SIMPOSIO “APLICACIÓN DIAGNÓSTICA Y TERAPÉUTICA DE LOS GLICANOS”

COMITÉ ORGANIZADOR

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RED TEMÁTICA GLICOCIENCIA EN SALUD

2018

Octubre 22/23

Auditorio del Centro de Química
de la BUAP, Puebla

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APLICACIÓN DIAGNÓSTICA Y TERAPÉUTICA DE LOS GLICANOS

INFECCIONES - INFLAMACIÓN Y METABOLISMO

PROGRAMA

OCTUBRE 22

8:00 - 9:00
Registro

9:00 - 9:20
Inauguración
Dr. Iván Martínez Duncker Ramírez

9:20 - 10:10
"CIBIOR research towards personalized medicine"
-Julio Reyes Leyva

10:10 - 11:00
"Role of galectins in the pathology of Alzheimer" *-Daniel Limón Pérez*

11:00 - 11:30
Coffee break

11:30 - 12:20
"Galectin-1-driven regulatory circuits in autoimmunity" *-Marta Toscano*

12:20 - 13:10
"O-GlcNAcylation and inflammation in physiological and pathological processes" *-Tarik Issad*

13:10 - 14:00
"O-GlcNAcylation: a new hallmark of colorectal cancer" *-Vanessa Dehennaut*

OCTUBRE 23

9:00-9:50
"Expression of Galectin-9 in cervical cancer" *-Verónica Vallejo Ruiz*

9:50-10:40
"Dysregulation of glycosylation observed in colorectal cancer" *-Tony Lefebvre*

10:40-11:30
"The therapeutic interest of the targeting of the sodium channels in the treatment of the cancers" *-Sebastien Roger*

11:30-12:00
Coffee break

12:00-12:50
"Studying the inmmunoglycobiology of fungal pathogens to find alternatives for diagnosis and treatment" *-Héctor Mora Montes*

12:50- 13:40
"Characterization of influenza virus strains resistant to neuraminidase inhibitors" *-Gerardo Santos López*

13:40-14:30
"Development of new trans-sialidase inhibitors" *-Gildardo Rivera* Sánchez

REGISTRO INDISPENSABLE

ESTE EVENTO ES SIN COSTO GRACIAS A NUESTROS PATROCINADORES

IMSS · Universidad Autónoma del Estado de Morelos · Centro de Investigación en Dinámica Celular · Red Glicociencia en Salud · CONACYT · CCG Centro de Ciencias Genómicas · Francia México

LA REALIZACIÓN DE ESTE EVENTO FUE POSIBLE GRACIAS AL APOYO FINANCIERO RECIBIDO POR PARTE DEL CONSEJO NACIONAL DE CIENCIA Y TECNOLOGÍA A TRAVÉS DE LA RED TEMÁTICA GLICOCIENCIA EN SALUD 293399 Y A NUESTROS PATROCINADORES.

CIBIOR research towards personalized medicine

Julio Reyes Leyva

East Biomedical Research Center (CIBIOR), Mexican Institute of Social Security (IMSS).

Research at the Mexican Institute of Social Security (IMSS) is done at Biomedical Research Centers (CIB) and Medical Research Units (UIM) they generate knowledge to understand the health-sickness process, oriented to the medical practice, for prevention, diagnosis, evolution and treatment of the diseases. Searching of new compounds and therapeutic procedures that improve the quality of life in health and disease, as well as, development of new technologies and innovations applicable to screening, diagnosis, treatment and follow-up of the disease processes. Research at CIBIOR (located at Metepec, Puebla) has as the main achievements: Research on health problems of a national priority.

Signed agreements that link scientific research with healthcare services. Construction of the biological containment laboratory and the instrumentation center. Plan of creation of the Translational Medicine Unit. CIBIOR's main research lines: Emerging viral diseases. Human papillomavirus and cervical cancer. Cell and molecular biology of breast cancer.

Transplant Immunology. Preclinical pharmacological research. CIBIOR has a "Translational Research Program on Cancer" that integrates both basic and clinical aspects of medical research since its origins. Analyzes all aspects of cancer from a multidisciplinary perspective. Progresses through convergence of several lines of research that are sequential in nature. Insides positively in cancer prevention, diagnosis and treatment in a personalized approach. Studies done in CIBIOR groups are resumed as: Breast Cancer Group: Molecular epidemiology of breast cancer, Identification of new biomarkers for early diagnosis and therapeutic targets, Search for liquid biopsy biomarkers, Evaluation of new anticancer drugs, Studies on invasion and metastasis process. Oncogenic Viruses Group: Design and development of diagnostic tools for viral infections. Molecular mechanisms of virus pathogenicity. Clinical response to antiviral therapy in HCV infected patients. Identification of occult HBV infections. Modulation and evasion of antiviral immune response. Development of new antiviral drugs. Oncoimmunology Group: Biology of bone marrow transplant. Characterization of hematopoietic stem cells, Hematic & immune post-transplant reconstitution. Personalized preclinical trials of drug susceptibility in bone marrow organoids. Cytomics of child and adult leukemias. Multiparametric flow cytometry of leukemias of unfavorable prognosis.

Cervical Cancer Group: Glycobiology of cervical cancer. Identification of glycan and tumoral antigens for early diagnosis of cervical cancer. Role of HPV oncogenes as inducers of glycosylated tumor antigens. Molecular epidemiology, persistence and genotyping of HPV CIBIOR current nanotech goals: Knowledge of human papillomavirus oncogenesis and cell transformation. Goal 1: Development of a quantitative nanostructured biosensor for early detection of cervical cancer cells in scraping samples. Goal 2: Development of highly specific nanocarriers for delivery of cytotoxic drugs into cervical cancer cells.

Role of the Galectins in the pathology of Alzheimer's.

I. Daniel Limón, C. Sánchez-Maldonado, E. Ramírez-Hernández and A. Patricio-Martínez

Recent evidences have shown that the administration of Amyloid-β 25-35 (Aβ25-35) into the hippocampus of rats increases the inflammatory response that causes memory impairment and neurodegeneration. The galectins could be included in the modulation of the neuroinflammation induced by the Aβ25-35. Galectins are animal lectins that bind to β-galactosides contained in glycoproteins or glycolipids, such as lactose and N-acetyllactosamine. Galectin-1 (Gal-1) and Galectin-3 (Gal-3) are involved in pathologies associated with the inflammatory process, cell proliferation, cell adhesion and migration, and apoptosis. It has been shown that galectins (lectins that recognize N-acetyllactosamine [Galβ (1-4) -GlcNAc; LacNAc]) exert an effect on the modulation of the immune and inflammatory response. In the nervous system it has been shown that Gal-1 and Gal-3 participate in the regulation of the activity of astrocytes and microglia. Gal-1 acts as a negative regulator of inflammation, since it promotes apoptosis, induces the expression of IL-10 and down-regulates proinflammatory cytokines. Gal-3 modulates the adaptive immune response and induces the activation of microglia and astrocytes. However, it is not yet known whether the neuroinflammation induced by the Aβ25-35 peptide modifies the expression of Gal-1 and Gal-3, and its relationship with the damage on the substrates required in the formation of memory.

The aim of this study was to the presence of Gal-1 and Gal-3 during the neuroinflammation by administration of Aβ25-35 induced into the hippocampus, decrease spatial memory in the Morris water maze. Furthermore we study that peptide Aβ25-35 induces changes glycosides, mainly on the sialylation patterns, which is associated with a process of response to neurodegeneration. After the administration of Aβ25-35, animals were tested for learning and spatial memory in the Morris water maze. Behavioral performance showed that Aβ25-35 did not affect spatial learning but did impair memory, with animals taking longer to find the platform.The hippocampus was examined for astrocytes (GFAP), microglia (Iba1), Gal-1 and Gal-3 via immunohistochemical analysis, and the cytokines IL-1β, TNF-α, IFN-γ by ELISA. This study's results showed a significant increase in the expression of Gal-3 in the microglia and astrocytes, while Gal-1 increased only in microglia of dorsal hippocampus. The expression of galectins is associated with the increased cytokines in the hippocampal formation of Aβ25-35 treated rats. These findings suggest that Gal-3 participated in the inflammation induced by administration of Aβ25-35 and could be involved in the neurodegeneration progress and memory impairment. An increase of sialic acid in α-2,6 binding was observed in the form of sialic acid in α-2,3 binding to the neurotoxic effect of Aβ25-35 peptide. It is proposed that before damage induced by the Aβ25-35 peptide, the expression of Gal-1 could be associated with an anti-inflammatory response; however, the effect of the Aβ25-35 peptide maintains the overactivation of the glial cells. An increase in the expression of Gal-3 in GFAP and Iba1 was observed in the formation of the hippocampus after the damage induced by the Aβ25-35 peptide. Understanding the interactions within the nervous system between Gal- 1, Gal-3 and the immune system, could be the key to prevent or delay neurodegeneration.

Galectin-1-driven regulatory circuits in autoimmunity

Marta Toscano

Galectin-1 (Gal-1), an endogenous glycan-binding protein, plays a critical role in immune cell homeostasis. In the past few years, several reports indicate that administration of recombinant Gal-1 can suppress clinical signs of several experimental models of immune mediated diseases. In addition, mice deficient in Gal-1 (Lgals1-/-) develop increased severity in experimental models of autoimmune neuroinflammation and arthritis. However, the contribution of Gal-1 to the control of peripheral tolerance has not yet been fully addressed. Here we analyzed the effect of Gal-1 deficiency in the development of spontaneous autoimmunity and evaluated the cellular mechanisms associated to its appearance. We report that disruption of the Gal-1-N-glycan axis leads to the development of an autoimmune inflammatory process characterized by enhanced CD8+ T cell activity, increased CD11c+ immunogenic dendritic cells with greater co-stimulatory potential and reduced frequency of regulatory T cells. In conclusion, these results describe the immune mechanisms triggered by endogenous Gal-1 to promote immune tolerance and prevent the development of spontaneous autoimmunity.

O-GlcNAcylation and inflammation in physiological and pathological processes

Tarik Issad

Alterations in protein O-GlcNAc-glycosylation (O-GlcNAcylation) have been involved in glucotoxicity associated with chronic hyperglycaemia. Only two enzymes, OGT and OGA, regulate addition and removal of O-GlcNAc on proteins, respectively. Recent studies suggested that OGA expression level in circulating blood cells could serve as a diabetes biomarker. The aim of this work was to evaluate whether OGA enzymatic activity could serve as a more rapidly accessible biomarker in diabetic patients.

Leucocytes were prepared from blood of healthy volunteers and of diabetic patients recruited at the Cochin Hospital. OGA activity, measured using a fluorogenic assay, was higher in leucocytes from diabetic patients compared to control individuals. However, OGA activity did not correlate with hyperglycaemia markers (blood glucose, HbA1C, fructosamine) but positively correlated with the inflammatory marker CRP. Therefore, OGA activity may constitute a biomarker of inflammation rather than of hyperglycaemia in diabetic patients.

In addition, mRNA levels of OGA were positively correlated with TNFalpha and TxNIP mRNA levels, suggesting again a link between inflammatory processes and O-GlcNAc cycling in diabetes. Finally, using mouse macrophages, we also provide extensive data indicating that O-GlcNAcylation participates in pro-inflammatory signal induced by LPS stimulation.

Therefore, altogether, our work suggests important links between O-GlcNAcylation and inflammation, both in mice and humans.

O-GlcNAcylation: a new hallmark of colorectal cancer.

Amélie DECOURCELLE, Ingrid LOISON, Dominique LEPRINCE and Vanessa DEHENNAUT

CNRS-UMR 8161, Mechanisms of Tumorigenesis and Targetd Therapies, « Institut de Biologie de Lille », Lille Nord de France University, Pasteur Institute of Lille, IFR 142, 1 rue Calmette, BP447, 59017, Lille Cedex, France.

Colorectal cancer (CRC) is one of the leading causes of mortality and morbidity by cancer - the second for women and the third for men - and is often associated with metabolic disorders (obesity, diabetes…). Largely spread in the Western societies, these two groups of pathologies are tightly linked. It is now widely accepted that the occurrence of CRC is dependent on the interplay between the genome and the epigenome, which together interact with environmental factors, including nutrition.

O-GlcNAcylation is a post-translational modification (PTM) belonging to the large group of glycosylations. Unlike to the other types of glycosylation, protein O-GlcNAcylation is confined within the cytosolic, nuclear and mitochondrial compartments. O-GlcNAcylation is highly dynamic like phosphorylation with which it can either act in concert or conversely compete for the same or the adjacent serine/threonine residues. The addition and the removal of the GlcNAc residue is mediated by the O-GlcNAc transferase (OGT), using the nutrient-sensor UDP-GlcNAc as the sugar donor, and the O-GlcNAcase (OGA) respectively. The level of UDP-GlcNAc, supplied by the hexosamine biosynthetic pathway (HBP), tightly correlates to the cell nutrient status since many metabolic pathways are required for the biosynthesis of the nucleotide-sugar. Accordingly, due to its crucial position, it has been suggested that O-GlcNAcylation regulates cell metabolism and functions in a nutrient-dependent manner. So we hypothesized that O-GlcNAcylation could relay the effects of an excessive food supply, malnutrition, obesity, and other metabolic problems that represent high risk factors of CRC.

In this sense, in a precedent set of studies we observed increased contents of O-GlcNAcylation and OGT in human colon cancer samples in comparison with normal tissues thus defining aberrant OGT and O-GlcNAcylation levels as new CRC hallmarks. Regarding the underlying mechanisms linking aberrant O-GlcNAcylation to CRC, we demonstrated that O-GlcNAcylation stabilizes β-catenin, the key regulator of the Wnt signaling pathway and whose aberrant stabilization is found in 90% of CRCs, through direct competition with phosphorylation at Thr41. In this context, we also showed that colons from mice fed high-carbohydrate diets exhibited higher amounts of O-GlcNAcylation and of □-catenin relative to mice fed a standard diet. These results thus sustain the hypothesis that nutritional disorders, even of short duration, are able to disrupt the dynamic of O-GlcNAcylation leading to the modulation of the expression of an oncogene, without any mutation, that could predispose to the emergence of CRCs.

Currently, we focused on the regulation of the expression of the members of the UNC5 gene family by O-GlcNAcylation. The UNC5 gene family consists of four related genes: UNC5A, UNC5B, UNC5C and UNC5D that act as receptors of Netrin-1. These genes belong to the family of dependence receptors that share the ability to regulate apoptosis positively or negatively, respectively in the absence or presence of their ligand and thus are defined as conditional tumor suppressors. The expression of some of these genes is

frequently down-regulated in colorectal cancer (CRC) in part through epigenetic mechanisms not fully understood. In recent years, O-GlcNAc transferase (OGT) has emerged as an important regulator of chromatin dynamic and gene expression notably by regulating the function of the histone methyltransferase EZH2, the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2) (Dehennaut el al., 2014). In this context, we hypothesized that the OGT-EZH2 axis could play a role in the epigenetic downregulation of the UNC5 gene family in CRC. First, we observed that the knockdown of OGT by RNAi in the colon cancer cell line HCT116 leads to an increase of UNC5A mRNAs but has no effect on the expression of the other members of the UNC5 family. By a combination of pharmalogical inhibitions and RNA interference approaches coupled to RT-qPCR analyses and promoter activities studies, we demonstrated that OGT/O-GlcNAcylation and EZH2 were both involved in the repression of UNC5A transcription in colon cancer cells. By lectin enrichment experiments, we confirmed the O-GlcNAcylation of EZH2 in HCT116 cells. Overexpression of the core PRC2 complex in these cells reduces UNC5A expression but inhibiting OGT activity with Ac5S-GlcNAc alleviates this PRC2-mediated repression of UNC5A. Taken together, these data demonstrate that the O-GlcNAcylated form of EZH2 represses the transcription of UNC5A and further support the hypothesis that hyper-O-GlcNAcylation could contribute to aberrant EZH2 activity leading to the repression of key tumor suppressor genes governing the cancerization of the colonic mucosa.

Expression of Galectin-9 in cervical cancer

Verónica Vallejo Ruiz

Cervical cancer is one of the malignant tumors with high incidence and mortality in developing countries, in Mexico represents the second cancer most common in women.

Galectins are a group of proteins known for their ability to bind to β-galactoside sugars, while having selective preferences for complex glycan structures, these proteins have been associated with cancer. Galectins can be found intracellularly, both in the cytoplasm and the nucleus and extracellularly. Galectin-9 have been implicated in tumor growth, metastasis, immune response and its expression level has been related to tumor aggressiveness and response to therapy.

Galectin-9 is a tandem repeat galectin, consisting of two carbohydrate-binding domains covalently bound by a flexible linker peptide. The roles of galectin-9 have been evaluated in different cancer cells. Galectin-9 can decrease invasion and metastasis of colon, melanoma and hepatic cells. In breast cancer has been reported a decreased expression associated with poor prognosis. In cervical cancer tissue, has been reported a decreased expression of Galectin-9, the tumor expression level of galectin-9 showed a trend towards improved survival. The decreased expression of this protein in cervical cancer tissue is related with the diminished levels of mRNA detected by our research group in cervical tissue. We have determined the galectin-9 concentration in serum of women with normal cytology, low grade squamous intraepithelial lesions, high grade squamous intraepithelial lesions and cervical cancer. The concentration of galectin-9 was increased in women with cervical cancer with respect to control group. It is important to mention that some women with high grade squamous intraepithelial lesions had increased levels of this protein.

Galectin-9 is a ligand of the immune receptor Tim-3, and this receptor participates in the secretion of galectin-9. Galectin-9 could be affecting immune response because this protein is able to impairs the anticancer activity of cytotoxic lymphoid cells, so the changes in galectin serum concentration could be modulating immune response. Galectin-9 may become a biomarker for the prediction of progression of cervical lesions and a valuable marker of prognosis for cervical cancer.

Dysregulation of glycosylation observed in colorectal cáncer

Tony Lefebvre

Colorectal cancer (CRC) is the third most frequent cancer worldwide, its incidence and mortality being particularly high in industrialized countries. Metabolic disorders such as diabetes, obesity or metabolic syndrome increase the risk of CRC. There are several methods used to diagnose and follow the progression of CRC, this includes fecal occult blood test, PET scan, endoscopy and also the use of more or less specific markers among which glycomarkers are found (CA 19-9 and CEA). Unfortunately, serum glycoprotein biomarkers show insufficient sensitivity and organ specificity. In that sense, glycosylation is often altered in cancer, this is the case in CRC. Glycosylation abnormalities promote tumor growth, metastasis and affect response to chemotherapy and immune response. Unlike complex glycosylations, O-GlcNAcylation consists in the addition of a single N-acetylglucosamine unit to serine and threonine residues of target proteins and is confined within the nucleocytoplasmic and mitochondrial compartments. Nevertheless, a number of clues tend to show that O-GlcNAcylation is a pivotal regulatory element of its complex counterparts. We propose different levels of regulation that encompass the control of the wide class of glycosylation enzymes via their expression, catalytic activity and trafficking. Our preliminary data indicate that silencing OGT deregulates the cancer cells specific GTase MGAT5 expression. The questions we would like to respond in a near future are: is O-GlcNAc a potential candidate to diagnose and monitor CRC cells, is it measurable in body fluids, are E-cadherin’s N-glycans modified in siOGT conditions, does it impact EMT.

The therapeutic interest of targeting voltage-gated sodium channels in the treatment of cancers

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Voltage-gated sodium channels (Na_v) are plasma membrane ion channels that physiological activity is critical for cellular electricity and the functioning of cells characterized as being “excitable”, such as neurons, muscle or cardiac cells. However, it appeared that these proteins are aberrantly expressed in cancer tissues and cells from different tissue origins such as in breast, lung, prostate, colon, cervix, stomach cancers, while they are not expressed in cognate non-cancer tissues. We have demonstrated that the expression and function of these ion channels in cancer cells are associated with invasive properties and the progression towards metastatic stages.

In breast cancer cells, the $Na_v1.5$ channel is critical to the “Mesenchymal invasion”. The $Na_v1.5$ channel is expressed in specific structures of aggressive cancer cells, called invadopodia, where it favours the activity of the Na+-H+ exchanger type 1 (NHE1), the efflux of protons, and the activity of cysteine cathepsins, thus promoting the degradation of the extracellular matrix. The activity of $Na_v1.5$ channel also sustains the Src kinase activity, the phosphorylation of the actin-nucleation factor cortactin and the polymerisation of the actin cytoskeleton. In vivo, the pharmacological targeting of of $Na_v1.5$, using the FDA-approved anti-arrhythmic drug ranolazine, prevented the metastatic colonization of organs.

In conclusion, Na_v channels could represent new important prognostic factors of cancers as well as pharmacological targets for new or repurposed Na_v-inhibiting drugs.

Studying the immunoglycobiology of fungal pathogens to find alternatives for diagnosis and treatment

Héctor M. Mora-Montes

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The cell wall of the opportunistic pathogen *Candida albicans* is composed of chitin, β-glucans, and glycoproteins, which are enriched with *N*- and *O*-mannans, and these are considered the main pathogen-associated molecular patterns that the innate immune system recognizes to establish a protective anti-*Candida* immune response. It has been demonstrated that *C. albicans O*-mannans are recognized by TLR4, *N*-mannans by mannose receptor, DC-SIGN, and dectin-2, and β1,3-glucan by dectin-1. Despite this knowledge, little is known about the relevance of cell wall components during the immune recognition of *Candida non-albicans*, and other fungal pathogens such as the causative agent of sporotrichosis, *Sporothrix schenckii*. Here, we studied the relevance of cell wall components of *S. schenckii*, *C. parapsilosis, C. tropicalis, C. guilliermondii* and *C. krusei* during cytokine stimulation and phagocytosis by human monocytes and monocyte-derived macrophages, respectively. Our results showed that these organisms have similar cell wall composition, but different degrees of cell wall porosity, and this physical parameter correlates with the ability to stimulate cytokine production. Removal of *O*- or *N*-glycans affected the stimulation of cytokines in a species specific-manner. As previously reported in *C. albicans*, *S. schenckii* stimulated cytokine production in a morphology-dependent way.

Moreover, the characterization of the cell wall from these organisms has led us to find immunoreactive molecules that we are currently investigating as potential candidates for the development of vaccines and diagnostic kits.

This work is supported by CONACyT, México (grant numbers PDCPN2014-247109 and FC 2015-02-834) Universidad de Guanajuato (ref. 1025/2016; CIIC 95/2018), and Red Temática Glicociencia en Salud (CONACYT-México).

Characterization of influenza virus strains resistant to neuraminidase inhibitors

Gerardo Santos López

There are of two types of antivirals against influenza viruses which act on two different viral targets: adamantanes, which block the M2 ion channel and the neuraminidase (NA) inhibitors. The reason of this drug resistance are the genetic variability of influenza viruses. In Mexico, the available studies on the antiviral resistance of circulating influenza strains are scarce, so in this work it has been performed an analysis of the Ina recent study on Mexican sequences between 2000 and 2017 we analyzed the antiviral resistance markers on both M2 and NA sequences. The resistance markers to M2 blockers were present in 100% of H1N1 pdm2009, in 83.6% of H3N2 and in 5.8% of seasonal H1N1 sequences. Two resistance markers conferring resistance to NA inhibitors were present in seasonal H1N1 sequences, H275Y and N70S. None of these viruses had both resistance markers, which are associated to oseltamivir resistance. The more frequent resistance marker in H1N1 pdm2009 NA sequences was H275Y. Only one of the resistance-associated markers (Q136K) in NA was present in the analyzed H3N2 sequences, while sequences of influenza B virus did not present resistance markers to NA inhibitors. Because of this, and the limited availability of influenza drugs, it is necessary to increase the epidemiological surveillance, including molecular analysis, which will provide data such as the presence of changes associated to antiviral resistance. This problem also compels us to investigate new resources to fight the infection, which includes the development of new drugs. Our group is working with other research groups to obtain new molecules with antiviral activity, particularly neuraminidase inhibitors to have other therapeutic options to use in case the active drugs lose effectiveness for the treatment of influenza.

Development of new trans-sialidase inhibitors

Gildardo Rivera Sánchez

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Chagas disease or American trypanosomiasis remains as an important public health problem in developing countries. In the last decade, *trans*-sialidase enzyme has become a pharmacological target for new anti-Chagas drugs, due to its vital role for the transfer of sialic acid from host to parasite surface. In a continuous effort to discover new potential *trans*-sialidase inhibitors, our research group has designed, synthetized and biological evaluated *in silico* and *in vitro* new series of benzoic acid derivatives, 3-amino-3-arylpropionic acid derivatives and novel phthaloyl derivatives as *trans*-sialidase inhibitors and anti-trypanosomal agents.

Results shown that three compounds (**14**, **18** and **19**) sharing a *para*-aminobenzoic acid moiety have more potent trypanocidal activity than the commercially available drugs nifurtimox and benznidazole in NINOA and INC-5 strains: the lysis concentration of 50% of the population (LC_{50}) was <0.15 µM on the NINOA strain, and LC_{50} <0.22 µM on the INC-5 strain. Additionally, compound **18** showed a moderate inhibition (47%) on the *trans*-sialidase enzyme and a binding model similar to DANA.

On the other hand, compound **D-11** had the highest binding affinity value (-11.1 kcal/mol) compared to reference DANA (-7.8 kcal/mol), a natural ligand for TS enzyme. Furthermore, the molecular docking study of compound D-11 showed interactions with all important amino acid residues (Arg35, Arg245, Arg314, Tyr119, Trp312, Tyr342, Glu230 and Asp59) on the active site of *trans*-sialidase. Additionally, D-11 showed the highest *trans*-sialidase enzyme inhibition (86.9% ± 5) by high-performance ion exchange chromatography. Finally, D-11 showed better trypanocidal activity than the reference drugs nifurtimox and benznidazole with an equal % lysis (63 ± 4 and 65 ± 2 at 10 µg/mL) and LC_{50} value (52.70 ± 2.70 µM and 46.19 ± 2.36 µM) on NINOA and INC-5 strains, respectively. Therefore, compounds **18** and **D-11** are small-molecules with inhibitory effects on *trans*-sialidase and a strong trypanocidal effect that could help in the development of new anti-Chagas agents.