Leptin plays an important role in the regulation of a variety of physiological functions, including food intake, body temperature and body weight maintenance. Tertiary structure of the leptin molecule reveals the existence of a four-helix bundle that is characteristic of the short-helix cytokines. In order to identify regions of the leptin molecule responsible for its bioactivity, a new series of decapeptides encompassing the region of fragments 98–122 [1] were synthesized and their effects on body weight and food intake were assessed when administered into the lateral cerbroventricle of normal rats. Peptides were synthesized by SPPS, purified by RP-HPLC and characterized by LC/ESI-MS. We also performed a conformational study of the peptides by circular dichroism in order to correlate the biological activity and secondary structure of the leptin fragments. Among the fragments tested, we found that Ac-hLEP110-119-NH2 was able to induce a significantly reduction in both body weight (>10%) and food intake (>39%). Interestingly, with fragment Ac-hLEP113-122-NH2 we observed a significative increased in the food consumption (>25%) but without any change in the body weight in comparison to the control. The use of synthetic leptin-derivate fragments may offer the basis for the development of compounds with potential application in human obesity or to its related metabolic dysfunctions. *Supported by FAPESP, CNPq, CAPES and UNIFESP/FADA.*

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P 214 A TETRAMERIC PEPTIDE-DRUG CONJUGATE TARGETING THE INTEGRIN $\alpha_V\beta_6$-POSITIVE NON-SMALL CELL LUNG CANCER

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Most chemotherapeutics exert their effects on tumor cells as well as their healthy counterparts, resulting in dose limiting side effects. The selective delivery of therapeutic agents to receptors over-expressed in cancer cells without harming the rest of the body is a major challenge in clinical oncology today. We have previously identified a peptide, named H2009.1 (sequence: RGDLATLRQL), from a phage displayed peptide library which binds to a large number of human lung adenocarcinoma cell lines. The cellular receptor for this peptide has been identified as the integrin $\alpha_v\beta_6$. Paclitaxel (Taxol) is a potent antitumor agent commonly used in the treatment of ovarian, breast and lung cancers, yet patients have to suffer some side effects caused by its normal tissue toxicity and aqueous insolubility. Here we report the design and synthesis of Taxol conjugated with H2009.1 tetrameric peptide via an ester linkage. The appropriate control-scrambled peptide conjugate has been synthesized as well. Characterization of tetrameric peptides and their conjugates has been determined by NMR, RP-HPLC and MALDI-MASS. H2009.1 tetrameric peptide-Taxol conjugate is more cytotoxic towards H2009 cells than scrambled peptide conjugate indicating that cellular uptake is mediated by H2009.1 peptide. Importantly, H2009.1 tetrameric peptide-Taxol conjugate is more cytotoxic towards a targeted cell than a cell line that does not express $\alpha_v\beta_6$ integrin. Finally, the peptide conjugate is highly water soluble which is a great advantage considering the severe hypersensitivity reactions experienced by patients treated with free Taxol in a Cremophore/ethanol emulsion.

P 215 USE OF PEPTOID-PEPTIDE HYBRIDS IN THE DEVELOPMENT OF Shc SH2 DOMAIN –BINDING INHIBITORS

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Src Homology 2 (SH2) domains participate in oncogenic cell signaling by recognizing and binding to specific phophorylated tyrosine sequences. Recently, using solid-phase derived peptides containing fluorescein isothiocyanate (FITC), we developed a fluorescence anisotropy (FA) competition - based binding assay for the Shc SH2 domain. We then employed this assay to examine several open - chain bis-alkenylamide containing peptides that were originally prepared as precursors for the planned synthesis of ringclosing metathesis (RCM) derived macrocycles. Unexpectedly, high binding affinities were observed for some of these open-chain peptides in which the original Gly was replaced with *N*a-substituted Gly (NSG) "peptoid" residues. Certain of these "peptoid-peptide hybrids" of the form, Ac-pY-Q-[NSG]-L-amide showed up to 40-fold higher Shc SH2 domain-binding affinity than the parent Glycontaining peptide. This presents the first application of peptoidpeptide hybrids to the design of SH2 domain-binding antagonists. Work is currently in progress to examine structural modifications that will lead to further enhancement of binding affinity.

P 216 THE USE OF PARAMAGNETIC AND FLUORESCENT-QUENCHING AMINO ACID TOAC FOR EVALUATING OF ANGIOTENSIN I- CONVERTING ACTIVITY

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Advantageously to other fluorescent quenching probes, the TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid), introduced earlier by us in chemistry [*J. Am. Chem. Soc.* (1993) 115, 11042] can be inserted at any position of an enzyme substrate. This work examined the specificity of angiotensin I-converting enzyme (ACE) that cleaves the angiotensin I (AI, DRVYIHPFHL) to produce the angiotensin II and inactivates bradykinin (BK, RPPGFSPFR). TOAC-attaching AI analogues at positions 0, 1, 3, 5, 8, 9 and 10 indicated that the first four analogues are substrates for ACE with k_{cat}/k_m values of 11.9, 9.2, 3.2 and 2.0 $\mu M^{-1}.min^{-1}$, respectively, in comparison with 15.4 $\mu M^{-1}.min^{-1}$ of the native AI. These results confirm that greater the proximity of the unnatural probe to the cleavage site (8-9), the smaller is the substrate specificity of analogues. Greater decrease in the substrate activity occurred with BK, where $TOAC^0$-BK and $TOAC^3$-BK presented k_{cat}/k_m of 20.9 and 38.9 $\mu M^{-1}.min^{-1}$, respectively (against 202 $\mu M^{-1}.min^{-1}$ for BK). Other analogues were devoid of substrate activity. EPR spectra indicated greater mobility for those analogues that were ACE substrates. A clear quenching property of TOAC affecting the Tyr^4 and $Phe^{5;\,8}$ residues in AI and BK, respectively was detected. The fluorescence of labeled substrates decreased with increasing distance between both residues, thus suggesting them extended structures. Differences between EPR spectra of TOAC-containing AI and BK substrates and cleavage products allowed the monitoring of ACE enzymatic activity.

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P 217 DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEW UROTENSIN II-RELATED PEPTIDES (URP)

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Urotensin-II (UII), a disulfide bridged peptide, is currently considered as one of the most potent mammalian vasoconstrictor identified. Recently, a UII paralogue, named urotensin II-related peptide (URP), was discovered and it was suggested that URP rather than UII may be the biologically active peptide in the regulation of autonomic, cardiovascular and reproductive functions. Both peptides exert their action through the activation of a specific membrane-bound UT receptor. The multiple effects of U-II and the broad expression pattern of its receptor indicate that U-II may be involved in physiopathological processes. In this study, we have designed and synthesized new URP analogs in which Trp-4 was replaced with natural, unnatural, constrained and charged amino acids in order to determine important physicochemical features for receptor binding and activation. Using different pharmacological models, we assessed the impact of these modifications on binding affinity and Ca^{2+} mobilization using UT-transfected cells, as well as in a contraction of aortic ring assay. Preliminary binding results demonstrated that analogs bearing an aliphatic side chain ([Cha^4]URP) or a turn inducing residue ([Tiq^4]URP) retained good binding affinity whereas other modification (Deg, Tic or Sar) led to inactive analogs. These data will give us new insights regarding the biological conformation of URP and will be used for the rational design of drug candidates potentially useful for the treatment of cardiovascular, endocrine and/or neurodegenerative disorders.