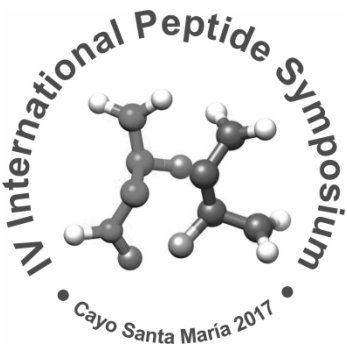
Synthetic Peptides 2017

June 22nd-25th Cayo Santa María, Cuba

Scientific Program

4th International Symposium on Synthetic Peptide as Human and Veterinary Pharmaceutical Products



June 22nd-25th
Cayo Santa María Hotel, Cuba

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SCIENTIFIC PROGRAM

ORAL PRESENTATION

Thursday June, 22nd

OPENING SESSION

18:30-18:40	Welcome remarks **Dra. Hilda Elisa Garay Pérez**
18:40-19:40	**Opening lecture:** Biomedical Research at CIGB and the development of drug peptides and peptide based vaccines. **Dr. Gerardo Guillén (Director of Biomedical Research at CIGB, Cuba)**
19:40-21:00	**WELCOME COCKTAIL**

Friday June, 23rd

ORAL PRESENTATION

Chairpersons:	**Dr. Istvan Toth (The University of Queensland, Australia)/Dr. Vladimir Besada (CIGB, Cuba)**
8:30-09:15	**Plenary Lecture:** Positional Scanning Libraries: Concepts, Elucidation of T cell Specificity and Drug Lead Discovery **Dr. Clemencia Pinilla (Torrey Pines Institute for Molecular Studies, USA)**
09:20-09:45	Unraveling Functional Sites on Domain III of the Envelope Protein of Dengue Virus: Design of Antiviral Peptide Inhibitors **Dr. Glay Chinea (CIGB, Cuba)**
09:50-10:15	Growth hormone releasing peptide-6 (GHRP-6) and other related secretagogue synthetic peptides: A mine of medical potentialities for unmet medical needs. **Dr. Jorge Berlanga (CIGB, Cuba)**
10:20-10:45	Epidermal growth factor and growth hormone-releasing peptide-6: combined therapeutic approach for neurodegenerative diseases **Dr. Diana Garcia del Barco (CIGB, Cuba)**

10:50-11:05	
11:05-11:30	Peptide/biomarker isolation using MALDI Imaging **Dr. Richard Naidoo (University of Cape Town, South Africa)**
11:35-12:00	Characterizing of peptide toxin diversity libraries using chemical derivatization and mass spectrometry **Dr. Beatrix Ueberheide (New York University School of Medicine, USA)**
12:05-12:30	Characterization by ESI-MS of low-abundance modified variants of peptides generated under stressed conditions and chemical synthesis **Dr. Luis J. González (CIGB, Cuba)**
12:35-13:35	**POSTER SESSION**
13:45-14:45	
Chairpersons:	**Dr. Richard Naidoo (University of Cape Town, South Africa)/Dra. Vivian Huerta (CIGB, Cuba)**
15:00-15:25	CIGB-814, a new therapeutic peptide for rheumatoid arthritis, from the bioinformatic design to the phase I clinical trial **Dr. María del C. Domínguez (CIGB, Cuba)**
15:30-15:50	Clinical trials with Heberprovac, a vaccine candidate

	against prostate cancer. Assessment of their safety and effectiveness over time **Dr. Jesús Junco (CIGB, Cuba)**
15:55-16:15	Cell Penetration and Secondary Structure of a Synthetic Peptide with Anti-HIV Activity **Dr. Anna Ramirez (CIGB, Cuba)**
16:20-16:40	CIGB-300: A peptide-based drug with perspectives for cancer targeted therapy **Dr. Dania Vázquez (CIGB, Cuba)**
16:45-17:05	Peptide purification by industrial Centrifugal Partition Chromatography **Dr. Laszlo Lorantfy (RotaChrom Technologiai, Hungary)**

Saturday June, 24th

ORAL PRESENTATION

Chairpersons:	**Dr. Clemencia Pinilla (Torrey Pines Institute for Molecular Studies, USA)/Dr. Gerardo Guillén (CIGB, Cuba)**
8:30-09:15	**Plenary Lecture:** Lipophilic peptide and vaccine delivery systems. **Dr. Istvan Toth (The University of Queensland, Australia)**
09:20-09:45	Liposomes containing Cobalt-Porphyrin-Phospholipid (CoPoP) as a vaccine adjuvant for his-tagged MPER peptides **Dr. Jonathan Lovell (State University of New York, USA)**
09:50-10:15	Multiantigenic peptide-polymer conjugates as therapeutic vaccines against cervical cancer **Dr. Mariusz Skwarczynski (The University of Queensland, Australia)**
10:20-10:45	Peptide nanostructures for biomedicine and bionanotechnology **Dr. Mariano Venanzi (University of Rome Tor Vergata, Italy)**
10:50-11:05	

11:05-11:30	A new strategy for the synthesis of proteins **Dr. Hironobu Hojo (Institute for Protein Research, Japan)**
11:35-12:00	Isolation and identification of biologically active conopeptides combining transcriptomics and proteomics tools **Dr. Alexei Licea (CICESE, México)**
12:05-12:30	Cutaneous secretions of amphibians: source of peptides with important biological activities **Dra. Miryan Rivera (Pontificia Universidad Católica del Ecuador, Ecuador)**
12:35-13:35	**POSTER SESSION**
13:45-14:45	
15:00-17:00	**Bilateral meetings** **(CIGB- RotaChrom Tech Company, CIGB- University of Rome, CIGB-University of Queensland, CIGB-University of Cape Town, CIGB- Universidad Nacional del Litoral, Others)**

Sunday June, 25th

ORAL PRESENTATION

Chairpersons:	**Dr. Hironobu Hojo (Institute for Protein Research, Japan)/Dr. Luis J. González (CIGB, Cuba)**
8:30-09:15	**Plenary Lecture:**
	Peptides as powerful tool in Drugs and Vaccines for Veterinary use
	Dr. Mario Pablo Estrada (Director of Agricultural Biotechnology Research at CIGB, Cuba)
09:20-09:45	Synthetic peptides as "magnifying glasses" for designing a vaccine against Equine Infectious Anemia Virus**.**
	Dr. Adriana Soutullo (Universidad Nacional del Litoral, Argentina)
09:50-10:15	Peptide used in the immunological control of ticks
	Dr. Alina Rodríguez (CIGB, Cuba)
10:20-11:00	Closing remarks, Selection of outstanding posters/presentations
	Dr. Melyssa Yaugel

SCIENTIFIC PROGRAM

ELECTRONIC POSTER PRESENTATION

P-01 One-bead-one-compound (OBOC) combinatorial peptides libraries: design, synthesis and application in cancer. *Masforrol Y., Gil J., González L.J., Garay H.E., Pérez-Riverol Y., Fernández-de-Cossío J., Sánchez A., Betancourt L., Cabrales A., Albericio F., Yang H., Zubarev R., Besada V., Perera Y., Caballero E., Perea S. and Reyes O.*

P-02 Obtention of functionalized magnetic iron oxide nanoparticles for retains beads of tentagel S NH2 resin with a peptide coupled in the presence of a magnetic field. *Masforrol Y., Bejarano J., Inostroza M., Torres R., Salas E., Albericio F., Fernandez J.R., Pentón-Rol G., Garay H.E. and Kogan M.J.*

P-03 Obtaining a combinatorial synthetic peptide library one-bead-one-compound (protgel-10) designed as a support for equalization of protein complex mixtures. *Masforrol Y., Gil J., González L.J., Besada V., Acosta R. and Reyes O.*

P-04 Positional Scanning Libraries: Concepts and Applications for Basic Research and Drug Discovery. *Appel J. and Pinilla C.*

P-05 Solid-phase chemical synthesis of monomeric and chimeric peptides from Trypanosoma cruzi. *Hernández M., Zulueta O. and Gómez I.*

P-06 Synthesis of chimeric peptides from the HIV-1 envelope gp120 protein and its application in the immunodiagnosis. *Hernández M., Zulueta O. and Gómez I.*

P-07 Synthesis of bicyclic peptide by two regioselective disulfide bonds formation. *Martínez J.C., Diago D., Masforrol Y., Perez E., Guzman L., Estrada C., Murillo D., Arencibia M., Abreu K., Hernández J., Garay H.*

P-08 Parallel Cyclic Homodimer Formation by Air and Iodine Oxidation of S-Trityl- and S-Acetamidomethyl-cysteine-peptides. *Diago D., Arencibia M., Martínez J.C., Abreu K.M., Pérez E., Masforrol Y. and Garay H.*

P-09 Rational design, synthesis and biological evaluation of analogues of antifungal peptide Cm-p5: importance of Glu and His residues. *Morales F.E., Pietro R., Otero-González A.J., González M., García-Rivera D., Jiménez A.M., Weber-Paixao M. and Garay H.E.*

P-10 Development of purification methodology of a synthetic peptide by reversed-phase chromatography. *Pérez E., Garay H., Arencibia M., Pérez Y., Hernández J., Diago D., Alvarez A., Masforrol Y., Abreu K., Antequera A., Martínez J.C., Támbara Y., Reyes O.*

P-11 Purification, characterization and biological evaluation of a peptide mixture of Rophalurus junceus venom. *Rodríguez A., Méndez M.C, Junco A.J., Álvarez A.*

P-12 Safety, pharmacokinetic evaluation and efficacy signs of intravenous CIGB-300 in patients with solid tumors, refractory to oncospecific treatments. *González L., Noyde B., Soriano J., García R., García I., Reyes V., Méndez L., Raíces I., Valenzuela C., Álvarez L., Fernández E., Perera Y., Martin Y., de la Torre A., Crespo J.C., de la Torre J, Catalá M., Izquierdo M., López P., Acevedo B., Muzio V.L. and Perea S.E.*

P-13 Quantifying therapeutic peptides in human plasma by MS: our experience applied to PK studies in clinical trials. *Cabrales A., Ramos Y., Gil J., Garay H., Gómez J., Audain E., Berlanga J., Perera Y., Perea S., Domínguez M., Besada V. and González L.J.*

P-14 Two synthetic antimicrobial peptides and their therapeutic potential in topical infections. *Ibarra Valencia M.A. and Corzo G.*

P-15 Panusin and its analogues exposed preferential binding to negative lipids composition. *Montero-Alejo V., Vázquez A., Perdomo-Morales R., Ortiz-Castro D. and Garay H.*

P-16 GHRP-6 induces beta-oxidation and mitochondrial biogenesis in healthy rat cardiac tissue. *García-Ojalvo A., Mendoza-Marí Y. and Berlanga-Acosta J.*

P-17 Growth Hormone-Releasing Peptide 6 Enhances the Healing Process and Improves the Esthetic Outcome of Wounds. *Mendoza Y., Urquiza A., Betancourt A., Fernández M., Hernández F., Aguilera A., García A., Bermúdez Y., Bermúdez C., Martín Y., Mir A.J. and Berlanga J.*

P-18 Comparison between chimeric synthetic peptides and their application in the diagnosis of Chagas disease. *Hernández M., Gómez I., Zulueta O., Hernández I. and Ramos G.*

P-19 Stability Studies of Pyr-GnRHm1-TT Drug Substance Storage at – 20 ± 5 ^{o}C during 12 months and at 5 ± 3 ^{o}C during 6 months. *Arencibia M., Diago D., García G., Sagardoy C., Abreu K.M., Pérez E., Hernández J., Antequera A., Pérez Y. and Garay H.E.*

P-20 Stability of HCV synthetic peptides. *Gómez I., Hernández M., Zulueta O. and Ortega D.*

P-21 Nanostructured peptides at high concentration and physiological conditions-some CIGB case studies. *Santana H., Ávila C.L., Falcón V., Guerra M., Páez R., Cabrera I., Ventosa N., Veciana J., Itri R. and Barbosa L.R.S.*

P-22 Formulation development of a lyophilized peptide (Heberprovac) for the treatment of prostate cancer. *López M., Garay H., Junco J., Caballero L., Zárate Y. and Castro F*.

P-23 Amino Acid Analysis for the determinations of: extinction coefficients, peptide content and amino acid composition. *Támbara Y, Alvarez A., Alvarez K. Pupo M. and Garay H.*

P-24 Enzymatic activity regulation of peptides derived from the Leishmania braziliensis NMNAT N-terminal region. *Ávila Jiménez S., Contreras L.E., Benítez C., Díaz G.J. Rivera Z., Granados C.G. and Ramírez M.H.*

P-25 Bioinformatic identification of crocodylians -defensins. *Santana F.L., Estrada K., Hernández-Vargas M.J., Milián-García Y., Montero-Alejo V., Morera V. and Corzo G.*

P-26 Photosubstitution of Monodentate Ligands from Ru(II)-dicarboxybipyridine complexes. *Caraballo R.M., Rosi P., Hodak J.H. and Luis M. Baraldo.*

P-27 Peptides improve cellular penetration of gold nanorods for biomedical applications. *Morales-Zavala F., Velasco C., Palma S., Sanchez-Navarro M., Giralt E. and Kogan M.*

P-28 Structural determinants for antimicrobial activity of panusin. *Vázquez A., Montero-Alejo V., Perdomo-Morales R., Ortiz D. and Garay H.*

P-29 Fish PACAP: its role in teleost immunity and potential applications in Aquaculture. *Carpio Y., Lugo J.M., Gorgoglione B., Tafalla C., Secombes Ch., Estrada M.P.*

P-30 Comparative analysis reveals amino acids critical for anticancer activity of peptide CIGB-552. *Maribel G Vallespí, Yolanda Gomez, Soledad Astrada, Exequiel Barrera, Gonzalo Oval, Otto Pritsch, Sergio Pantano and Mariela Bollati-Fogolín.*

P-31 Enhancement of the inhibitory effect of an IL-15 antagonist peptide by alanine scanning and D-amino acids substitutions. *Rodríguez Y., Reyes O., Garay H., Cabrales A., Gerónimo H., Chico A., Estévez M., Martínez K. and Santos A.*

ABSTRACT

ORAL SESSION

Biomedical research at CIGB and the development of drug peptides and peptide based vaccines.

Gerardo E Guillén Nieto

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The bioinformatics tools together with the development of molecular biology and instrumentation allowed an accelerated targets identification and validation increasing the possibility of drug development based on synthetic peptides.
The existing vaccines are mainly limited to the microorganisms we are able to cultivate or to those whose killing is mediated by humoral response (antibody mediated). It has been more difficult to develop vaccines capable to induce functional cellular response needed to prevent or cure chronic diseases.
With understanding of the immunological system together with the achievements at preclinical levels a great interest appears on therapeutic vaccination to treat chronic diseases including cancer.
This work is aimed to present the scope of the biomedical research at the Center for Genetic Engineering and Biotecnology (CIGB) including the preclinical and clinical results with peptide based drugs and therapeutic vaccines.
Studies in animals and humans with peptide vaccines evidenced that it is possible to induce functional immune response against prostate and cervix cancer and develop promising drugs based on antitumoral peptides. Peptides drugs are also under development at CIGB against cardiovascular, autoimmune and neurological diseases.

Positional Scanning Libraries: Concepts, Elucidation of T cell Specificity and Drug Lead Discovery

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The concepts of synthesizing and testing mixture-based synthetic combinatorial libraries containing thousands to millions of compounds for the identification of active drug lead compounds were presented over 25 years ago. Positional scanning libraries were initially developed in a format in which each position of a peptide was defined with one of the naturally occurring amino acids while the remaining positions contained equimolar mixtures of the same amino acids. The positional scanning library concept has been further applied to other synthetic chemistries that can create heterocyclic and small molecule scaffolds and incorporating a variety of building blocks. Identification of active compounds from the screening of positional scanning libraries in a given assay is straightforward by simply synthesizing a set of molecules that contain the combination of the building blocks found in the active mixtures. Examples of the many different libraries that have been synthesized and their uses in a variety of assays will be presented. The integration of a biometrical analysis for the identification of peptides from positional scanning peptide libraries will also be presented in examples using T cell clones of known and unknown specificities.

Unraveling functional sites on domain III of the envelope protein of dengue virus: design of antiviral peptide inhibitors

Chinea G.[1], Huerta V.[1], Pupo D.[1], Garay H.[2], Ramos Y.[1], Fleitas N.[1], Martin A.[1], Toledo P.[1], Vidal Y.[1], Falcón V.[2], Sarría M.[1], Guirola O.[1], Yero A.[1], Reyes O.[2] and González L.J.[1]

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Dengue virus (DV) is an important human pathogen which causes annually between 100 and 400 million infections, about half a million severe disease manifestations and 10 000 fatalities worldwide. DV is a complex consisting in four genetically and antigenically closely related viruses (or serotypes) DEN1-4, which elicit a neutralizing homotypic and a non-neutralizing (enhancing) heterotypic antibody response. Domain III of the envelope protein of DV is involved in key virus-host protein interactions and it is also the target of several neutralizing antibodies. In order to get insights into the location of functional sites on the surface of DIII, we have analyzed the sequence conservation patterns of the surface patches of DIII and compared it to the serotype reactivity patterns of DIII-reactive serum proteins identified by an affinity-chromatography/mass spectrometry proteomic study. Furthermore, we synthetized lineal and cyclic/topographic peptides mimicking different surface patches of the DIII and evaluated its antiviral activity in vitro. Some peptides were also evaluated by assessing its binding properties to a serum protein and a membrane receptor. The results indicate that the beta-hairpin FG of DIII is an important functional site of the envelope protein and it is the basis for the design of potent antiviral peptides which affect the interaction with cell receptor(s) and inhibit the viral infection by DV.

Growth hormone releasing peptide-6 (GHRP-6) and other related secretagogue synthetic peptides: A mine of medical potentialities for unmet medical needs.

Berlanga J.[1], Abreu A.[2], Garcia-del Barco D.[1], Mendoza Y.[1], Rodríguez A.[1], Garcia A.[1], Falcon V.[1], Hernandez F.[1] and Guillen G.[1]

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Growth hormone-releasing peptides (GHRPs) constitute a group of small synthetic peptides that stimulate the growth hormone secretion and the downstream axis activity. Mounting evidences since the early 1980s delineated unexpected pharmacological cardioprotective and cytoprotective properties for the GHRPs. Despite intense basic pharmacological research, alternatives to prevent cell and tissue demise before lethal insults have remained as an empty niche in the clinical armamentarium. GHRP-6, a member of the GHRPs family of peptides binds to two different receptors (GHS-R1a and CD36), which redundantly or independently exert relevant biological effects. GHRP-6 binding to CD36 activates pro-survival pathways such as PI-3K/AKT, thus reducing cellular death. Furthermore, GHRP-6 decrease reactive oxygen species (ROS) spillover, enhance the antioxidant defenses, and reduce inflammation. These cyto- protective abilities have been revealed in cardiac, neuronal, gastrointestinal, and hepatic cells, representing a comprehensive spectrum of protection of parenchymal organs. Antifibrotic effects have been attributed to GHRP-6 by counteracting fibrogenic cytokines. In addition, GHRP family members have shown a potent myotropic effect by promoting anabolia and inhibiting catabolia. Finally, GHRP-6 exhibit a broad safety profile in preclinical and clinical settings. Taken together these lines of evidence incite to envision multiple pharmacological uses for GHRP-6, especially as a myocardial reperfusion damage-attenuating candidate. GHRPs are a family of “drugable” peptides that waits for a definitive clinical niche.

Epidermal growth factor and growth hormone-releasing peptide-6: combined therapeutic approach for neurodegenerative diseases

Garcia del Barco D.[1], Pérez-Saad H.[1], Subirós N.[1], Aldana L.[1], Berlanga J.[1], Vesada V.[1] and Palomares S.[1]

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In the biology of brain damage, neuroproteccion is a limited concept, so it is necessary to think in a more complete praxis involving brain-protection. Some of the pathophysiological phenomena produced during brain damage are targeted by the cytoprotective effects of both Growth Hormone Releasing Peptide-6 (GHRP6) and Epidermal Growth Factor (EGF). The most relevant of these effects act on oxidative stress-induced damage, on mitochondrial dysfunction and on glutamate-induced excitotoxicity. All these properties can explain the salutary therapeutic effects of EGF+GHRP6 co-administration observed in different experimental models of amyotrophic lateral sclerosis, multiple sclerosis and stroke during preclinical evaluations. Considering the multiple pathophysiological mechanism involved in neurodegenerative diseases we sustain the convenience of combined therapy to induce brain protection. Such strategy could allow a concerted blocking of key points of the complex pathophysiology characteristic of neurodegenerative diseases. The combined therapy of EGF and GHRP6 simultaneously targets different pathophysiologic key points involving not only neurons, but also glial cells and vascular endothelium. The results of this work could be considered as an example of therapeutic alternative holistically directed to brain protection.

Peptide/biomarker isolation using MALDI Imaging

Richard Naidoo

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MALDI imaging allows for the investigation of the molecular signatures directly on tissue. The technology enables quantification of peptides/proteins and their spatial distributed directly on tissue sections. The technology has tremendous potential for biomarkers discovery in cancer. These investigations interrogate the use of MALDI-TOF to determine the level and extent of peptide/protein profiles on tissue. Cancer tissue samples were obtained from the archives of the Division of Anatomical Pathology. Formalin fixed paraffin embedded (FFPE) tissues were sectioned at 6u and picked up on ITO slides. The tissues were then antigen retrieved before MALDI preparation. The corresponding H&E sections were also scanned before image preparation. The tissue was coated with trypsin followed by the addition of the matrix coating. This step was done on the Bruker Imageprep Instrument. The slides were then analysed in the Bruker Autoflex 111 instrument. The spectra were captured automatically, followed by analysis using the Fleximaging software. The peaks from the spectra generated were assigned different colours which could be imaged directly on the tissue section. We were successful in identifying specific peptide which was overexpressed in certain areas of the tissue. We have shown that FFPE tissue can be used for MALDI imaging. This method is suitable for the identification and distribution of potential cancer biomarkers in association with the histological phenotype.

Characterizing of peptide toxin diversity libraries using chemical derivatization and mass spectrometry

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Animal venoms represent one of nature's great diversity libraries of bioactive molecules and have long been viewed as a rich depository of biomedically active and insecticidal peptides. However, characterizing the primary amino acid sequence and post translational modification accurately is challenging, due to limitations in database availability and production. Sequence determination has traditionally relied on low-throughput techniques, such as Edman Degradation, that require large quantities of purified compounds, which may be difficult or impossible to obtain. Here we combine simplified chemical derivatization of cysteines, electron transfer dissociation mass spectrometry and a flexible search engine to reveal the true diversity found in animal venoms. Our technique allows to sequence crude venoms directly using mass spectrometry without fractionation or digestion. We demonstrate the accurate sequencing of heavily modified *Unedogemmula bisaya* venom and toxins from *Hottentota judaicus* and *Leiurus quinquestriatus hebreus* scorpion as well as a population study of venom variability in *Hadronyche infensa*.

Characterization by ESI-MS of low-abundance modified variants of peptides generated under stressed conditions and chemical synthesis

Espinosa L.A, Santana H., Besada V., Sánchez A., Garay H.E, González L.J.

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According to ICHQ3A, for a new drug substance produced by chemical synthesis, and administered at a maximum daily dose equal or lower than 2 g/day, those impurities that exceed 0.05 %, 0.10 % and 0.15 % in abundance should be reported, identified and qualified, respectively. We synthesized a cell-penetrating cyclic peptide, CIGB-300, that is able to inhibit selectively the phosphorylation of casein kinase-2 substrates and triggering a cell-death mediated apoptosis process. The reverse phase chromatography shows the presence of low-abundance impurities (> 0.3 %) that were characterized by ESI-MS/MS and biological activity. Most abundant impurity with no biological activity was CIGB-300 with a sulfoxide Met[21]. Two variants of dimmers with a biological activity higher than the monomer as well as trimmer and tetramers with very low abundance (> 0.05 %) were detected.
On the other hand, during the development of a formulation, stressed conditions are evaluated in order to characterize the degradation pathways and stability. We have used CIGB-500 peptide for the treatments of acute myocardial infarction and hypertrophic queloid scars. The stability of this peptide was evaluated at different pH and buffers under stressed conditions and the degradation products were characterized by ESI-MS/MS. Major products resulted from binding of citric acid and its cyclization. Also head-to-tail cyclic peptide was favored in phosphate buffer. At basic pH, deamidation of the C-terminal amide, as well as a modified variant with a mass 12 Da higher than expected, were detected. Other peptides ragged at both ends and their cyclization products were observed.

CIGB-814, a new therapeutic peptide for rheumatoid arthritis, from the bioinformatic design to the phase I clinical trial

Prada D.[2], Gómez J.[2], Lorenzo N.[1], Corrales O.[1],González E.[1], Cabrales A.[1], Reyes Y.[2], Molinero C.[2], Mantecó A.M.[2], Torres A.M.[2], Hernández M.V.[2], Garay H.E.[1], López M.[1], Pérez E.[1], Reyes O.[1],Gonzalez L.J.[1], Domínguez M.C.[1]

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Induction of peripheral tolerance has long been considered a promising approach to the treatment of rheumatoid arthritis (RA).
We aimed to evaluate the therapeutic potentialities of an altered peptide ligand (APL) derived from human heat-shock protein 60 (hHsp60) for the treatment of RA. A novel epitope of T cells located in the N terminal region of hHsp60, an autoantigen involved in the pathogenesis of autoimmune arthritis, was identified by bioinformatics tools and an APL (called CIGB-814) was designed starting from this epitope. We evaluated the therapeutic effect of this peptide in two animal models for RA (AA in Lewis rat and CIA in DBA1 mice) and in ex vivo assays using PBMC isolated from RA patients.
CIGB-814 therapy reduced significantly the course of RA in both animal models and induced proliferation of regulatory T cells in ex vivo assays using PBMC from RA patients.
In addition, we performed Phase I Clinical Trial with CIGB-814 in RA patients. This study was designed according three dosage levels of CIGB-814. The Schedule included 9 doses per patient in six months.
Phase I Clinical Trial concluded showing safety of CIGB-814. Treatment reduced Il-17 and IFN- levels in patients. 17 patients achieved ACR 70, when they finished therapy. At 6 months, synovitis and edema score by magnetic resonance imaging from baseline was significantly lower. These results reinforce the therapeutic possibilities of CIGB-814 as a novel candidate for treatment of RA.

Clinical trials with Heberprovac, a vaccine candidate against prostate cancer. Assessment of their safety and effectiveness over time.

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GnRH-based vaccines represent a promising anti-hormonal treatment alternative in prostate cancer, because they can reduce serum testosterone to castrating levels, avoid the "hot flushes" produced by GnRH analogues and can be administered in acute and complicated forms of prostate cancer. The present study assesses the application of Heberprovac, a GnRH based vaccine candidate for patients suffering from advanced prostate cancer and their following up to ten years in 2 clinical trials.
The main objective of the first clinical trial was to evaluate the safety and possible efficacy indicators and the second, Phase II clinical trial, to evaluate the safety and efficacy of this vaccine candidate in 4 levels of dosage.
To this aim, 6 patients affected by advanced prostate cancer diagnosed by biopsy, were included in the first clinical trial and 56 were treated in the second one. As result of the first clinical trial it was generated a 100% of effective anti GnRH immune response that corresponded with a significant testosterone reduction until castration levels, normalization of PSA and full clinical response in the whole patients subset. On the other hand, the Phase II clinical trial revealed that after the first immunizations, the patients exhibited anti GnRH antibodies and in turn, testosterone levels reduction. In concordance with the hormonal and immunological response, the patients exhibit a decrease of both; the number of obstructive symptoms as well as the severity of them. There was also a normalization of the prostatic specific antigen (PSA) in the 80% of the patients after they finished the last immunization and the clinical evaluation demonstrated the significant reduction of the primary tumor from grades III/IV to I/II in all the patients that respond biochemically.

Cell Penetration and Secondary Structure of a Synthetic Peptide with Anti-HIV Activity

Paneque T.E.[1], Ramírez A.C.[1], Casillas D.[1], Duarte C.A.[1], Chinea G.[2]; Espinosa C.[1]; Garay H.E.[3], Gómez L. and Fernández-Ortega C.[1]

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Cellular proteins have been proposed as therapeutic targets in HIV infection to solve one of the most important disadvantages of current antiretroviral therapy, the emergence of viral resistance. Our group has identified the cellular protein vimentin as a potential target to inhibit HIV replication in MT4 cells. Experiments with vimentin knockdown MT4 cells showed that a reduction in vimentin expression levels led to a drastic inhibition of viral replication. A synthetic peptide, previously reported to promote the disassembly of vimentin IFs network, was capable of inhibit HIV replication in the nanomolar range. Since this peptide coined CIGB-210, is thought to target an intracellular protein it is most likely that it must cross the plasmatic membrane of the cell to mediate these effects. In the present work, the capacity of CIGB-210 to penetrate in human cell lines of different origins was evaluated including the MT4 cell line. The cell entry of CIGB-210 in the cell lines was directly proportional to peptide concentration and incubation times. Despite the differences detected in the uptake kinetics, CIGB-210 was capable of transducing the human cell lines of different origins without any help. Finally, a mostly disordered structure in aqueous solution, with an estimated alpha helical content of less than 5% was indicated by circular dichroism spectrometry analysis. All this results together contribute to the characterization of CIGB-210 as a novel drug candidate against HIV/AIDS.

CIGB-300: A peptide-based drug with perspectives for cancer targeted therapy

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CIGB-300 is a first-in-class and hypothesis-driven peptide initially discovered by screening a peptide phage display library using a CK2 phosphoaceptor site. Previous in vivo findings in solid tumor cell lines indicated that CIGB-300 preferentially binds to B23/NPM protein and inhibits its CK2-mediated phosphorylation leading to apoptosis. Interestingly, in blood cancer cells like Chronic Lymphocytic Leukemia with higher sensitivity toward CIGB-300, it prevented the phosphorylation of the direct CK2 target residue Ser129 on Akt/PKB and also downregulated Ser380 PTEN phosphorylation. Accordingly, phosphorylation of PI3K downstream targets Akt/PKB, PKC and GSK-3 decreased in a dose-dependent manner after CIGB-300 treatment. In line with this, CIGB-300 reduced the tumor growth in a CLL animal model. Importantly, CIGB-300 has also showed both antiangiogenic and antimetastasic effect in animal models in separated experimental settings. This peptide is being explored as a therapeutic candidate and different Phase 1 clinical studies have already shown safety, tolerability and pharmacokinetic of CIGB-300 using either local or systemic administration. Recently, in a dose-finding Phase 2 study with 15-70 mg of CIGB-300 concomitant to chemoradiotherapy in cervical cancer, the combining setting exhibited higher antitumor response than chemoradiotherapy alone. These findings provide perspectives for CIGB-300 as potential adjuvant therapy to chemoradiotherapy in cervical cancer and useful clues for the next Phase 3 clinical trial. Collectively, our data outline important new clinical and non-

clinical insights that reinforce CIGB-300 a novel peptide-based candidate with perspectives to treat cancer.

Peptide purification by industrial Centrifugal Partition Chromatography

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Peptides either produced by fermentation or synthesis pose major challenges for production specialists, especially when purification requires an expensive reverse phase chromatographic technique.
Traditional liquid chromatographic (LC) techniques for many peptides require a selected bonded phase (C4, C8, C18) for molecule hydrophobicity, endcapping to eliminate tailing, and an optimised buffer – gradient selection. The gel must also have sufficiently large pores to accommodate the size of the peptide molecules. For these reasons, scaling to preparative levels utilizing traditional LC techniques takes many months and sometimes years.
Centrifugal Partition Chromatography (CPC) is a method of peptide purification which does not utilize any solid stationary phase (such as expensive bonded silica) to achieve high resolution downstream processing. In this technique, both the mobile and stationary phases are liquid solvents, and the liquid stationary phase is immobilized by a centrifuge. This process eliminates many challenges associated with RP peptide purification including pore size, bonding, endcapping, and many more.
Purification of a peptide only requires a liquid-liquid two phase solvent system composed of organic solvents such as water, salts, buffers, and sometimes ionic-liquids or hydrophilic polymers.
During my presentation, I will discuss the application of an industrial scale Centrifugal Partition Chromatography (iCPC) platform for different peptide purification challenges. Specifically, I will discuss isomer separation, where the only difference between the target molecule and the impurity is a missing bond, or different amino acid. A simple solvent system composed of hexane, acetone and water can easily purify lipopeptides and depsipeptides, with a high yield (>80%) and purity (>98%), on a kg scale.
In addition, I will also discuss iCPC solutions for hydrophilic peptides and proteins utilizing Aqueous Two-Phase Solvent systems as an excellent tool for separating kDa molecules.

Lipophilic peptide and vaccine delivery systems

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Peptide Delivery. We have developed a drug-delivery system for the oral administration of drugs and peptides. The method involves combining the peptide or drug with a lipoamino acid (LAA) or a lipopeptide (LP), which acts as a carrier. LAAs combine the properties of amino acids (NH2 and COOH groups) with those of lipids (hydrophobic side chains). Combining a LAA with a peptide or drug provides a means of getting the compound into the body in a stable and biologically active form. The LAAs and their homo-oligomers were covalently conjugated to or incorporated into LHRH or endomorphine-1 with either amide bond or prodrug type linkages. We report biological activity assessments of the conjugates.

Vaccine Delivery. Subunit vaccines that contain the minimal microbial components necessary to stimulate appropriate immune responses have the potential to overcome strong allergic response or autoimmunity that can result from using classical vaccines. We developed new delivery systems by combining the adjuvant and antigenic peptide epitopes derived from group A streptococcal proteins, into one chemically bonded entity and engineered the constructs to form dendritic nanoparticles. The compounds could be administered mucosally to generate protective immune response in mouse models.
We extended our vaccine delivery platform investigations by using lutenizing hormone-releasing hormone (LHRH) as antigen. An anti-LHRH vaccine aims to control the level of sex hormones FSH and LH by generating antibodies against LHRH in murine and ovine models. We have observed significant IgG antibody response after primary immunization without the use of additional adjuvant.

Liposomes containing Cobalt-Porphyrin-Phospholipid (CoPoP) as a vaccine adjuvant for his-tagged MPER peptides

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A number of neutralizing antibodies that are broadly effective across numerous HIV strains target the membrane proximal external region (MPER) of the gp41 envelope protein. MPER is a conserved, short and linear epitope which makes it an attractive vaccination target. Unfortunately, vaccines that induce potent, broadly neutralizing antibodies have not yet been developed. A promising subset of MPER vaccine strategies have emerged that involve presenting the MPER in the context of a lipid membrane. We have discovered that liposomes that include a novel lipid (Cobalt (III) porphyrin phospholipid; CoPoP) can coordinate with his-tag ligands within the bilayer.[1] This lends to stable and rapid antigen decoration of liposomes, following simple aqueous incubation with his-tagged proteins or peptides. In preliminary results, we have shown this facile approach is effective with conventionally-synthesized his-tagged MPER peptides and Co-PoP liposomes embedded with lipid Toll-like receptor (TLR) ligands. A potent anti-MPER titer was induced in mice. In proof-of-principle studies, we showed the IgG produced in vaccinated mice inhibited HIV-1 entry in vitro.

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Multiantigenic peptide-polymer conjugates as therapeutic vaccines against cervical cancer

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Cervical cancer is caused by human papillomavirus (HPV) and remains third only to breast and ovarian cancer in terms of female cancer mortality worldwide. Prophylactic HPV vaccines were commercialized and are clinical effective in preventing HPV infection but do not have a therapeutic effect against established cervical cancer. The development of therapeutic vaccines that eliminate established HPV-associated tumors would therefore be beneficial and desirable.[1] Among the various immunotherapeutic delivery systems, peptide-based vaccines are simple, stable, well tolerated and can be tailored to produce the desired immunogenic effects.[2] However, such vaccines need delivery system and immunostimulant (adjuvant) to trigger desired immune responses. Not only are there a limited number of adjuvants approved for human use, but many of these adjuvants are associated with toxicity and low efficacy. To overcome obstacles associated with the use of classical adjuvants, we have designed new vaccine delivery system based on the polyacrylate polymer conjugated to peptide epitope.[3] The synthesis of peptide epitopes was greatly improved by the change of standard SPPS procedure and application of the isopeptide method under microwave irradiation condition.[4] The new HPV-related epitopes were designed, synthesized, conjugated to polyacrylate polymer, and self-assembled into the microparticles.[5] The lead vaccine candidate from the first series produced desired therapeutic effect against established tumor without help of any external adjuvant. In the second series dendritic and linear polymers were conjugated to the most promising antigen.[6, 7] The tumor-bearing mice treated by leading vaccine candidates showed high survival rate which was

significantly better than antigen co-administered with commercial adjuvant. Moreover, most of mice were tumor free at the end of the experiments. This vaccine delivery system was further optimized and its potency to eliminate existing tumor were tested in mice.[8, 9] Thus, we have developed the first self-adjuvanting delivery system for a therapeutic peptide-based vaccine against cervical cancer.

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Peptide nanostructures for biomedicine and bionanotechnology

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Since the last decade, peptide nanostructures, i.e. peptide fibrils, nanotubes, nanowires, nanoparticles, have been found application for biosensing, catalysis, medical imaging, drug delivery, tissue engineering, stem cell research. The crucial issue is the control of the growth of peptide-based supramolecular structures, both in size and morphology, to get the desired functional structure. In this regard, hierarchical self-assembly, i.e. the ordered aggregation of subunits, presenting at the different levels of organization diverse functionality, seems the most appropriate approach. In this contribution, we will discuss on a molecular basis the driving forces that determine the morphology of aggregates of model peptides in solution and on surfaces. Particular attention will be dedicated to the factors that lead to the formation of proto-fibrils, the toxic elements in many neurodegenerative diseases, and mature fibrils, in competition with the formation of globular structures. We will show that a single-residue substitution can dramatically affect such competition.
The morphology of peptide films, covalently linked on a metal surface, i.e. self-assembled monolayers, or adsorbed on the substrate by Langmuir-Blodgett deposition, has also been characterized by spectroscopy and microscopy techniques with nanometric resolution. The possible application of peptide films for antimicrobial coating will be also described at the Conference.

A new strategy for the synthesis of proteins

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We have been engaged in the chemical synthesis of proteins and glycoproteins by the ligation methods, such as the native chemical ligation [1] and the thioester methods [2], for their biological and structural studies. One of the remaining issues in these methods is the necessity of the purification of the intermediate segments during ligation reactions, which leads to the low recovery yield from the HPLC column as well as the low efficiency of the entire synthetic process. Thus, currently, many researchers are engaged in developing the one-pot ligation method, which can eliminate this problem. We also have been developing novel one-pot three and four segment ligation methods using our thioesterification devices [3].
In this presentation, we will talk about the synthesis of histone H4 [4] and superoxide dismutase using these methods.

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Isolation and identification of biologically active conopeptides combining transcriptomics and proteomics tools

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Several authors have claimed that proteomics or transcriptomics have transformed the identification of active peptides, this in deed is true, however, there is a huge disadvantage regarding these techniques, you are not able to know the activity or the specific target of the *de novo* identification of peptides. By using classical biochemistry, you can identify only one peptide at a time, and by using proteomic or transcriptomic, you can identify thousands at a time. In our lab, we have an eclectic mixture of classical biochemistry and proteomic and transcriptomic in order to identify active peptides knowing at the same time the target and the biological activity of a peptide of interest. In this work, we present an example of three different peptides isolated and characterized using our strategy. With this, we can identify the peptides, and all the posttranslational modifications present in the conotoxins.

Cutaneous secretions of amphibians: source of peptides with important biological activities

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The skin of amphibians constitutes a true chemical arsenal because, they produce in their skin, a high number of molecules among which the antimicrobial peptides stand out. Considering Ecuador has the highest number of frogs per square meter, studies about the bioactivity of cutaneous secretions of anurans in search of peptides with possible biomedical applications were performed. This led to detect that the cutaneous secretion of one species of Hylidae (*Agalychnis spurrelli*) are able to control the growth of multiresistant bacteria, and of five yeast species of the genus *Candida*, as well as lymphoblasts from blood of patients with acute lymphoblastic leukemia and acute myelocytic leukemia. Additionally, hemolytic tests were performed revealing that the concentration in which pathogens and leukemic cells are destroyed (500 g/ml), does not harm human erythrocytes. Once the bioactivity of total cutaneous secretion is detected, the responsible peptides are begining isolated and characterized chemically and molecularly. So far, ten peptides have been identified including one tryptophyllin, five dermaseptins, one phylloseptin, and three orphan peptides. It is important to emphasize that these results were detected from a single species of frog. Therefore, more research is needed in other amphibian species to continue the analysis of peptides secretions in search of new molecules that allow, in the long term, generate alternative drugs.

Peptides as powerful tool in Drugs and Vaccines for Veterinary use

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To date, many technologies have been developed to produce molecules in the era of the Molecular Biology Revolution. Platforms using prokaryotic and eukaryotic cells have been developed through the genetic engineering to produce proteins with different biological functions. However, in the case of small proteins or peptides minor than 40 amino acids, the chemical synthesis has won this niche. In the Center for Genetic Engineering and Biotechnology of Havana, we had isolated, characterized and performed "proofs of concept" with small peptides for their used in animal health. Vaccines, nutraceutical supplements, growth stimulators, immune-stimulators and molecular adjuvants have been tested and developed to commercial products. The results obtained have demonstrated that the chemical synthesis of peptides is a powerful, profitable and exciting system to obtain biomolecules for veterinary use

Synthetic peptides as "magnifying glasses" for designing a vaccine against Equine Infectious Anemia Virus.

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Equine Infectious Anemia is a disease caused by a lentivirus (EIAV), provoking a lifelong persistent infection only in Equidae family, characterized by frequent febrile episodes and intense viral replication, which diminish at the asymptomatic carrier stage, when the immunological mechanisms can reduce viral load.
EIAV has three structural proteins, being capsid p26, the predominant protein and envelopes glycoproteins, gp90 and gp45, involved in the entrance of viron.
At present there are no effective commercially available vaccines against EIAV. Therefore, our main purpose was to design a vaccine against EIAV, using synthetic peptides as “magnifying glasses” to define the candidate immunogens.
First, we selected relevant B and T conserved epitopes within the structural proteins. We employed blood from carrier horses in the libraries of overlapping peptide synthesized on a membrane, in ELISA and lymphoproliferation assays, using synthetic peptides as antigen. We detected five sequences that mimic B and T cell epitopes from conserved regions. The most frequently recognized sequences were peptides A (gp90/45), less peptides B (gp90/45) and the minor peptide was p26 region. Then we analyzed their immunogenicity in mice Balb/c, showing that peptides A gp90/45 produced the most levels of antibody. Indeed, peptide gp90-A induced the production of IFNγ; by contrast, the peptide p26 inhibited it.

Based on our findings, we design a DNA vaccine, packaging within the plasmid vector the *env* gene sequences that code these four peptides gp-90/45 selected. We hope to induce a protective inmmune response, associated with high levels of antibody and the IFNγ production.

Peptide used in the immnulogical control of ticks

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Ticks are ectoparasites that can transmit pathogens, which cause human and animal illnesses. The use of chemicals constitutes the primary measure to control ticks. However, the intensive use of these acaricides produces food contamination, environmental pollution and development of chemical resistant tick strains. Vaccination is considered a promising alternative. However, the progress of anti-tick vaccines has been slow; the limiting step is the identification of new antigens with an important biological function within the arthropod. Recent data provides an interesting foundation demonstrating that the housekeeping-like proteins were immunogenic despite being highly conserved across taxa.
The ribosomal protein P0 is a multifunctional protein. It is an essential structural component of ribosomes and it is also involved in DNA repair and apoptosis. The presence of this protein on the cell surface of parasites, yeasts and mammalian cell lines and more recently in the tick saliva has been demonstrated. However, the development of a vaccine candidate based on this antigen against ticks has as a drawback the high degree of identity between the reported tick P0 sequences respect to the host orthologous proteins. Recent experiments in controlled conditions with a synthetic 20 amino acid peptide of the protein P0 from ticks that is not very conserved, used as immunogen against different tick species have shown efficiencies around 90%. As is known, peptides are generally less immunogenic, requiring more booster immunizations. So, the challenge to convert this peptide into a tick vaccine is to achieve its high immunogenicity using different formulations.

ABSTRACT
ELECTRONIC POSTER SESSION

One-bead-one-compound (OBOC) combinatorial peptides libraries: design, synthesis and application in cancer.

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Combinatorial chemistry holds the promise of minimizing what constitutes the longest stage during the development of a new drug: its initial discovery. There are several advantages to the use of peptides in preparing combinatorial libraries for drug discovery, including their exquisite selectivity when used as drugs, and the fact that solid-phase peptide synthesis is a robust, well-known technology. At CIGB we designed, obtained and used a combinatorial synthetic hexapeptide library "one-bead-one-compound" (Masforrol Y. et al.; ACS Comb Sci., 2012; 14(3):145-9). The library containing sixteen aminoacids (different sequences) was synthesized on a TentaGel resin previously modified with a dipeptide linker (Asp-Pro). This peptide bond is highly susceptible to cleavage under mild acidic conditions in a salt-free solution prepared with $H_2{}^{16}O/H_2{}^{18}O$ (60/40 %v/v). In the hydrolysis hexapeptides are released with an additional Asp residue partially labeled with 18O at the C-terminus. The hydrolysis conditions release enough peptide for characterization by MS and complementary analyses such as stepwise Edman degradation. This OBOC library was employed in different projects, such as: SIDA, CANCER, for the search of bioactive molecules. A few peptides was passed to optimizations steps of sequence by the recognition signals obtained in the biological assays with NCI-H125 cell line in lung cancer researches.

Obtention of functionalized magnetic iron oxide nanoparticles for retains beads of TentaGel S NH_2 resin with a peptide coupled in the presence of a magnetic field

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The TentaGel S NH2 resin has been widely used in the synthesis of "one-bead-one-compound" (OBOC) peptides combinatorial libraries for drug discovery. The polyethylene glycol on the surface of the resin allows the peptides synthesis in organic medium and, also bioassays in aqueous medium. At the CIGB we obtained an OBOC combinatorial synthetic hexapeptides library (Masforrol Y;ACS Comb Sci.,2012;14(3):145-9) with TentaGel S NH2 resin (130µm). The selection of active molecules coupled to the resin for posterior identification of its structure is an important step to finding a new pharmaceutics candidate. The use of magnetic nanotechnology has been a significantly technological advance in chemistry combinatorial. This work describes the obtention of magnetic iron oxide nanoparticles (MNp): synthesis, stabilization, functionalization, physicochemical characterization, and demonstration of its biological applications. MNp were prepared by coprecipitation of Fe2+ and Fe3+ with amonia. Surface coated with APTES was the best stabilization option obtained in the study. Streptavidine-alkaline phosphatase conjugate was used for the functionalization. Two peptide-resin models was synthesized by SFFS on the same resin, with and without biotin molecule coupled to the sequences. By this mean, the potentialities of MNp to retain beads of TentaGel S NH2 resin (130 µm) with a peptide coupled in the presence of a magnetic field after positive recognition bioassay was demonstrated. These MNp appropriately functionalized allows the future innovations in screening strategies for our OBOC library.
This work was partially funded by Fondazione Clinico Humanitas and Italy-Cuba Friendship Association.

Obtaining a combinatorial synthetic peptide library one-bead-one-compound (PROTGEL-10) designed as a support for equalization of protein complex mixtures

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One of the challenges in the proteomic studies is the wide dynamic range that has complex mixtures of proteins that are necessary to characterize. Proteins whose concentrations can range from g/L and ng/L coexist in biological fluids. The synthetic peptide combinatorial libraries can equalize complex protein mixtures by reducing the dynamic range and facilitate detection of protein underrepresented was indicated by previous studies. Is needed synthesis into polymeric matrices very small diameter to achieve a very high combinatorial in a very small volume for to use this methodology in proteomics. In this paper, we designed and synthesized ProtGel-10, a Combinatorial Hexapeptide Library of the type "one-bead-one-compound" with more than 24 million different sequences. To generate the combinatorial were used 17 natural amino acids and the TentaGel resin (10 µm) as the solid phase. The divide-couple-recombine synthesis methodology combined with the stir-centrifuge-decant operations was used to obtain the library. The ProtGel-10 library was obtained in a small volume packaging of 3.5 mL which makes possible handling in proteomics experiments. Its usefulness was demonstrated to detect contaminating proteins in samples of commercial proteins. This approach equalizes the concentration of proteins as well as it simplifies considerably the number of peptides to be analyzed by mass spectrometry rendering a greater chance to identify low abundance species in vaccines and complex mixtures.

Positional Scanning Libraries: Concepts and Applications for Basic Research and Drug Discovery

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The concepts of synthesizing and testing mixture-based synthetic combinatorial libraries containing thousands to millions of compounds for the identification of active drug lead compounds were presented over 25 years ago. This presentation will illustrate how positional scanning libraries have been successfully used for the identification of active novel compounds.

Solid-phase chemical synthesis of monomeric and chimeric peptides from *Trypanosoma cruzi*

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The detection of specific antibodies to *T. cruzi* is of great importance due to the risk of transmission of this parasite by the parenteral route, through blood and its derivatives. The objective of this work was the solid phase chemical synthesis of monomeric and chimeric peptides of immunodominant sequences of *Trypanosoma cruzi*, which causes Chagas' disease and its subsequent application in an immunoassay. Synthetic peptides were synthesized by the solid phase method described by Merrifield in 1963 and their biological activity was tested in a UMELISA assay. Eight synthetic peptides, two monomeric peptides corresponding to *T. cruzi* immunodominant repeating regions, and six chimeric peptides were synthesized. The reactivity of the peptides was evaluated using samples from Colombia and the specificity against healthy donor samples from a blood bank. The specificity in all was 100% and the antigenicity of the chimeric peptides was superior to that of the monomeric peptides. Chimeric synthetic peptides can be used as antigens in assays for the detection of specific antibodies against more than one protein simultaneously from *Trypanosoma cruzi*, in donor blood certification and in epidemiological surveillance.

Synthesis of chimeric peptides from the HIV-1 envelope gp120 protein and its application in the immunodiagnosis

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For the serological diagnosis of HIV infection immunoassays are widely used for the detection of antibodies against the different antigenic viral proteins. Antibodies directed against the envelope protein gp120 of the virus play an important role in the early detection of HIV-1 infection. In this work we present the synthesis of two monomeric synthetic peptides and two chimeric peptides, representative of two immunodominant sequences corresponding to the C-terminal region and the V3 loop of said protein, located in two possible orders, forming a single highly peptidic sequence Antigenic and specific. The antigenicity of the peptides was evaluated in an indirect type UltramicroELISA assay, using positive samples from panels PRB-931 (Seroconversion), PRB-942 (Seroconversion), PRB-917 (M) (Seroconversion), PRB -107 (M) (Under Title) and PRZ-205 from Boston Biomedica Inc. and 24 samples of HIV-1 Cuban seropositives. Specificity was assessed with negative samples from healthy donors. Chimeric peptides (Qm1 and Qm2) showed the highest antigenicity compared to the monomeric peptides. The specificity was 100% for all synthetic peptides evaluated. The results demonstrate the possible utility of these synthetic peptides as antigens in the immunodiagnosis of HIV-1, in blood certification and epidemiological surveillance.

Synthesis of bicyclic peptide by two regioselective disulfide bonds formation

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Bicyclic peptides have received interest as anticancer agents, protease inhibitors, antibiotics, receptor antagonists, artificial receptors and models of various structural motives of proteins. The formation of disulfide bridges is often a crucial final stage in peptide synthesis. There is compelling evidence that the disulfide pattern can be critical in the folding and structural stabilization of many natural peptide and protein sequences, while the artificial introduction of disulfide bridges into natural or designed peptides may often improve biological activities/specificities and stabilities. We report the experimental details of the syntheses in solution of cysteine(Cys)-containing peptide (obtained by Fmoc SPPS) using regioselective synthesis, firs by air oxidation by simple aeration under gentle stirring at pH7.5-8.5 of two Cys with trityl (Trt) as acid-labile protecting group, and then iodine oxidation for the conversion of S-acetamidomethyl (Acm) protection. We used 80% (v/v) acetic acid and iodine in simultaneous removal of the sulfhydryl protecting group and disulfide formation. The excess iodine needs to be quenched or adsorbed as quickly as possible after completion of the disulfide bond formation and ascorbic acid was added. The peptide was purified on a semi preparative HPLC using gradient method. The collected fractions are assayed by HPLC to determine the purities. The purified peptides were analyzed by RP-HPLC and mass determined by mass spectrometer.

Parallel Cyclic Homodimer Formation by Air and Iodine Oxidation of S-Trityl- and S-Acetamidomethyl-cysteine-peptides

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Even though the orthogonality of the thiol-protecting groups and the methods developed for their oxidative deprotection allow for regioselective formation of two and more disulfide bridges, such syntheses require careful planning because of sequential deprotection/ oxidation and purification steps. Taking in account the protection scheme, the power-law dependence on loop length during the early oxidation, reorganizing steps and final oxidation, formation of parallel cyclic homodimers from two disulfide bonds by a combination of two different oxidative procedures, air oxidation of two Cys residues, S-Trityl-cystein-peptide, followed by one regioselective iodine-mediated disulfide formation, S-Acetamidomethyl-cysteine-peptide (ACM) can be predicted and achieved. In this work is shown the strategy for the synthesis and purification of two cyclic homodimers (dimer A and dimer B) which the primary loop length and in the position of ACM in their respectively monomers were different. The purity of both parallel cyclic homodimers was higher than 95 %.

Rational design, synthesis and biological evaluation of analogues of antifungal peptide Cm-p5: importance of Glu and His residues

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Peptides used as drugs is very common nowadays because of their great advantages as prophylaxis and therapy agents. Chemical synthesis is a valuable tool for obtaining analogs of biologically active peptides found in natural sources. This work describes the synthesis of analogs of the antifungal peptide Cm-p5 with the aim with the aim of determining the importance of several amino acid residues. It was necessary a rational design to implement some changes in the structure of Cm-p5 based in the characteristics of known AMPs. Analogs were obtained by solid phase peptide synthesis employing the Fmoc/t-Bu methodology. Compounds were characterized by ESI-MS and analytical RP-HPLC and purification was achieved by preparative RP-HPLC. In this work, we report the synthesis of several analogues of cmp5 which did not show biological activity, evidencing the importance of certain amino acid residues, especially Glu and His residues.

Development of purification methodology of a synthetic peptide by reversed-phase chromatography

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The preparations of the sample to be injected and slopes of elution gradient have an important role in peptides separations by reserved-phase high-performance liquid chromatography (RP-HPLC). The present work describes improvements introduced in RP-HPLC purification process of a synthetic peptide that is an Altered Peptide Ligand (APL) from a CD4+ T-cell epitope of human heat shock protein 60 (HSP60), an autoantigens involved in the pathogenesis of rheumatoid arthritis (RA). Were studied different organic solvent for preparation of the sample and slopes for the acetonitrile gradient used for elution, obtaining the best results when we used 10 % of methanol for the preparation of the sample and a slope of 0,33 in acetonitrile gradient. With that were increase yields in a 20 % compared with the previous methodology.

Purification, characterization and biological evaluation of a peptide mixture of zophalurus junceus venom

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In the current research, we describe the purification and characterization of a complex mixture of proteins and peptides derived from the poison of the Rophalurus junceus, popularly known as blue scorpion, using analytical and cellular cytotoxic method.
To carry out this work, fluid extracts of the venom of the Rophalurus junceus from Labiofam laboratories in the province of Camaguey were used.
The biochemical characterization of the fluid extract was performed through the purification of the crude by a high pressure chromatography (HPLC) system as well as Acrylamide gel electrophoresis. For the cellular cytotoxicity assays, the metabolic damage method (MTT) and neutral red staining were employed.
As result, it was obtained that the fluid extract of Rophalurus junceus contains at least 13 fractions, within which about 50% consist of polypeptides of less than 14 KDa. In vitro evaluation of cytotoxic effect of the fluid extract in the Dunning R3327-G prostaglandin, revealed that the dose of 100 g produced the greatest inhibition of tumor growth, while in the murine myeloma model P3-X63 / AG8 / 653, doses of 1 and 10 g produced the greatest cytotoxic effect. We conclude that the fluid extract of the venom of Rophalurus junceus contains a mixture of polypeptides and proteins responsible for its cytotoxic effect, which support its therapeutic effect on cancer.

Safety, pharmacokinetic evaluation and efficacy signs of intravenous CIGB-300 in patients with solid tumors, refractory to oncospecific treatments

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CIGB-300 is a novel synthetic peptide that induces apoptosis in malignant cells and elicits antitumor activity in cancer animal models. Based on CIGB-300 could represent a candidate drug for cancer therapy, we investigated its tolerability, efficacy and pharmacokinetics in patients with solid tumors, resistant to previous oncotherapy. Material and Methods: An open-label, sequential, dose-scaling Phase I clinical trial was carried out. Doses of 0.2, 0.4, 0.8 or 1.6 mg/kg of CIGB-300 were administered intravenously, once per day, during five consecutive days. This cycle was repeated in alternate weeks to complete 3 cycles. The adverse events were identified in general physical exam and by means of parameters hematological. Pharmacokinetics of plasmatic CIGB-300 was developed in four patients, using a system ELISA. Therapeutic response was mainly defined by the overall survival. Results: Sixteen patients were included, lung cancer prevailed (10 patients), followed by breast cancer (3 patients). The most frequent adverse events were mostly mild (61.6%). Twelve patients remained alive for more than 6 months; one of them, with lung cancer, is currently alive (3 years after treatment).

Pharmacokinetics profile: half-life time about 45 minutes while the value of the distribution volume in stationary state (Vss) was of 9,58 L. Conclusions: We demonstrated the safety and tolerability of CIGB-300 by intravenous infusion. The CIGB-300 exhibits an adequate pharmacokinetic profile which makes it a good candidate to explore further clinical phases of its development. Global survival after CIGB-300 administration tended to be greater that one expected for patients with advance cancer.

The TS-FARMEV-300 Group: Baladron I., Campa I. Melo G., Cruz K, Bermúdez C., López M., Garay H., Reyes O., Nodarse C, Delgado Y., García E. Barcelona S., Delgado M. Brea Y., Herrera A, Lourdes H, Cruz M.

Quantifying therapeutic peptides in human plasma by MS: our experience applied to PK studies in clinical trials

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Mass spectrometry has become in a very powerful analytical tool in discovery and development stages of a drug in the pharmaceutical industry due to its impressive capabilities in terms of structural elucidation, resolution, and mass accuracy. However, a quantitative determination of therapeutic peptides in biological samples still remains as an analytical challenge for mass spectrometry in terms of selectivity and sensitivity due to the huge complexity of biological fluids. The availability of the proper internal standards; the strategies for their selection and synthesis; as well as the choice of a suitable method for detection and quantitation are key elements to obtain successful and reliable results. Peptides are molecules with a wide diversity of physicochemical properties, and the development of methodologies cannot be implemented automatically as generic approaches, they need to be tailored. We will present our experiences in the development and validation of mass spectrometry-based methods for peptide absolute quantitation in human plasma and their applications to pharmacokinetic studies in phase I clinical trials of three novel therapeutic peptide candidates used for the treatment of rheumatoid arthritis (CIGB-814), cardiovascular diseases (CIGB-500) and cancer (CIGB-300). In this work it had been used Signal-Ion-Monitoring in combination with MALDI-TOF (CIGB-300) and QTOF (CIGB-500) mass spectrometers for the absolute quantification of two peptides that have been administered by intravenous

route. Also, we used Single Reaction Monitoring in a triple quadrupole tandem mass spectrometer to determine the PK profile of a peptide administered subcutaneously (CIGB-814).

Two synthetic antimicrobial peptides and their therapeutic potential in topical infections

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The emergence of multidrug-resistant (MDR) microorganisms has become a public health problem exacerbated by the slow development of new antibiotics, and the indiscriminate use of them in livestock. The increase in numbers of antimicrobial-resistant bacteria is ultimately compromising the treatment of various human bacterial infections. For example, the group of immunosuppressed people such diabetic patients often present skin damages, which facilitates bacterial infections mainly caused by strains of *S. aureus* and *P. aeruginosa*. Strains of this two microorganisms are repeatedly classified as MDR. On the other hand, one possibility to content with MDR strains could be the use of antimicrobial peptides (AMP), which present an alternative mode of action to inhibit the growth of bacteria because of their amphipathicity, structural diversity and cationic strength. In this work, we challenge two AMPs, Pin2 [G] and FA1, against two MDR bacteria denominated *S. aureus* UDP13 and *P. aeruginosa* UDP3, which were isolated from diabetic foot-ulcers. The antimicrobial activities of Pin2 [G] and FA1 was tested *in vitro* and *in vivo* experiments. The *in vivo* effects were assayed in infected wound models using New Zealand rabbits. The *in vivo* results corroborate that observed *in vitro* for Pin2 [G] against the two MDR strains; however, FA1 didn't inhibit the growth of any of the strains in the rabbit model. Finally, fluorescence analyses suggest a membrane-level effect of Pin2 [G]. The results allow us to conclude the possible therapeutic use of Pin2 [G] to treat topical infections.

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Panusin and its analogues exposed preferential binding to negative lipids composition

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The -defensins like peptides from invertebrates have been recently reported, revealing a wide spectrum of antimicrobial activity and low cytotoxic effect over red blood cells. The cytotoxic specificity in their mechanism of action over cell membrane structures is central to evaluate the potential of these peptides as lead (o template) in the design of new molecular entities with novel mechanism of action for the treatment of infectious diseases. Panusin is the first member of the new family of -defensins like peptides from the hemocytes of spiny lobster *Panulirus argus*. Analogues variant of panusin were designed and synthesized using the solid-phase peptide synthesis with Fmoc (fluoren-9-ylmethoxycarbonyl)-protected amino acids. The binding capacity to membranes model was evaluated through peptide´s intrinsic fluorescence in presence of different vesicles compositions. Quenching experiments were carried out using the collisional quencher acrylamide in order to compare the accessibility of tyrosine´s to liposomal vesicles mimetizing membrane composition of bacteria or erythrocytes. A comparative analysis of secondary structure was conducted for all analogues by circular dichroism, in order to assess if the inserted variations were able to alter the native arrangement of panusin. The results point out that panusin analogues lacking disulfide bridges kept the ability of binding to negative membranes, indicating that this structural feature is not a determinant for peptide-membrane interaction. Binding experiments also revealed that analogues variants conserved specificity towards bacterial mimetic membranes, with lower affinity for erythrocyte mimetic membranes.

GHRP-6 induces beta-oxidation and mitochondrial biogenesis in healthy rat cardiac tissue

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Growth hormone releasing peptide 6 (GHRP-6) is a six-amino acid synthetic peptide that belongs to the growth hormone secretagogues (GHS) family. Besides its first described GH-releasing activity, mounting evidences substantiate that GHRP-6 is endowed with myriad of pharmacological effects including cytoprotection. GHRP-6 is able to amplify cellular cytoprotective mechanisms during hepatic and cardiac ischemia/reperfusion episodes in which surgically-relevant times were used. The peptide also proved to prevent and reverse heart failure in a model of dilated cardiomyopathy. Of note, GHRP-6 binds to two different receptors (GHS-R1a and CD36), which redundantly or independently exert relevant biological effects. GHRP-6 binding to CD36 activates pro-survival pathways such as PI-3K/AKT, thus reducing cellular death. In all the lethal models involved, GHRP-6 significantly stimulated animals' survival. We have recently identified that GHRP-6 stimulates the transcription of critical myocardial genes involved in myocardial cells survival and energy homeostasis. In a time point kinetic study in healthy rats we showed that GHRP-6 occupation increases the expression of multiple genes involved in fatty acid mobilization toward the mitochondrial oxidative phosphorylation, and many of these up-regulated genes are known targets of peroxisomal proliferator-activated receptor (PPAR)-gamma. Consistent with this, compelling data suggest the existence of a basic phosphorylative and mitochondriogenic mechanism in the heart, via GHRP-6 as an ATP emergency remedial.

Growth Hormone-Releasing Peptide 6 Enhances the Healing Process and Improves the Esthetic Outcome of Wounds

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Despite the multitude of therapeutic strategies to prevent or reduce hypertrophic scarring, this condition remains as orphan clinical niche of ultimately effective interventions. Growth hormone-releasing peptide 6 (GHRP-6) previously proved to reduce liver fibrotic induration. Regarding this, the anti-fibrotic effect on cutaneous wounds was investigated in two animal models: excisional full-thickness lesions in rats and rabbit's ears hypertrophic scarring (HTS). Topical administration of GHRP-6 accelerated wound closure and reduced the number of inflammatory cells in rats and prevented the onset of HTS in rabbits. GHRP-6 modulated the fibrotic response by reducing transcriptional expression of TGFB1 and CTGF and increasing PPARG and MMP13. Based on these pre-clinical results, we recently completed a proof of concept in humans. GHRP-6 gel formulation was administered to patients with personal antecedents of HTS or keloids. Treatment was applied twice (group I, n=11) or once (group II, n=10) a day, during 4 weeks. Safety and efficacy variables were analyzed weakly during the application period and monthly during the following 5 months. Wound healing quality was evaluated according to Vancouver's and Manchester's scales. The treatment was well-tolerated, with local temporary irritation as main adverse effect. At the end of the study, 7/11 (63.6%) and 9/10 (90%) of patients (group I and II, respectively) showed good esthetic quality of the scars, with no signs of HTS. These results allowed us to conclude that topical application of GHRP-6 represents a novel and attractive proposal for the prevention of keloids and HTS.

Comparison between chimeric synthetic peptides and their application in the diagnosis of Chagas disease

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Chagas disease or American Trypanosomiasis is one of the most important endemic problems in South and Central America. Its etiological agent is the flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*). A solution to the problem of the serological diagnosis of Chagas disease is the use of synthetic peptides and recombinant proteins, designed to obtain a diagnostic test that guarantees results with a high level of sensitivity and specificity. In the present work, six chimeric peptides (Q-1, Q-2, Q-3, Q-4, Q-5 and Q-6) were obtained by chemical synthesis in solid phase incorporating antigenic sequences from two repetitive B cell epitopes of *T. cruzi* and two monomeric synthetic peptides P-1 and P-2. The antigenicity of monomeric and chimeric synthetic peptides was evaluated in an indirect UMELISA assay against Chagas positive samples (n = 82) from Bolivia and Brazil, while specificity was evaluated with donor samples from a blood bank (N = 44) and positive samples to other infectious diseases (n = 86). The results showed that the Q-5 chimeric peptide was the most antigenic, detecting all the positive samples evaluated, so it is considered very useful for the immunodiagnosis of Chagas' disease.

Stability Studies of Pyr-GnRHm1-TT Drug Substance Storage at – 20 ± 5 °C during 12 months and at 5 ± 3 °C during 6 months

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Through the life cycle of product the manufactures would demonstrate that the final drug keeps the quality attributes, especially those relate to safety and efficacy. The stability studies are carrying out to accomplish this purpose and will be an important documented knowledge to submit the dossier to the National Regulatory Authority to obtain the Sanitary Register of pharmaceutical product. The Pyr-GnRHm1-TT is a synthetic peptide for therapy against prostate cancer. In this work, is shown the methodology and the results of stability studies of Pyr-GnRHm1-TT drug substance. The quality attributes fulfilled the expected results demonstrating the stability of Pyr-GnRHm1-TT drug substance storage at – 20 ± 5 °C and at 5 ± 3 °C during 12 months and 6 months, respectively.

Stability of HCV synthetic peptides

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Hepatitis caused by hepatitis C virus (HCV) has become one of the main problems of emerging infectious diseases, and is recognized as the main cause of chronic hepatitis, accounting for 70% of them and 20% of hepatitis Acute (1). For the diagnosis, the most used methods are the immunoassays that use like recombinant proteins antigens or synthetic peptides of both the structural and nonstructural region of the virus. By the solid-phase Merrifield method, 1963, synthetic peptides of high specificity were obtained for the core regions and a chimeric region of the NS4 and NS5 regions. The objective of the work was to evaluate the stability over time of these peptides, preserved at 4 ºC for which the same basis of the UMELISA HCV assay was used. Samples were from blood donors, some of them confirmed by PCR and by Hepanostika. Using a newly synthesized peptide as control, a correlation of the fluorescence values with the peptides synthesized 9 years previously was performed. The peptide of the nucleus region maintained its stability at 9 years, with a correlation higher than 0.85. In the case of the chimeric the coefficients of correlations were superior to 0,7; However, it could be verified that an increase in the lyophilization time caused affectations in its behavior. Mixtures of these peptides, per year, showed a correlation higher than 0.75. The results of this work demonstrated that the stability of these peptides, preserved at 4 ° C and in hermetically sealed vials, is 5 years.

Nanostructured peptides at high concentration and physiological conditions-some CIGB case studies

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Peptides have increasingly gained wider attention in drug development as judged by over 80 ongoing clinical trials and significant approvals. These molecules are powerful drugs, but specific formulation issues should be addressed, such as solubility and stability. Peptides are probable self-organization at high concentration (over 20 mg/ml) and in the physiological environment, phosphate buffer saline pH 7.4 and room temperature. In this context, in the current study we focus our attention on the properties of two peptide selfassembly in aqueous solution investigated by cryogenic and conventional transmission electron microscopy. The results revealed that depending on peptide sequence they were nanostructured as globular micelles, or form elongated linear structures which on increasing peptide concentration self-arrange in solution displaying a bi-dimensional hexagonal array.

Formulation development of a lyophilized peptide (Heberprovac) for the treatment of prostate cancer

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Heberprovac is a therapeutic vaccine for the treatment of advanced prostate cancer. The active pharmaceutical ingredient is a peptide obtained by chemical synthesis, designed by bioinformatics, and analogue of Gonadotropin releasing hormone (GnRH). Antibodies generated against vaccines based on synthetic GnRH peptides neutralize endogenous GnRH, hence their use in the immunotherapy of prostate cancer. The formulation development was carried out according the intrinsic properties of the peptide. The solubility was the main challenge for the formulation. There are several factors that can affect the solubility of peptides, which include pH, ionic strength, peptide concentration, excipients, and temperature. The peptide was not solubilized in phosphate buffer or citrate at pH between 5.8 and 7.2. The acetate, glycine, tartrate and succinate buffers, all at pH 4 and 5, were evaluated. The highest solubility was obtained in glycine-HCl buffer, pH 4. It was determined the molarity of glycine for its buffering effect in the formulation, before and after of lyophilization process. The glycine 200 mol/L guarantees the pH within the desired limits, at acidic pH. On the other hand, it is greater physical stability of the peptide, reaching complete solubility. During the lyophilization process and storage, the physical and chemical integrity of the peptide is not affected. Stress studies showed that the purity of the peptide, determined by chromatography (RP-HPLC), is greater than 90% when stored for 77 days at 45°C. Glycine also imparted good mechanical properties to the lyophilized cake. An elegant cake was obtained without collapse in its structure during storage.

Amino Acid Analysis for the determinations of: extinction coefficients, peptide content and amino acid composition

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Several techniques are used frequently to determine protein/peptide's content or solution concentration´s; like: amino acids analysis (AAA), total nitrogen determination by Kjeldahl, dry weight method, Edelhoch method and colorimetric methods such as Bradford techniques or BCA. From all of them, the amino acid analysis is the most accurate; another advantage of this method is its independence from the abundance of aromatic residues in the sample analyzed and it´s precision at low concentrations.
The AAA procedure includes hydrolysis, derivatization, separation, detection and quantification. Hydrolysis is typically achieved under acid conditions (HCl 6M; 24 hours, 110°C). Fragile amino acids, especially tryptophan and cysteine, will be partially destroyed. Then, hydrolyzed samples (amino acids) are derivatized for sensitive detection and separated by HPLC/GC. The use of internal and external standards of known amount is crucial for accurate quantification of each amino acid.
The EZ:faast amino acid analysis procedure (Phenomenex, US) consists of a solid phase extraction step followed by derivatization and liquid/liquid extraction; derivatized samples are quickly analyzed by gas chromatography (GC) with FID detection.
The quantitative analysis of the data allows calculating the relative amino acid composition of the peptide sample, the peptide content, the concentration and also the extinction coefficient even at 226nm if the samples do not contain aromatic amino acids. Some examples of different applications to synthetic peptides like: CIGB-228, CIGB-210, NPs-PLGA-PEG-CIGB 55 will be provided.

Enzymatic activity regulation of peptides derived from the *Leishmania braziliensis* NMNAT N-terminal region

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Nicotinamide mononucleotide adenylyltransferases (NMNATs) are enzymes that catalyzing the reaction of nicotinamide mononucleotide (NMN) or nicotinic acid mononucleotide (NaMN) and ATP to produce NAD or NaAD. NAD is a leading cofactor for many of the crucial enzymes in the energetic metabolism of organisms. The region N-terminal of 43 residues of this enzyme is present exclusively in trypanosomatids, and is absent in the NMNAT human isoforms. Because of this, this segment was chosen as a template to design two peptides corresponding to regions 39-43 (pep5) and 26-43 (pep18), to compare their action on the enzyme activity, due to their possible non-covalent association. In assessing its effect, it was found that pep18 is able to inhibit enzyme activity, and the other hand the short peptide increases the enzyme activity against the positive control. This behavior could be associated with conformational changes of the protein when interacting with itself to form oligomers or with other regulatory proteins. These results open the possibility of using peptides derived from this protein as inhibitors, as well as to explore into the molecular mechanisms that regulate their action.

Bioinformatic identification of crocodylians β-defensins

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Antimicrobial resistance against all different classes of antibiotics used for treatment of several pathologies is a major world health concern. Antimicrobial peptides have emerged as an attractive alternative for development of novel therapeutics. These molecules generally interact with highly conserved microbial targets; therefore, the development of resistance is less likely compared to conventional antibiotics. **β**-defensins are naturally occurring host defense peptides which play an important role as components of the innate immune defense against infection. In addition to its potent microbicidal properties, defensins may act as immune modulators, chemoattractants, and also are involved in other physiological processes in the organisms. All these properties make **β**-defensins extremely attractive candidates for novel therapeutics. Crocodylians are evolutionarily ancient animals who are territorial and live in microbially challenging environments; so, they appear to have a robust immune system. In this work we identified putative **β**-defensins sequences from four species of crocodylians using bioinformatic approaches. Our results suggest that these molecules are widely represented in crocodylians with several paralogs and orthologs

among the studied species, as well as with sequences of birds and reptiles defensins. To assess and confirm the biological activity of the putative crocodylian **β**-defensins, we chemically synthesized and evaluate the antimicrobial activity of some representative variants against medically important pathogens.

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Photosubstitution of Monodentate Ligands from Ru(II)-dicarboxybipyridine complexes

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In this work, we report the photophysical and photochemical properties of Ru(II) polypyridine complexes $[Ru(bpy)(dcbpy)py_2)]^{2+}$ $(\mathbf{1})^{2+}$ and $[Ru(dcbpy)_2py_2)]^{2+}$ $(\mathbf{2})^{2+}$ (bpy= 2,2'-bipyridine, dcbpy= 4,4'-dicarboxy-2,2'-bipyridine, py= pyridine). These complexes combine a monodentate ligand with a chelate bipyridine substituted with carboxylate groups. At low pH both complexes present metal-to-ligand charge transfer (MLCT) absorption bands in the visible region and room temperature photoluminescence (PL) with long excited state lifetimes (> 200 ns). At physiological pH their absorption and emission maxima are displaced to higher energies with a significant reduction of their emission lifetime. These species show photosubstitution of the monodentate pyridine upon irradiation at 450 nm. At low pH the quantum yield for this process is very low, but at physiological pH they are very active, with a $_{PS,450}$ of 0.14 for $(\mathbf{1})^{2+}$ and 0.17 for $(\mathbf{2})^{2+}$. The products of photosubstitution were identified as the monoaquo complexes. Both, the reactants and the products of the photosubstitution show photoluminescence, but with very different lifetimes making it possible to follow the reaction by the time constant of their decay. The ability of complexes $(\mathbf{1})^{2+}$ and $(\mathbf{2})^{2+}$ to photorelease monodentate ligands at physiological pH makes them attractive candidates for the delivery of biomolecules linked to more complex structures through the carboxylate functional group. New perspectives of applications are also presented in this work.

Peptides improve cellular penetration of gold nanorods for biomedical applications

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Gold Nanorods (GNRs) are interesting nanomaterials due for their use in biomedical applications such as drug delivery systems, imaging devices and/or therapeutic agents. However, for this nanosystem to have all these possible applications, it is necessary to improve the ability to penetrate cellular barriers. To accomplish the latter, we used peptides with biological activities improving their stability, biocompatibility and uptake into the bEnd.3 cell line. We used Angiopep 2 and ApoE peptides developed as a shuttle to different cargoes to the central nervous system (CNS) and the peptide D1 used as a beta sheet breaker for Alzheimer disease. We evaluated the ability of these peptides to improving the uptake of GNRs in the brain endothelial cell line bEnd.3. These nanosystems were characterized by dinamic light scattering, zeta potential, electron microscopy and Uv-Vis-NIR spectroscopy. The cytotoxicity was evaluated by using MTS assay and diffusion capacity was evaluated by PAMPA assay. Flow cytometry evaluated cell uptake. We observed that these nanosystems were not cytotoxic at the concentrations and period that they were used. The diffusion capacity of these nanosystems was low when compared to smaller molecules. Finally, we observed that the cellular uptake of the GNRs change depending on the peptide that is used to modify the surface of GNRs, indicating that the uptake is drived by the GNR's surface modification with the different peptides used.

Structural determinants for antimicrobial activity of panusin

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The -defensins are amongst the most studied and characterized of host defence peptides. Initially presumed to exist in vertebrates only, a family of -defensin like peptides was recently reported in the spiny lobster *Panulirus argus*, being panusin its representative member. Panusin is a cationic antimicrobial peptide with wide and unspecific antibacterial and antifungal activity, with a predicted structural pattern and arrangement of disulfide bridges well conserved among other vertebrate -defensins. Also, all panusin-like peptides show a distinctive abundance of tyrosine residues. To assess the importance of these structural characteristics, we have designed and synthetized analogue variants of panusin lacking the disulfide bridge formation and/or containing alanine mutations in one or several tyrosine residues. Antimicrobial activity of these variants was evaluated against Gram + and Gram – bacteria, showing a significant reduction of effect for the linear analogues. The tyrosine mutants show a dramatic descent of activity for the variants with less abundance of this particular aminoacid. Summarizing, we can conclude that disulfide bridge formation is relevant but not determinant for antimicrobial activity of panusin; on the other hand, the conserved tyrosine residues in the peptidic sequence seems to be central for the microbicidal effect of this -defensin like peptide.

Fish PACAP: its role in teleost immunity and potential applications in Aquaculture

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Recent findings added PACAP and its receptors to the growing list of mediators that allow cross-talk between the nervous, endocrine and immune systems in fish. Our studies demonstrated that PACAP has an important role not only as a growth promoter factor but also in fish immunity. These findings are supported by the different expression patterns found as a result of viral and bacterial septicaemic infections in two teleost fish models, its direct antimicrobial actions against several fish pathogens and its modulator activities on innate and acquire immunity. All the experiments were conducted with the synthetic peptide corroborating the relevance of chemical synthesis as an invaluable tool to elucidate the biological function of small molecules. Taking into account the overall evidences obtained, PACAP could be a promising molecule to develop a commercial product for Aquaculture.

Comparative analysis reveals amino acids critical for anticancer activity of peptide CIGB-552

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Because of resistance development by cancer cells against current anticancer drugs, there is a considerable interest in developing novel antitumor agents. We have previously demonstrated that CIGB-552, a novel cell-penetrating synthetic peptide, was effective in reducing tumor size and increasing lifespan in tumor-bearing mice. Studies of protein–peptide interactions have shown that COMMD1 protein is a major mediator of CIGB-552 antitumor activity.
Objectives: Structure- Activity relation (SAR). In this study, we compare the physicochemical properties, pro-apoptotic effects, COMMD1 and lipid binding capacities of CIGB-552 and its main derived metabolites.
Methods: We made a comparative analysis between CIGB-552 and its main metabolites regarding physicochemical properties, cellular internalization, and their capability to elicit apoptosis in MCF-7 cells. Molecular dynamic simulations were performed in aqueous solution and in the presence of a phospholipid's bilayer using a random conformation for each peptide. All simulations were performed at the coarse-grained level, and nearly atomistic detail was recovered using SIRAH tools.
Results: In the present study, we show the results obtained from a comparative analysis between CIGB-552 and its main metabolites. None of the analyzed metabolites proved to be as effective as CIGB-

552 in promoting apoptosis in MCF-7. We also examine their cell-penetrating capacity and interaction with COMMD1. We show that internalization, a lipid binding-dependent process, is impaired as well as metabolite–COMMD1 interaction, key component of the apoptotic mechanism. Molecular dynamics simulations performed here suggest that a looped conformation conserved in the two longest peptides offers a putative explanation for the similar characteristics displayed in contrast with shorter metabolites in terms of enhanced membrane interaction, internalization, and cytotoxic activity.
Conclusion: Our results suggest that features conferred by the amino acid sequence are decisive for CIGB-552 biological activity, turning it into the minimal functional unit.

Enhancement of the inhibitory effect of an IL-15 antagonist peptide by alanine scanning and D-amino acids substitutions

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IL-15 has been proposed as a therapeutic target in Rheumatoid Arthritis (RA). Previously, we reported a peptide named P8, which specifically binds to IL-15R and inhibits IL-15 biological activity in CTLL-2 cells with an IC50 of 130 M. In order to improve binding of peptide P8 to the receptor, we used an Ala scan strategy to study the contribution of each amino acid to the peptide's antagonist effect. To evaluate the effects of the peptides, CTLL-2 cells were incubated with serial dilutions of peptides plus 300pg/mL of IL-15 by 72h. Proliferation was measured by MTT mitochondrial staining. Additionally, cells from patients with RA were incubated with 50µg/mL of peptide and TNF- concentration was determined by ELISA. To improve the stability of the P8 peptide, we substituted each amino acid by D-aa. The binding to IL-15R alpha was evaluated by ELISA and the biological activity was determined using the CTLL-2 cell proliferation assays. We found that F and C are important for peptide binding to IL-15R . We also investigated other single site mutations and substituted the K by T. The resulting peptide exhibited a higher activity than P8 with an IC50 of 24 M. We also found that this peptide was more active than peptide P8 in the inhibition of TNF secretion by synovial cells from RA patients. The D-aa substitutions study

allowed us to identify a peptide more active than the original peptide. The peptide described here is a new type of IL-15 antagonist peptide with potential applications in AR.

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