems was evaluated by immunological assays. Long-term stability was evaluated using UV-vis. Results: Our study verified the successful synthesis of gold spheres characterized by a hydrodynamic diameter of 59.8 ± 26.9 nm, a Z-potential of -32.6 ± 13.5 mV, and a STEM diameter of 41.1 ± 4.1 nm. Subsequently, we confirmed the conjugation of two different nanosystems with antibodies, resulting in hydrodynamic diameters of >100 nm, Z-potentials of approximately -20 mV, and a UV-vis red shift of 3 nm. Notably, our nanosystem showed antigen-specific recognition as demonstrated by immunological assays. Conclusion: We've achieved a functional nanosystem with gold nanospheres through cooperative adsorption. While further studies are needed to optimize protocols and evaluate efficacy in animal models, our findings show that the functionalization of nanoparticles with antibodies is feasible and could potentially be used as an effective delivery tool.

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6.
EVALUACIÓN DE DERIVADOS MITOCONDRIALES DEL ÁCIDO GÁLICO Y GENTÍSICO SOBRE ADIPOCITOS DE RATÓN. Evaluation of mitochondrial derivatives of gallic and gentisic acid in mouse adipocytes.

Obesity has become the pandemic of the last century due to the Western diet and sedentary lifestyle, triggering chronic diseases associated with obesity such as type 2 diabetes mellitus, hepatic steatosis and cardiovascular disorders. The pharmacological treatments for these pathologies are varied, however, for obesity there are very few therapeutic strategies. At the cellular level, white adipose tissue contains fat-storing adipocytes, these cells being the most involved in the progress of the condition. Targeting the reduction of lipid content in these cells could generate a therapeutic alternative. Within this aspect, several alternatives have been evaluated to reduce the fat content in adipocytes. Additionally, the mitochondrial activity of adipocytes positively influences lipid metabolism, where white adipocytes become a beige phenotype (browning of white adipocytes), and using lipids to generate energy, which is often the dissipation of heat by the uncoupling protein 1 (UCP-1) activity. In this context, we have designed lipophilic cations derived from gallic acid (TPP+C10) and gentisic acid (GA-TPP+C10) capable of concentrating into mitochondria and generating the uncoupling effect of the electron transport chain, similar to the effect of UCP1. In this work we used 3T3-L1 cells differentiated into adipocytes and evaluated the effect of these compounds on the triglyceride content through Oil O red staining, the expression of proteins such as UCP-1 and PGC1-α through of western blot and RT-qPCR, to determine the consequence of the mitochondrial effect of the cations, as well as we evaluated the activity of the AMPK protein, through western blot, an essential protein in metabolic control. The results showed that both cations reduce the lipid droplets of adipocytes as well as increase the expression of UCP-1, PGC1-α and the activity of AMPK. In conclusion, these results suggest that the compounds TPP + C10 and GA-TPP + C10 could brown white adipocyte through a mitochondrial mechanism.

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7.
ACTIVIDAD ANTIBACTERIAL Y ANTITUMORAL DE ALCALOIDES DE ZEPHYRANTHES MONANTHA. Antibacterial and antitumor activity of alkaloids from Zephyranthes monantha.

Amaryllidaceae es una familia de plantas productoras de alcaloides con activad farmacológica como licorina y haemantamina, los cuales han sido estudiados como potentes agentes antitumorales. Por otra parte, numerosos estudios han respaldado la idea, que diferentes bacterias están asociadas con el cáncer colorectal humano y son capaces de inducir tumorigénesis en modelos murinos genéticamente susceptibles. Los biofilms bacterianos son comunidades microbianas, en las que las células bacterianas en estado sésil están protegidas mecánica y químicamente, mejorando la resistencia a los compuestos antibacterianos. Zephyranthes monantha es una planta endémica de Chile y hasta la fecha no hay reportes de su composición alcaloidea o su actividad biológica, por lo tanto, el objetivo de nuestro estudio fue identificar los alcaloides de Z. monantha y evaluar su actividad antimicrobiana y citotóxica sobre células Caco 2. Zephyranthes monantha fue colectada en matanzas, Región de O´Higgins (-33.907742, -71.837973). El extracto metanólico (Z.MeOH) se realizó con la planta seca y molida (1:20). Z.MeOH se disolvió en H2SO4 (2%) y se extrajo con AcEt, posteriormente se llevó a pH 10 con NH4OH (25%). La fracción alcaloidea se extrajo en CHCl3. El crudo alcaloideo se analizó por GC-MS. Células Caco 2 se cultivaron en medio DMEM suplementado con SBF (10%), antibiótico (1%). El ensayo se realizó en microplaca placa a 24 y 48 horas de exposición del extracto alcaloideo. El ensayo de susceptibilidad bacteriana se determinó por difusión en agar para Listeria monocytogenes (formadora de biofilm) y E. coli. Se identificaron los alcaloides galantamina, papilina, haemantamina entre otros. El extracto fue citotóxico y antibacterial en concentraciones menores a 125 mg/L. Z. monantha constituye una fuente importante de alcaloides con actividad antitumoral y antibacterial.

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8.

LA INHIBICIÓN QUIMIOGENÉTICA DE LAS NEURONAS DOPAMINÉRGICAS MESOLÍMBICA CONDUCA A LA SENSIBILIZACIÓN LOCOMOTORA AL QUINPIROL EN RATONES. The chemogenetic inhibition of mesolimbic dopamine neurons leads to locomotor sensitization to Quinpirole in mice.

In rats, the repeated administration of Quinpirole (QNP), a dopamine D2 receptor agonist, induces compulsive-like behaviors and locomotor sensitization, a sustained increase in horizontal locomotor activity. Although QNP-induced locomotor sensitization is very robust in rats, in mice, this effect is poorly observed, with reports showing a mild increase and others not affecting locomotion. The mesolimbic dopaminergic system, composed of ventral-tegmental Área (VTA) dopamine neurons projecting to the Nucleus Accumbens (NAc), underlies the induction of locomotor sensitization. In this system, QNP activates D2R in the indirect medium spiny neurons, promoting locomotion, and D2R in dopamine neurons, decreasing dopamine release. Previously, we found that rats showing QNP locomotor sensitization have decreased basal dopamine release in the NAc. We hypothesize that the chronic reduction of extracellular dopamine levels facilitates QNP-induced locomotor sensitization. To induce a chronic decrease of NAc dopamine levels independently of D2R activation, we expressed the inhibitory DREADD, hM4Di, selectively in the mesolimbic dopamine pathway in mice and administered intraperitoneally its agonist C-21 for nine days. After this period, we administered QNP for another nine days and evaluated locomotor activity after each injection. We found that mice repeatedly treated with QNP did not develop locomotor sensitization. However, QNP administration in CP-21 pre-treated mice induced sustained and enhanced locomotion, suggesting that chronic reduction of tonic dopamine in the NAc facilitates QNP-induced locomotor sensitization. Currently, we are setting fast-scan cyclic voltammetry (FSCV) and western-blot analysis to assess whether the repeated inhibition of mesolimbic dopamine neuron activity impacts D2R presynaptic function and protein levels, respectively. We intend to contribute to understanding the mechanisms that underlie OCD by studying the involvement of D2R in dopamine neurons.

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9.

RESVERATROL Y SU IMPACTO EN LA FUNCION DEL RECEPTOR DE GLICINA. Resveratrol and its Impact on Glycine Receptor Function.

Glycine receptors (GlyRs) are anion-permeable pentameric ion channels (pLGICs). The GlyR activation is critical for the control of key neurophysiological functions, such as motor coordination, respiratory control, muscle tone and pain processing. The relevance of the GlyRs function is further highlighted by the presence of abnormal glycinergic inhibition in many pathophysiological states, such as hyperekplexia, epilepsy, autism and chronic pain. In this context, searching for molecules capable to modulate the GlyRs function have gain an important attention last years. Several molecules with different chemical features, such as alcohols, general anesthetics and cannabinoids have been reported as a positive allosteric modulation. In this line, recently, compounds of natural origin such as Gelsemin and Resveratrol have shown modulatory effects on the GlyRs function. In the case of resveratrol, a phytopolyphenol extract from grape seeds have shown that inhibited the glycinergic transmition in the inferior colliculus and auditory cortex. Unfortunately, the molecular determinants and specificity of this modulation is still unknown. In the present work, we investigated, by using in silico molecular docking assays combined with electrophysiological recordings, which are the putative binding site in the alpha1β and alpha2β GlyR subunits. Our results obtained by in silico studies shows that resveratrol is able to bind discrete pocket in all subunits tested. Remarkably, alpha1β and alpha2β subunits shared homologous putative pockets in the close conformation. Interestingly, functional studies show that the function of the alpha1β and alpha2β GlyR subunits was inhibited in a concentration dependent manner by Resveratrol. Thus, our in silico and functional combined data, shows that Resveratrol is capable to bind to putative binding site, different to the orthosteric site and inhibited the glycine activated currents in two subunits heteromeric in a allosteric fashion.

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10.

CARACTERIZACIÓN DE LA CAPACIDAD DE UNIÓN DE UN ANTICUERPO MONOCLONAL ANTI-MICA TOTALMENTE HUMANO PARA LA INMUNOTERAPIA DEL CÁNCER. Characterizing the binding capacity of a fully human anti-mica monoclonal antibody for cancer immunotherapy.

MICA is a protein expressed on the membrane of cancer cells that interacts through its α1 and α2 domains with the NKG2D receptor, present on natural killer (NK) cells. This interaction triggers the cytotoxic anti-tumor response of NK cells, constituting a defense mechanism against cancer. However, when MICA is released from cancer cells, it acts as an inhibitor of NK cell cytotoxic activity, thereby promoting the proliferation and survival of cancer cells, thus contributing to tumor progression. This study focused on the development of a human antibody targeted against the α1 domain of MICA to enhance the immunological response against Gastric Cancer (CG). Employing phage display technology, we selected a single-chain variable fragment (scFv) to generate the antibody AcHuαMICA. The antibody was synthesized in CHO-S cells and subsequently purified via high-resolution chromatography. AcHuαMICA affinity for MICA and its capacity to block MICA-NKG2D interaction were assessed by Enzyme-Linked Immunosorbent Assay (ELISA). Additionally, the antibody binding to various gastric cell lines (GES-1, MKN-45, AGS), which carry different MICA variants (MICA*008, *009, *010) was evaluated by flow cytometry. The results illustrate that AcHuαMICA effectively neutralizes the soluble form of MICA, inhibits its interaction with NKG2D, and binds to various MICA variants in cell lines. This broad binding capacity can be attributed to AcHuαMICA targeting a non-polymorphic site on the α1 domain of MICA.

These findings provide support for the therapeutic potential of AcHuαMICA in cancer immunotherapy and in other tumors expressing MICA. In conclusion, our monoclonal antibody holds promise as a valuable tool in the treatment of MICA-expressing cancers, particularly in the context of immunotherapy.

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11.
DIETA ALTA EN GRASA Y MEMORIA ESPACIAL EN RATAS MACHO SPRAGUE DAWLEY. HIGH FAT DIET AND SPATIAL MEMORY ON MALE SPRAGUE DAWLEY RATS.

Modern western society has embraced the use of highly processed, and hypercaloric diets as a main source of nutrition. This has led to the occurrence of an ever-increasing number of associated pathologies, including mental health. For example, it has been shown that high fat diet (HFD) on pregnant rats impairs hippocampal functions in the offspring. However, little is known on the effects of juvenile exposure to HFD on memory tasks. Aim: To evaluate the impact of HFD on juvenile rats on a spatial memory task Methods: To test this HFD (5.13 kcal/g, 60% from fat) was given to a group male Sprague-Dawley rats from post-natal day (PND) 21 to PND 68, while a control group were given regular rat chow (3.0 kcal/g, 14.3% from fat). At PND 60 Olton radial arm maze win-shift procedure was carried out, as an outcome of memory learning Results: A two-way ANOVA analysis shows that there is a statistical difference ($p < 0.05$) in the learning through time between HFD fed rats and control animals. Moreover, HFD fed rats do not achieve 100% rate of success throughout the period of testing, while control animals do. Conclusion: Feeding rats with HFD from weaning onwards impairs spatial memory, in comparison to control fed male rats. These results highlight the impact of nutrition on a hippocampal related task, further suggesting the importance of diet on mental health.

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12.
DESARROLLO DE UNA CADENA SIMPLE DE REGION VARIABLE (SCFV) DIRIGIDA CONTRA LA PROTEÍNA MICA. Development of single-chain fragment variable (scFv) targeting the MICA protein.

The soluble MICA protein is recognized as an oncotarget because of its potential to promote immune evasion by internalizing the NKG2D receptor on Natural Killer (NK) cells, a key component of antitumor cytotoxicity. In this context, the neutralization of soluble MICA, using antibodies, represents an interesting strategy to counteract this mechanism, which has been shown to favor tumor progression. The aim of this study was to generate a single chain fragment variable (scFv) antibody capable of binding to the α2 domain of MICA. Phage display technology was used to generate an anti-MICA scFv antibody. The transfected E. coli DH5α clones were evaluated by PCR and phage ELISA. Sequence homology comparisons between the selected scFv antibodies and germline antibody genes were performed using IgBlast. The scFvs were expressed in the pET_52b vector using E. coli BL21 for production, followed by refolding and size exclusion column analysis. Two scFvs targeting the α2 domain of MICA were successfully generated. These scFvs shared 98.5% of sequence identity with the IGKV2-30*01 and IGHV1-46*01 antibody families. As expected, the complementarity determining regions (CDRs) of the scFvs showed significant differences. The recombinant scFvs achieved a yield of 1 mg/mL with a purity of 90%, and their folding was optimized in a buffer containing L-arginine. Furthermore, the scFvs demonstrated high affinity, as confirmed by ELISA analysis. In conclusion, phage display has proven to be a successful approach for generating scFv antibodies against MICA. This innovative technology holds great promise for the development of high-affinity antibodies that can be directed against multiple molecular targets, thereby advancing the field of therapeutic antibody research.

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13.
LA MICROBIOTA INTESTINAL COMO MARCADOR TEMPRANO DE CAMBIO METABÓLICO INDUCIDO POR LA DIETA: UN ESTUDIO EN RATAS PRE-OBESAS JÓVENES. The gut microbiota as an early marker of diet-induced metabolic shift: a study in pre-obese young rats.

The occurrence of overweight and obesity in youth has increased globally, with growing evidence supporting the relevance of the gut microenvironment as pathophysiological factor. The time preceding the establishment of obesity is particularly relevant to therapeutic management, however most studies linking diet-induced dysmetabolism and gut microbiota investigate animal models that are already obese. We have fed young rats with high fat diet (HFD, 62% calories from fat) to model pre-obesity status, showing that within 15-30 days these animals -not yet overweight- have higher adiposity and liver lipid content than rats fed regular chow (14% calories from fat). Caecal microbiota transplantation from pre-obese rats induced higher glycaemia levels in healthy receptors, in comparison to those recipients treated with heat-inactivated caecal material. To shed some light on the nature of early microbiome changes induced by HFD, we aimed to broadly characterize caecal bacterial families in young rats exposed to HFD. Rats received HFD from postnatal day (p) 30 onwards. Caecal content of HFD and control rats was obtained at p45, p60 and p90. Bacterial DNA was isolated and the V3-V4 region of 16S rRNA gene was sequenced using an Illumina MiSeq platform. The abundance of Tanerellaceae and Bacteroidaceae was increased early (p45) in HFD rats. On the other hand, Muribaculaceae and Lactobacillaceae abundances were significantly lower in HFD rats, but only at p60 and p90, respectively. Gut microbiota

composition can not only contribute to health and/or pathology, but may also be used as marker for early disease stages involving metabolic shifts, improving the chances for successful interventions. A detailed description on Tanerellaceae and Bacteroidaceae at the genus level may potentially provide useful markers for a pre-obese status.

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14.
DIMORFISMOS SEXUALES SOBRE EL COMPORTAMIENTO SOCIAL DE RATAS EXPUESTAS DE MANERA TEMPRANA A ANTIBIÓTICOS. Sexual dimorphisms on social behaviour of juvenile rats exposed early in life to antibiotics.

Early life exposure to antibiotics (ELEA) has been shown to affect the mesocorticolimbic circuit in a sex-dependent manner. This dopaminergic circuit is also affected in individuals with autism spectrum disorder (ASD). Additionally, ASD traits manifest differently between males and females. Aim: To evaluate whether ELEA impoverishes social behaviour of juvenile rats, in a sex dependent manner, associated with the reward circuit. Methods: ELEA was achieved, through oral administration of a mixture of bacitracin, neomycin, vancomycin (100 mg/kg each) and pimaricin (5µg/kg) to pregnant Sprague-Dawley dams, from embryonic day 18 to post-natal day (PND) 7. Behavioural analysis was carried out on male and female offspring on PND 35 to 37, where half of the subjects underwent gonadectomy (GDX). Results: GDX in ELEA rats increases immobility regardless of sex. On open field test, time spent on the periphery is lower in control females than control males, contrary to what happens in GDX rats. Frequency of rearing is higher in control females than control males, but the opposite happens in GDX rats. Frequency of interaction with a novel object is decreased by GDX in both sexes, yet has no effect when an unknown rat is present. Time spent interacting with a strange rat differs between males and females, regardless of GDX. No differences were observed on rough-and-tumbling and elevated plus maze tests. Conclusions: GDX in ELEA rats did not alter social behavior. However, it seemed to evoke anxious-type behaviours and hypervigilance in a sex dependent manner and a reduction in search for novelty and mobility regardless of sex.

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15.
TRIAZOLOPIRIDINAS: COMPLEJOS DE INCLUSIÓN CON CICLODEXTRINAS Y SU EVALUACIÓN BIOLÓGICA COMO POTENCIALES AGENTES TRIPANOCIDAS. Triazolopyridines: inclusion complexes with cyclodextrins and their biological evaluation as potential trypanocidal agents.

The synthesis of bioactive compounds emerges as a pharmacological alternative for the treatment of neglected diseases such as Chagas disease. Triazolopyridine derivatives have shown great potential in medicinal chemistry and recently our group has described the development of a new base structure [1,2,3] Triazolol [1,5-a]pyridine, whose biological activity is related to the Inhibition of the TcCYP51 enzyme in Trypanosoma cruzi. In this work, physicochemical properties of triazolopyridine derivatives included in three models of cyclodextrins that would improve trypanocidal activity were studied. The results obtained through the use of spectroscopic techniques (fluorescence and molecular absorption) indicated that inclusion constants of all the complexes studied have a 1:1 stoichiometry, with inclusion constant values for all the complexes above 200 M-1. These thermodynamic parameters were studied using the Benesi-Hildebrand methodology and the van't Hoff thermodynamic equation at different temperatures (20, 28 and 37 ° C). The values obtained indicated that the complexation is mainly controlled by Van der Waals type interactions and complemented by hydrophobic type interactions. Using the Autodock program, molecular modeling studies were carried out using docking methodologies with the purpose of optimizing the geometry of inclusion complex between cyclodrextrins and polynitrogenated heterocycles, and thus, estimating the contributions of most important interactions for the formation of the association complex between host molecule and guest molecule. In addition, cell viability studies were carried out for [TP2–M-β-CD] complex that presented trypanocidal activity against Trypomastigote form. In conclusion, by improving the potency of this family we will be able to generate new pharmacological tools to fight Chagas disease, a forgotten disease that silently attacks our population.

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16.
RESOLVINA D1 ATENÚA LA SENESCENCIA DE FIBROBLASTOS CARDIACOS DE RATA ADULTA INDUCIDA POR ANGIOTENSINA II. Resolvin D1 Attenuates Angiotensin II-Induced Senescence in Adult Rat Cardiac Fibroblasts.

The aberrant accumulation of senescent cardiac fibroblasts (CF) is associated with a poor prognosis in various cardiac diseases. Angiotensin II (AngII) accelerates the accumulation of senescent CF. However, the mechanisms by which AngII induces senescence in CF have not been fully described. Resolvin D1 (RvD1) has a significant anti-inflammatory effect on CF. Nevertheless, it is unknown whether this compound can attenuate AngII-induced CF senescence. Methodology. Adult rat CF primary cultures maintained in 1% FBS medium were stimulated with AngII for 24 and 72 hours. Subsequently, the following cellular senescence markers were evaluated: Histone γH2AX, p-p38, p53, p21, pRb, and senescence-associated β-galactosidase activity (SA-β-Gal). Senescent CF were then treated with RvD1 for 48 hours. After this time, pRb and SA-β-Gal were reevaluated. Results. AngII increased the levels of p-p38 at short times, with a peak at 30 minutes, which is lost at 72 hours. Additionally, γH2AX, p53, and p21 increased at 24 hours, with γH2AX and p21 remaining elevated at 72 hours. No increase in p16 and p53 was observed at 72 hours. However, pRb decreased and SA-β-Gal increased after 72 hours of AngII stimulation. On the other hand, RvD1 increased pRb and decreased SA-β-Gal in senescent CF stimulated for 72 hours with AngII. Conclusion. AngII induces senescence in CF, evidenced by cell cycle arrest and increased SA-β-Gal. Meanwhile, RvD1 reduces the number of senescent CF.

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17.

MADURACIÓN DE AFINIDAD DE UN ANTICUERPO ANTI-MICA HUMANO: DE LA PREDICCIÓN AL EXPERIMENTO. Affinity Maturation of a Human Anti-MICA Antibody: From Prediction to Experiment.

Affinity maturation is an essential step in antibody development which aims to enhance antibodies binding affinity to their targets. Traditionally, it has been carried out in vitro, requiring time-consuming and expensive experimental techniques. To overcome these challenges, new computational methods have emerged to speed up and to improve the antibody development process. In this study, we performed an affinity maturation process using both molecular modeling techniques and subsequent experimental validation to improve the interactions between a fully human anti-MICA antibody and its target protein MICA. Molecular dynamics simulations allowed us to identify 30 favourable sites within the CDRs for possible mutations; these sites were exhaustively mutated by 18 amino acids. The resulting Fv mutants were further evaluated by free energy binding calculations. From those Fv mutants, ones that showed enhanced in silico binding affinities were selected for subsequent expression as full antibodies in CHO-S cells. Using MICA-coated plates in an indirect ELISA assay, we compared the binding affinity of the Ile29Asp mutant to that of the wild-type anti-MICA antibody. Our results indicate that this mutant has at least a 50% increase in binding affinity compared to the wild-type antibody. This increase in affinity can be explained by molecular dynamics results, which showed that this mutant can form an ionic bridge with Arg38 of MICA that is not present in the wild-type antibody. In conclusion, we have characterized the molecular interactions between MICA and the anti-MICA antibody developed in our laboratory. We have also demonstrated the utility of computational affinity maturation for enhancing antibody binding affinity, offering the potential for more efficient antibody development.

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18.

EVALUACIÓN DE LA ACTIVIDAD TRIPANOCIDA Y MECANISMO DE ACCIÓN DE COMPLEJOS METÁLICOS DE LA PRIMERA SERIE DE TRANSICIÓN CON LIGANTES HÍBRIDOS CUMARINA TIOSEMICARBAZONA. Evaluation of trypanocidal activity and mechanism of action of metal complexes of the first transition series with coumarin-thiosemicarbazone hybrid ligands.

Chagas disease, caused by the protozoan Trypanosoma cruzi (T. cruzi), affects approximately 6 to 7 million people worldwide. Outside Latin America, where it is endemic, transmission is increasing due to migration to the European Union, the United Kingdom and the United States, among other countries. Currently, nifurtimox and benznidazole are clinically effective drugs in the acute phase, but not in the chronic phase, where they have serious side effects. In the design of more effective drugs, the coordination of transition metals with trypanocidal ligands is a promising and widely studied strategy for the second transition series. However, little information is available on the trypanocidal activity of metal complexes of the first series. Therefore, in this work we evaluated the trypanocidal activity of Zn(II) or Cu(II) complexes with coumarin-thiosemicarbazone hybrid ligands and phenanthroline as co-ligand. The trypanocidal activity was correlated with physicochemical parameters such as reduction potential and lipophilicity. A possible mechanism of action related to mitochondrial function was also determined. It was observed that the formation of complexes increased the trypanocidal activity compared to free binders. Thus, copper complexes showed higher trypanocidal activity (IC_{50} < 1 µM), which correlated with lipophilicity. The results also suggest that the complexes have a possible mechanism of action mediated by intraparasitic oxidative stress.

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19.

PREVALENCIA DE REACCIONES ADVERSAS A OPIOIDES Y SU RELACIÓN CON POLIMORFISMOS GENÉTICOS EN CYP2D6 Y CYP3A4, EN PACIENTES CON CÁNCER COLORRECTAL ETAPA AVANZADA. Prevalence of adverse reactions to opioids and their relationship with genetic polymorphisms in CYP2D6 and CYP3A4, in patients with advanced colorectal cancer.

Colorectal cancer (CRC) is one of the leading causes of death in Chile. Opioid drugs are used as treatment to provide analgesic action. However, they are not exempt from the occurrence of adverse reactions. Pharmacogenetics plays an important role in gene variation that can affect the pharmacokinetics of drugs, including toxicity. The main enzyme involved in the metabolism of opioid drugs is cytochrome P450 system. Two polymorphisms, namely rs1065852 and rs2740574, are associated with CYP2D6 and CYP3A5, respectively. These polymorphisms have been linked to opioid drug dosing. General Objective. To stablish an association between the genetic variations of the CYP2D6 (rs1065852) and CYP3A4 (rs2740574) genes, involved in the pharmacokinetics of opioid drugs, in relation to the response, in terms of toxicity, in patients with advanced colon cancer. Methodology. An ethics-authorized observational cross-sectional study was conducted on patients with advanced CRC who have been treated with opioid drugs at the National Cancer Institute of Chile and the Clinical Hospital of the University of Chile. Real-time PCR was used to determine the genotypes of CYP2D6 and CYP3A4. Clinical records were analyzed to assess the prevalence of adverse reactions, using descriptive statistical analysis. Results. Genetic variants in CYP2D6 and CYP3A4 were associated with an increase in toxicity in patients with CRC, with 5% significance. Higher frequencies of adverse reactions such as nausea and vomiting were observed. Both polymorphisms are in Hardy-Weinberg equilibrium, with a p-value of 1 and 0,0716, respectively. Conclusion. Genetic variants studied were significantly associated with an increase in

the frequency of adverse reactions to opioid drugs in patients with advanced CRC. Key words: opioids; pharmacokinetics; adverse reactions; CYP2D6 polymorphism; CYP3A4 polymorphism; cancer pain.

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20.

LA ACTIVACIÓN DE PPAR-GAMMA AUMENTA LA MIGRACIÓN DE QUERATINOCITOS IRRADIADOS CON UV-B, AUMENTANDO LA RESPUESTA ANTIOXIDANTE Y DISMINUYENDO LA RESPUESTA INFLAMATORIA. Activation of PPAR-gamma increases the migration of keratinocytes irradiated with UV-B, increasing the antioxidant response and decreasing the inflammatory response.

Keratinocytes are the most abundant cells in the epidermis and are responsible for skin regeneration and healing. UV-B rays induce adverse biological effects in keratinocytes, such as oxidative stress and inflammation. Furthermore, oxidative stress and inflammation are the critical factors attributed to the delay in the wound repair process. The PPAR-gamma receptor controls epidermal lipid synthesis, permeability barrier homeostasis, and inflammation and oxidative stress damage containment. However, the role of PPAR-gamma on oxidative stress and inflammation induced by UV-B radiation and its impact on wound healing is still unknown. This study aimed to evaluate the role of PPAR-gamma in keratinocyte migration and the effect on the oxidative and inflammatory response induced by UV-B radiation. The cells were irradiated with UV-B 45mJ/cm2 and treated with pioglitazone, a PPAR-gamma agonist, for 24 h. To antagonize pioglitazone-induced responses, keratinocytes were treated with GW9662. Cell migration was determined by in vitro scratch-wound assays and using transwell. The GCLC (Glutamate-Cysteine Ligase Catalytic Subunit), GSS (Glutathione Synthetase), and COX-2 levels were measured using RT-qPCR and western blot. Glutathione and PGE2 levels were quantified by commercial kit and ELISA, respectively. The results show that pioglitazone increases the migration of UV-B-irradiated keratinocytes and increases the content of antioxidant factors, such as glutathione, GCLC, and GSS. Furthermore, pioglitazone decreased the expression of COX-2 and the synthesis of PGE2. Pretreatment with GW9662 abolished the effects produced by pioglitazone. In conclusion, our findings present evidence of the beneficial role of PPAR-gamma on the migration of keratinocytes exposed to UV-B radiation due to an increase in the antioxidant response and a decrease in the inflammatory response.

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21.

EL AGONISMO DEL RECEPTOR GPR39 MODULA LA ACTIVIDAD DEL TRANSPORTADOR DE SEROTONINA. GPR39 receptor agonism modulates serotonin transporter activity.

GPR39 is a member of a large family A of 7-transmembrane segment G protein-coupled receptors activated by physiological concentrations of Zn+2. This receptor is expressed in various endocrine and metabolic tissues, as well as in the brain. In CNS, it has been demonstrated that a Zn+2-deficient diet, which leads to a depressive phenotype, is associated with decreased GPR39 in the hippocampus and prefrontal cortex of rodents. Additionally, an increase in GPR39 has been observed in the prefrontal cortex of mice treated with selective serotonin reuptake inhibitors. This evidence suggests that GPR39 may play a role in regulating serotonergic transmission through the modulation of the serotonin transporter (SERT). Thus, the present study aims to evaluate the effect of GPR39 receptor agonism on SERT activity in HEK cells that endogenously express the receptor. SERT activity was assessed through the reuptake of a fluorescent false neurotransmitter of serotonin (FFN246) in HEK cells overexpressing SERT-YFP (HEK SERT-YFP cells), using epifluorescence microscopy (EVOS FLoid; FFN246 excitation 390/emission 446; YFP: excitation 482/emission 532) and a microplate reader (TECAN Infinite M Plex plate reader with excitation and emission wavelengths set at 388 and 434 nm). FFN246 reuptake into HEK SERT-YFP cells was determined using the FFN246 signal to YFP-SERT ratio (YFP: excitation 490nm/emission 530nm). Our results show that GPR39 agonism (TC-G 1008: 100uM) decreases SERT activity by 53% ($p<0.05$) compared to the control, a result that aligns with what was observed with the antidepressant fluoxetine (0,5uM; 88% inhibition; $p<0.05$). Consequently, our findings suggest that GPR39 may have a physiological role in modulating serotonin homeostasis through the regulation of SERT, and its agonism could be a therapeutic target in the treatment of depression.

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22.

TRIÓXIDO DE ARSENIC (ATO) AUMENTA LA PROLIFERACIÓN CELLULAR MEDIANTE LA ACTIVACIÓN DE NHE1: EFECTOS DEPENDIENTES DE MTOR SOBRE LAS CÉLULAS HT-29 DERIVADAS DESDE CARCINOMA DE COLON. Arsenic trioxide (ATO) increases cell proliferation through NHE1 activation: mTOR-dependent effects on HT-29 cells derived from colon carcinoma.

It has been showed that arsenic trioxide (ATO) can regulate cell proliferation through mTOR. Also, ATO is known to participate in cell proliferation by increasing NHE1 activity. However, whether ATO-dependent cell proliferation is NHE1-mediated activity-dependent through mTOR in HT29 cells has not been described. Objective: to determine whether ATO increases cell proliferation by increasing NHE1 activity through mTOR. Methods: intracellular pH (pHi) and NHE1 activity (dpH/dt) were determined by radiometric fluorescence microscopy (fluorescent probe 2,7-bicarboxyethyl-5,6-carboxyfluorescein acetoxymethyl ester (BCECF-AM/12μM)), in the absence of NaHCO3. The BrdU Cell Proliferation ELISA kit determined cell proliferation. Values are mean ± S.E.M, and "n" indicates the number of experiments. ANOVA followed post hoc Tukey analyses by the multiple-comparison was used, and $p<0.05$ was considered significant. Results: ATO (0.05μM/48h) was able to increase basal pHi compared to the control (7.51±0.08, n=4 vs. 7.16±0.021, n=16), which was rescued by rapamycin (mTOR inhibitor). Besides, after acidification by an ammonium

pulse (20mM, pH 7.4), ATO markedly increased the velocity of cell recovery pH (0.41±0.015 vs. 0.14±0.014 pH units/min, respectively). Notably, in cells treated with rapamycin (with and without ATO), the dpH/dt were similar to the control condition (0.11±0.018, n=8 and 0.04±0.01, n=5 vs. 0.14±0.014 (n=16) pH units/min, respectively). Importantly, ATO was able to increase cell proliferation (D24.2±3.5%, n=6), which was abolished by zoniporide (100nM) (NHE1 inhibitor) and rapamycin (20nM). Conclusion: These results strongly suggest that ATO-dependent cell proliferation was associated with increased NHE1 activity and alkalinization mediated by mTOR. Thus, our results open new avenues for treating cell proliferative diseases with possible new therapeutic targets.

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23.

EFECTOS CITOTÓXICOS EN LÍNEAS CELULARES DE CÁNCER DE MAMA DE CHALCONAS DERIVADAS DE UN PRECURSOR NATURAL Y SU ANÁLISIS DE ACOPLAMIENTO MOLECULAR. Cytotoxic Effects on Breast Cancer Cell Lines of Chalcones Derived from a Natural Precursor and Their Molecular Docking Analysis.

This study aimed to determine the in vitro cytotoxicity and understand possible cytotoxic mechanisms via an in-silico study of eleven chalcones synthesized from two acetophenones. Five were synthesized from a prenylacetophenone isolated from a plant that grows in the Andean region of the Atacama Desert. The cytotoxic activity of all the synthesized chalcones was tested against breast cancer cell lines using an MTT cell proliferation assay. The results suggest that the prenyl group in the A-ring of the methoxy and hydroxyl substituents of the B-ring appear to be crucial for the cytotoxicity of these compounds. The chalcones 12 and 13 showed significant inhibitory effects against growth in MCF-7 cells (IC_{50} 4.19 ± 1.04 µM and IC_{50} 3.30 ± 0.92 µM), ZR-75-1 cells (IC_{50} 9.40 ± 1.74 µM and IC_{50} 8.75 ± 2.01µM), and MDA-MB-231 cells (IC_{50} 6.12 ± 0.84 µM and IC50 18.10 ± 1.65 µM). Moreover, these chalcones showed differential activity between MCF-10F (IC_{50} 95.76 ± 1.52 µM and IC_{50} 95.11 ± 1.97 µM, respectively) and the tumor lines. The in vitro results agree with molecular coupling results, whose affinity energies and binding mode agree with the most active compounds. Thus, compounds 12 and 13 can be considered for further studies and are candidates for developing new antitumor agents. In conclusion, these observations give rise to a new hypothesis for designing chalcones with potential cytotoxicity with high potential for the pharmaceutical industry.

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24.

OBTENCIÓN POR HEMISÍNTESIS DE DERIVADOS DEL ÁCIDO CEANÓTICO CON ACTIVIDAD INHIBITORIA DE ACETILCOLINESTERASA MEDIANTE INTERACCIÓN CON SU SITIO ANIÓNICO PERIFÉRICO. Semisynthetic derivates of ceanothic acid as acetylcholinesterase inhibitors via peripheral anionic site interaction.

Ceanothic acid (CA), a pentacyclic triterpene derived from Talguenea quinquenervia, is known for its significant competitive and reversible acetylcholinesterase (AChE) inhibitory activity through its peripheral anionic site (PAS). However, CA has not been able to outperform traditional AChE inhibitors such as galantamine and donepezil. The aim of this study was the structural modification of CA to refine its AChE inhibitory activity and selectivity for PAS. Methodology: Aerial parts and roots of T. quinquenervia were subjected to conventional extraction methods to obtain CA with high purity. From CA, six derivatives were obtained by semi-synthesis, purified by chromatographic methods and their structures were confirmed by spectroscopic methods (NMR, MS/MS). Their pharmacological activity was evaluated in vitro by Ellman's assay, enzymatic kinetics, propidium iodide displacement and molecular docking. Results: All derivatives increased their inhibitory activity on AChE compared to CA. Such inhibition was predominantly mixed, preferring to bind to the free enzyme interacting with PAS. Such inhibition could prevent the formation of the AChE-beta-amyloid peptide complex, an event that promotes senile plaque formation. This suggests that these molecules are novel for the development of targeted alternatives for the treatment of Alzheimer's disease. Further studies are required to better understand which modifications are more favorable for the purpose of enhancing the inhibitory activity of AChE and its derivatives, which could be the first step towards the development of new therapeutic strategies based on multi-target compounds to address several factors involved in AD.

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25.

DISEÑO, PRODUCCIÓN RECOMBINANTE Y OPTIMIZACIÓN DE LA EXPRESIÓN DE ASNASE_H_Q, UNA ASPARAGINASA QUIMÉRICA HUMANIZADA CON POTENCIAL BIOFARMACÉUTICO. Design, Recombinant Production and Optimization Expression of ASNASE_H_Q, a Humanized Chimeric Asparaginase with Biopharmaceutical Potential.

This study addresses the use of L-asparaginase (ASNase) in the chemotherapy of acute lymphoblastic leukemia (ALL). ASNase, with its antitumor properties due to its hydrolytic capacity, has played a significant role in ALL treatment. However, its bacterial origin makes it highly immunogenic, leading to various adverse reactions. To mitigate this issue, the study focuses on creating a recombinant humanized variant of ASNase and optimizing its expression in Escherichia coli's periplasm.The chimeric enzyme was designed by combining human and bacterial sequences, with its stability and activity evaluated using AlphaFold, docking, and molecular dynamics. Amplifying the chimeric ASNase gene was achieved through PCR, with specific primers adding a histidine sequence for purification and a signal sequence for periplasmic expression. Recombinant plasmids were introduced into E. coli cells, and the Rosetta strain was found to be more favorable for humanized protein expression. A response surface plot was used to optimize chimeric protein expression with minimal IPTG concentration (0.01 mM), minimal time (2 hours), and a maximum temperature (37°C), which led to maximum activity. The study demonstrated that the designed humanized chimeric enzyme maintains asparaginase activity (2.4 U/ml in the crude extract) and aligns with the response surface design results. This represents a significant advancement in ALL treatment by reducing immunogenicity and enhancing the potential for this vital enzyme in chemotherapy.

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26.
IDENTIFICANDO BLANCOS ASOCIADOS AL ALZHEIMER USANDO FARMACOLOGÍA DE REDES. Finding novel Alzheimer-disease targets using network pharmacology.

Annually, there are 10 million new Alzheimer's disease (AD) cases, a complex neurodegenerative illness. Despite available medications for AD treatment, their ineffectiveness against this multifactorial condition is apparent. In the information age, the overwhelming volume of publications related to AD and other illnesses exceeds our capacity for comprehensive assimilation, leading to the risk of missing important insights. Biological databases, housing data on proteins, compounds, and diseases, represent a vast information source to aid in the selection of protein targets for new compound development or the repurposing of approved drugs. Nevertheless, managing these databases becomes challenging, especially when handling extensive data and the need to cross-reference information across various sources. We have created a workflow within the data science platform Knime. This workflow simplifies the process of identifying fresh targets by harnessing databases like ChEMBL, the Therapeutic Target Database (TTD), the Open Targets Platform, String, Complex Portal and Uniprot. It takes into account approved AD compounds, compounds in clinical and preclinical stages, and proteins interacting with those already associated with AD. To prioritize potential targets, we employ a scoring system that places more importance on proteins already connected to approved AD compounds. Furthermore, we visualize protein-protein interactions as a network, providing a more comprehensive outlook on well-known and potential targets.

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27.
IDENTIFICACIÓN DE COMPUESTOS CON LA CAPACIDAD DE RESCATAR EL FENOTIPO PRIMARIO DE LA ENFERMEDAD NIEMANN- PICK TIPO C. Identification of compounds with the ability to rescue the primary phenotype of Niemann-Pick type C disease.

Niemann-Pick disease type C (NPC) has an Autosomal Recessive inheritance and an incidence of 1:120,000 live births. This pathology is mainly caused by mutations in the NPC1 or NPC2 gene, causing an alteration in lysosomal storage. 95% of cases are associated with mutations in the NPC1 protein, which is encoded by the NPC1 gene. NPC1 is responsible for transporting different types of lipids, essentially cholesterol, from late endosomes and lysosomes to the extracellular medium. There are more than 400 clinical mutations for the NPC1 protein, but the most common is NPC1|I1061T, which presents misfolding and therefore incomplete trafficking from the endoplasmic reticulum to the lysosomes. The primary phenotype presented by the disease is due to an abnormal accumulation of cholesterol in the lysosome, which causes deterioration of this organelle. Our analyzes were based on a repositioning of FDA-approved drugs consisting of 1582 molecules. A virtual screening was carried out with the wild-type NPC1 protein and with the I1061T mutation, which allowed us to obtain 8 candidate drugs that complied with the 5 Lipinski rules, a binding affinity to the mutated protein greater than -8.0 (kcal/ mol), also presented hydrogen bond type interactions with the amino acid threonine that is exchanged at position 1061 for isoleucine in the mutated protein. The molecules were tested on the human fibroblast cell line that has the I1061 mutation. Our findings aim to find one or more candidates that act as pharmacological chaperones and can reverse the misfolding that causes inadequate transport from the endoplasmic reticulum to the lysosome, which is what ultimately generates the primary NPC phenotype.

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28.
EFECTOS DE AMILOIDES INTRACELULARES DE AB EN CULTIVOS DE NEURONAS ACUMBALES DE RATONES 2XTG. Effects ointraneuronal amiloid aβ on cultured accumbal neurons from the 2XTg mice.

The classical clinical symptoms of Alzheimer's disease (AD) are cognitive impairments associated with cortical and hippocampal deficits. On the other hand, non-cognitive features, such as mood and personality disturbances, are described early in the disease progress, and they may be associated with changes in the mesolimbic reward system. A key region of this neural circuit is the nucleus accumbens (nAc). In addition, few reports have focused on the toxic effects of intracellular oligomers, that we postulate to serve as an early neurotoxic event in AD. In a previous study, we described the accumulation of intraneuronal Aβ oligomers and its synaptic toxicity in accumbal neurons in 6-month-old APP/PS1 mice. Material and methods: Culture neurons were prepared from embryos of 18.5 days of APP/PS1 and C57BL6 (WT) mice. After 10 days in vitro they were treated with oligomers of Aβ1-42 for 24 hours, then experiments using calcium imaging, electrophysiology, and immunocytochemistry were performed. Results: First, a methodology was developed to generate neuronal cultures derived from APP/PS1 mice accumulating intracellular Ab. Using an antibody that recognizes several conformations of Aβ, we found a significant level of these toxic components associated with intraneuronal deposition. Second, calcium imaging experiments showed increased calcium transients in APP/PS1 and WT for all the treatments with Aβ1-42. Third, the amplitude of AMPAergic miniature currents in APP/PS1 neurons treated with Aβ1-42 oligomers was increased, together with a decrease in the decay constant of the current. Discussions: We developed a protocol for generating an in vitro model of accumbal neurons derived from Alzheimer's disease model that presents intracellular accumulation of Aβ. The neurons from APP/PS1 mice are more susceptible to extracellular Aβ than native ones likely because of the damage generated by intracellular oligomers of Aβ. Synaptically, the excitatory AMPA receptor showed altered electrophysiological properties, suggesting they are important Ab targets. These results appear relevant to understanding early alterations in the mesolimbic system before cognitive alterations associated to Alzheimer's Disease.

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29.

DOSIS SUBTÓXICAS DEL ENTACTÓGENO MDMA (3,4-METILENDIOXIMETANFETAMINA, "ÉXTASIS") PERTURBAN EL PARADIGMA DE LA CONDUCTA DE AYUDA EN RATAS SPRAGUE-DAWLEY. Subtoxic doses of the entactogen MDMA (3,4-methylenedioxymethamphetamine, "ecstasy") disrupt the helping behavior paradigm in sprague-dawley rats.

Emphathy is a human capability associated with prosocial behavior, which includes social interaction and helping behavior. In rodents, whereas social interaction covers a stereotyped behavioral pattern, helping behavior is suitable to evoke more sophisticated human-like behaviors. In rats, administration of subtoxic doses of MDMA (3,4-methylenedioxymethamphetamine, "Ecstasy") has been demonstrated to enhance empathy-like behaviors, such as the so-called "adyacent lying" in the social interaction paradigm. In contrast, a preliminary evaluation suggests that MDMA might not enhance helping behavior. In the present work, the effects of five subtoxic dose levels (0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, 5 mg/kg and 10 mg/kg i.p.) of MDMA on helping behavior has been evaluated using a modified water-trap model developed ad hoc. Male Sprague-Dawley rat pairs (randomly assigned as "helper" rat or "soaked" rat) were placed separately in a box divided in 2 compartments ("wet"/"dry") by a transparent, acrylic wall with a circular door to go across the wall. 12 days-administration/training cycles lasting 5 minutes each were carried out to evaluate each drug dose level. Similar cycles were also carried out after interchanging roles ("helper" versus "soaked"). Helping behavior was verified when the helper rat opened the door to rescue the soaked rat from the water-trap. The results obtained indicated that MDMA (5 mg/kg and 10 mg/kg) fully abolished helping behavior (even after interchanging roles), whereas doses ranging from 0.25 mg/kg up to 1 mg/kg only restore the occurrence of helping behavior in a non-strict dose-dependent manner. Taken together, these data not only confirm that MDMA does not enhance helping behavior, but also suggest that the effects elicited by the drug might reflect an inhibitory mechanism mediated by serotonin.

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30.

EL ENTACTÓGENO ÉXTASIS INCREMENTA LA DEPRESIÓN DE LARGO PLAZO EN LA VÍA CÓRTICO-ACCUMBENS: POSIBLE ROL EN LA PERTURBACIÓN DE LA CONDUCTA DE AYUDA EN RATAS. The entactogen ecstasy increases long-term synaptic depression of the prefrontal cortex-accumbens pathway: possible role in MDMA-mediated disruption of helping behavior in rats.

Subtoxic doses of the entactogen MDMA (3,4-methylenedioxymethamphetamine, "ecstasy") enhance prosocial behaviors in rodents, its effect on the serotonin transporter within the nucleus accumbens (NAc) being necessary and sufficient to induce these effects. Nevertheless, the contribution of serotonin released by MDMA in eliciting helping behavior remains unknown. Since development and persistence of prosocial behaviors require reinforcement through complex reward mechanisms, including long-term depression (LTD) of excitatory synaptic transmission in the NAc, in the present work we studied the acute effects of MDMA (10 mg/kg i.p.) on the in vivo induction of LTD elicited in the NAc core of adult Sprague-Dawley rats by electrical stimulation of the dorsal raphe nucleus (DRN). Animals were anesthetized with 1.5 g/kg i.p. urethane, tracheostomized and placed in a stereotaxic frame under artificial ventilation. Field potentials were evoked in the NAc core by electrical stimulation of the medial prefrontal cortex (mPFC), and LTD was elicited with a 900-pulse protocol delivered at 5-Hz applied to the DRN. The results obtained showed that the stimulation protocol applied to the DRN produced an early increase (10 min long) in peak-to-peak amplitude of mPFC-NAc responses (likely of glutamatergic origin), followed by a long-lasting decrease (LTD) of field responses evoked in the NAc (likely of serotonergic nature). Following i.p. MDMA administration, a significant LTD increase could be recorded from the NAc core. Taken together, these data suggest that a MDMA-dependent increase in serotonin released in the NAc results in an increased LTD neuroplasticity process in spiny neurons that might be responsible for the disruption of the helping behavior paradigm at doses up to 10 mg/kg.

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31.

LA INHIBICIÓN FARMACOLÓGICA DE METALOPROTEASAS INDUCE LA LIBERACIÓN DE LA PROTEÍNA MICA EN VESÍCULAS EXTRACELULARES: UN NUEVO MECANISMO DE EVASIÓN INMUNE EN CÁNCER. Pharmacological inhibition of metalloproteinases induces the release of the MICA protein in extracellular vesicles: a novel immune evasion mechanism in cancer.

MICA is a membrane protein with multiple variants that is overexpressed in cancer. Upon binding to the NKG2D receptor on natural killer (NK) cells, MICA triggers the cytotoxic response, resulting in tumor lysis. However, as an immune evasion mechanism, MICA can be released in a soluble form (cleaved) by metalloproteinases or, in the case of MICA*008, as part of extracellular vesicles (EVs). Soluble MICA has a down-modulating effect on NKG2D, altering NK cell cytotoxicity. Thus, inhibition of metalloproteinases could be proposed as a pharmacological approach to restore NK cell cytotoxicity in cancer. Our main objective was to study the effect of metalloproteinase inhibition on the release profile of MICA variants (cleaved and as part of EVs) and their impact on the NKG2D receptor on NK cells. Methodologically, we used transfectant systems with five of the most prevalent MICA variants in the world population. The results indicate that several variants of MICA, other than the previously described MICA*008, can be recruited to EVs. Interestingly, MICA variants showed different release profiles upon inhibition of metalloproteinases. While MICA*008 was not affected, other variants were mainly recruited to EVs with a reduced

release of the cleaved form. Both cleaved and EV MICA induced downregulation of the NKG2D receptor. However, MICA-EVs were more potent and the variants more abundant in EVs had a stronger effect on NK function. In conclusion, we demonstrated that metalloproteinase inhibition induces the release of MICA as part of EVs, which affects NK functionality. This is critical if the therapeutic approach is to use inhibitors or antibodies that prevent the release of MICA from the membrane, as this exacerbates immune evasion.

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32.
EFECTO DE NAVITOCLAX SOBRE MIOFIBROBLASTOS CARDIACOS SENESCENTES DE RATA. Effect of navitoclax on senescent rat cardiac myofibroblasts.

Los miofibroblastos cardiacos son células que derivan desde los fibroblastos cardiacos por efecto del TGF-B1. Se ha demostrado que los miofibroblastos senescentes abundan en las zonas fibróticas del corazón y han demostrado ser un tipo celular de mal pronóstico asociado a fibrosis cardiaca. Navitoclax, es un fármaco senolítico que induce la muerte por apoptosis de células senescentes que expresan elevados niveles de las proteínas de la familia Bcl-2. Hasta la fecha se desconoce si los MFC senescentes inducidos con TGF-B1 expresan altos niveles de las proteínas de la familia Bcl-2 y si son sensibles a los fármacos senolíticos. Objetivo: Demostrar que Navitoclax induce la muerte por apoptosis de MFC senescentes de ratas neonatas. Materiales y Métodos: FC de ratas neonatas fueron cultivados por 7 días en presencia de FBS 10%+ TGF-B1 10 ng/mL, después de este tiempo se determinaron parámetros de senescencia (pRb, p53, p16/15, p21 y B-galactosidasa (b-gal)). También se evaluaron los niveles de expresión de las proteínas Bcl-XL, Bax y Bcl-2. Después de los 7 días de tratamiento con TGF-B1, los MFC fueron tratados con Navitoclax 1 uM por 48 horas. Resultados: Los FC se diferenciaron a MFC en 7 días (evaluado por la expresión y ensamblaje de α-SMA), y se consiguió el perfil de MFC senescente con TGF-B1 caracterizado por disminución de pRb, aumento de p21 y aumento de la actividad B-gal; mientras que no hubo disminución de p16. Además, los MFC senescentes no presentaron cambios en los niveles de expresión en las proteínas Bax, Bcl-XL ni Bcl-2. Conclusión: Navitoclax no modifica la viabilidad de los MFC senescentes inducidos por TGF-B1, sugiriendo que los MFC senescentes no son sensibles a Navitoclax.

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33.
DETERMINACIÓN DE LA COMPOSICIÓN FITOQUÍMICA Y ACTIVIDAD ANTIOXIDANTE DE EXTRACTOS DE HOJAS DE LAMPAYO MEDICINALIS (VERBENACEAE). Determination of the phytochemical composition and antioxidant activity of extracts of lampayo medicinalis leaves (verbenaceae).

Traditional medicine and the use of medicinal plants are fundamental to the treatment, disease prevention and the development of pharmaceutical products, thanks to the therapeutic properties attributed to their secondary metabolites. Lampayo medicinalis (Verbenaceae) is a small bush that grows in the Andean Altiplano, which is traditionally used to treat different ailments and illnesses. In this investigation Lampayo medicinalis leaves were used, obtaining extracts by static maceration and successive extractions with crescent polarity solvents (hexane, dichloromethane, ethyl acetate and ethanol) with the purpose of evaluating the phytochemistry composition through phytochemical screening and thin layer chromatography, and the antioxidant activity through the DDPH test. The phytochemical analysis indicated that the ethanol extract has the highest number of secondary metabolites, containing phenols, flavonoids, glycosides, quinones, saponins, tannins and terpenoids; while the hexane, dichloromethane and ethyl acetate extracts primarily contain terpenes and sterols. In all extracts there is an absence of coumarins and alkaloids. The antioxidant activity determined that the ethanol extract has a significantly higher antioxidant effect with an inhibition percentage of DDPH of 22,93 ± 4,81%, due to a higher quantity of phenolic compounds, in opposition to the other extracts that have minimal inhibition without major differences among them. The identification of secondary metabolites existing in the extracts provides an orientation regarding the biological activity attributable to the plant and validates its use in traditional medicine due to its wide range of compounds with biological activity.

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34.
SGK1 ES NECESARIA PARA LA DIFERENCIACIÓN DE LOS FIBROBLASTOS CARDIACOS INDUCIDA POR TGF-BETA1. SGK1 is necessary to cardiac fibroblast differentiation induced by TGF-beta1.

Cardiac fibroblasts (CFs) activation is a common response to most pathological conditions affecting the heart, characterized by increased cellular secretory capacity. Fibrotic activation of CFs induces the increase in tissue protein content, with the consequent tissue stiffness, diastolic dysfunction, and heart failure. Therefore, the search for new mechanisms of CFs activation is important to find novel treatments for cardiac diseases. In this regard, TGF-beta1, a cytokine with fibrotic properties, is crucial in the CFs activation and fibrotic diseases, whereas its molecular targets are not completely known. Serum and glucocorticoid-regulated kinase (SGK1) is a protein involved in various pathophysiological phenomena, especially cardiac and renal. Additionally, SGK1 phosphorylates and regulates the activity and expression of several targets, highlighting FoxO3a for its role in the regulation of oxidative stress and CFs activation induced by TGF-beta1. However, the regulation of SGK1 by TGF-beta1 and its role in CFs activation have not been studied. In this work, we evaluate the role of SGK1 in CFs isolated from neonatal rats. SGK1 participation in CFs fibrotic activation induced by TGF-beta1 was analyzed, using an inhibitor or siRNA of SGK1. In addition, the role of SGK1 on the FoxO3a regulation and oxidative stress

induced by TGF-beta1 was analyzed. TGF-beta1 increased both the activity and expression of SGK1 in CFs, while inhibition and silencing of SGK1 prevented TGF-beta1-induced fibrotic activation of CFs. In addition, SGK1 inhibition prevented FoxO3a inactivation and expression reduction, catalase and SOD2 expression decrease, and the increase of oxidative stress induced by TGF-beta1. Taken together, our results position SGK1 as an important regulator of CFs activation driven by TGF-beta1, at least in part, through the regulation of FoxO3a and oxidative stress.

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35.
ESTUDIO DEL SITIO DE UNIÓN DEL ANTIARRÍTMICO SELECTIVO AURICULAR AVE0118 EN EL CANAL NAV1.5 Studying the binding site of the atrial-selective antiarrhythmic drug AVE0118 in Nav1.5 channel.

Promiscuous drugs have emerged as suitable alternatives for treating complex diseases when acting on the right targets. An illustrative case of promiscuity is the antiarrhythmics used to treat atrial fibrillation (AF), those with selectivity for atrial ion channels are promising candidates to improve therapy without the risk of ventricular proarrhythmia. A well-known atrial-selective blocker is AVE0118, targeting potassium channels TASK-1, Kv1.5, and the sodium channel Nav1.5. Experiments and mathematical models have shown that simultaneous blocking of TASK-1/Kv1.5 and Kv1.5/Nav1.5 could offer advantages for AF. Functional mutagenesis revealed the binding site (BS) for AVE0118 in TASK-1 and Kv1.5, but no information exists about its BS in Nav1.5 channel. We explored AVE0118's binding site in the Nav1.5 channel using bioinformatics and functional mutagenesis. First, a molecular docking was carried out to predict AVE0118's BS in Nav1.5 and to identify putative key residues for drug binding. Three mutations, F1418A, F1465A, and F1760A, were generated in the Nav1.5 channel. The patch clamp technique was used for biophysical characterization of WT and mutants in the presence and absence of AVE0118. The docking analysis suggests that AVE0118 could bind in Nav1.5's central cavity, extending to the fenestrations. However, experimental results showed that the F1465 substitution with alanine increases drug block compared to WT. F1465 belongs to S6 transmembrane segment of DIII domain, in close proximity to the fenestration between DII and DIII. It has been previously suggested that DII-DIII fenestration in Nav1.5 is a key pathway for drug access. We presume that F1465A mutation opens the fenestration, favoring drug entrance to the central cavity. Bioinformatics analysis has been carried out to test this hypothesis.

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