

PRÊMIO JOSÉ RIBEIRO DO VALLE 2012

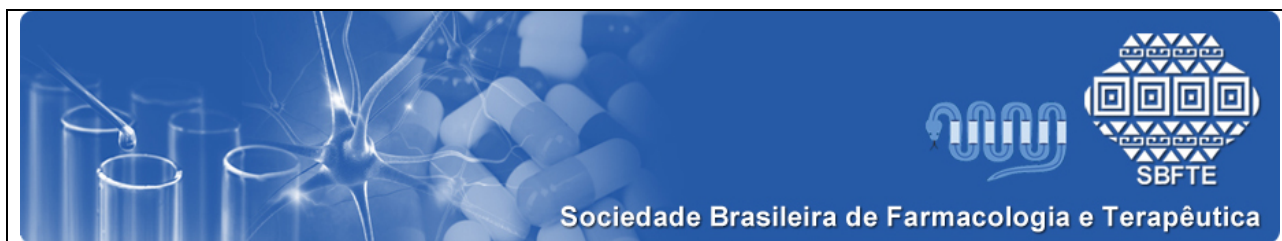
O prêmio José Ribeiro do Valle, oferecido a cada ano pela SBFTE, visa identificar a cada ano os melhores trabalhos científicos desenvolvidos por jovens investigadores na área da Farmacologia. Entre os trabalhos inscritos para esta décima-quinta edição do prêmio, foram selecionados cinco finalistas, que fizeram apresentações de seus respectivos trabalhos perante comissão julgadora, em sessão pública durante o 44^o Congresso Brasileiro de Farmacologia e Terapêutica Experimental, em Foz do Iguaçu, PR. O resultado foi o seguinte:

Primeiro prêmio

Maira Assunção Bicca

02.004 The role of kinin B₂ receptor on amyloid- β - induced neuroinflammation in vivo: Evidence for the modulation of PKC and MAPK pathways. Bicca MA, Loch-Neckel G, Figueiredo CP, Costa R, Calixto JB UFSC – Farmacologia

Introduction: Recent evidence suggests that the (amiloid- β) A β peptide, particularly soluble oligomers, has a central role in Alzheimer's disease (AD) (FERREIRA S.T., *Neurobiol Learn Mem*, 96, 4:529, 2011) and early studies have reported the involvement of the kallikrein-kinin system in the pathophysiology of AD in both humans and experimental models (VIEL T.A., *Curr Alzheimer Res*, 8, 1:59, 2011). The aim of this study was to investigate the mechanisms underlying the role of the kinin B₂ receptor in the A β -induced neuroinflammation in mice. **Methods:** Experimental procedures were carried out using male Swiss mice treated with the selective kinin B₂ receptor antagonist, HOE 140 (50 pmol/site, i.c.v.), given 2 h prior, or 24 h after the i.c.v. injection of A β ₁₋₄₀ peptide (400 pmol/site), mainly soluble oligomers, previously identified by both HPLC and microscopy electron transmission analyses. After 14 days, the animals were subjected to behavioral tests and on day 15 their brains were removed to perform Western blot analysis. The same treatment protocol was used to carry out tissue collections, at specific time points (6h, 24h or 8 days) for immunohistochemical analysis. These time point were chosen based on the maximum expression level of each protein. All procedures were approved by the ethical committee for the use of animals from UFSC (protocol number: PP00625). **Results:** The pretreatment (2 h prior) of animals with HOE 140 prevented the cognitive impairment induced by A β ₁₋₄₀ peptide when assessed using object recognition task. Conversely the posttreatment schedule (24h after A β ₁₋₄₀ peptide) did not result in any significant effect. The administration of HOE 140 prior A β ₁₋₄₀ peptide prevented A β -induced synaptic damage as assessed by the evaluation of synaptic proteins expression (synaptophysin and PSD-95). Furthermore, i.c.v injection of A β ₁₋₄₀ peptide increased B₂ receptor expression in the mice hippocampi after 24 h and 15 days, an action that was prevented by HOE 140. Also, the A β ₁₋₄₀-induced inflammatory process was evidenced by increased of both microglial activation (1 day) and the increased expression of COX-2 (1 day), iNOS and nNOS (both 8 days) in the mice hippocampi after A β ₁₋₄₀ administration as evaluated by immunohistochemistry (at specified time-points) and western blot (15 days) after A β ₁₋₄₀ injection. All these alterations were prevented by the pretreatment of animals with HOE 140. The neuroinflammatory effect caused by A β ₁₋₄₀ seems to be mediated by activation of protein kinase C (PKC) (δ and ϵ isoforms), and by Mitogen Activated Protein Kinase (MAPKs), namely p-38/MAPK, JNK and by transcription factors NF κ B and c-Jun as it injection of A β ₁₋₄₀ caused significant increase in the expression/activation of this pathways. Importantly, these events seem to be modulated by activation of B₂ receptor, since blocking this receptor with HOE 140 prevented all these changes. **Discussion:** Taken together, the present data provided consistent evidence that the A β -induced neuroinflammation is greatly



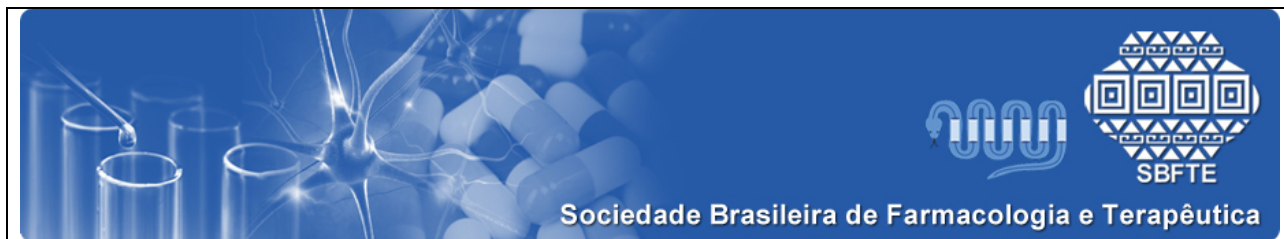
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mediated by activation of kinin B₂ receptor and the signaling pathways activated by it, such as PKC, MAPKs and their effectors. Research support: **Financial support** was provided by FAPESC, CNPq and CAPES.

Segundo prêmio

04.053 Role of CCR2 in neutrophil articular infiltration in arthritis. Talbot J¹, Bianchini FJ¹, Souto FOS², Nascimento DCB¹, Pinto LG¹, Peres RS², Oliveira RD³, Almeida SL³, Silva JR², Ferreira SH¹, Louzada-Junior P³, Cunha TM¹, Cunha FQ¹, Alves-Filho JC¹ ¹FMRP-USP – Farmacologia, ²FMRP-USP – Imunologia, ³HC-FMRP-USP – Clínica Médica

Background: Rheumatoid Arthritis (RA) is an autoimmune arthropathy characterized by joints pain, synovial hyperplasia and progressive damage of cartilage and bone joint. These features of arthritis have been associated with neutrophil infiltration on articular cavity. Blood neutrophils (PMN) trafficking during inflammation is a complex process which several types of chemotactic factors which may include lipids, complement activation products and especially CXC chemokines. In general, CXC chemokines, MIP-2 and KC, appear to be involved in mediating PMN influx into tissues, while CC chemokines (CCLs) interact predominantly with monocytes and lymphocytes. However, our group demonstrated that blood neutrophils from mice and patients with sepsis express de novo the CC chemokine receptor type 2 (CCR2). Even, it was demonstrated that CCR2 expression has an essential role in neutrophil tissue infiltration and multiple organ dysfunction in sepsis. Previously, we found high levels of CCR2 ligand (CCL2) in synovial fluid of Rheumatoid Arthritis Patients. Here, we investigate the expression of CCR2 on neutrophils from Rheumatoid Arthritis Patients and the role of CCR2 expression in neutrophils in a mouse model of experimental arthritis. **Methods:** Neutrophils from healthy or Rheumatoid Arthritis Patients were purified and used to chemotaxis assay and PCR evaluation of CCR expression. The same was done for neutrophils from experimental arthritis. WT and CCR2 deficient mice (CCR2^{-/-}) were sensitized (s.c.) with 500 ug of mBSA in a emulsion containing complete Freund's adjuvant. Booster injections of mBSA in incomplete Freund's adjuvant were given 7 and 14 days after the first injection. Arthritis was induced in the immunized mice 21 days after the initial injection by intra-articular (i.a.) injection of mBSA (10 µg/cavity) or MCP-1 (50ng/cavity). Neutrophil migration was assessed 6hs after i.a. challenges. Articular hypernociception was evaluated at this same time using an electronic version of the von Frey test. Cytokine determination in i.a lavage was realized by ELISA. In some experiments, mice were treated with CCR2 antagonist (30 mg/kg, RS504393) i.v. twice, 24 h and 1 h before the challenges. *In vitro* neutrophil chemotaxis in response to MIP-2 (30ng/ml) or MCP-1 (1ng/ml) was performed using a Boyden microchamber. CCR2 expression in neutrophils isolated from blood was determined by PCR-Real Time (m RNA) and immunofluorescence. All experiments were approved by HCFMRP/USP Human Ethics Committee (2981/2009) and Animal Ethics Committee from FMRP/USP (nº 181/2008). **Results:** Results show that human and murine neutrophils isolated from blood of arthritic mice express CCR2 and respond *In vitro* to CCL2 chemokine. Even more, neutrophils from bone marrow of arthritic mice do express a functional CCR2. Furthermore, CCL2 was able to induce neutrophil intra-articular infiltration in immunized mice. Neutrophil recruitment to joints and articular hyperalgesia induced by antigen challenge (mBSA) was significantly reduced in CCR2^{-/-} mice or by treatment with CCR2 antagonist. To ensure that CCR2 were directly responsible of neutrophil infiltration to joint cavity in arthritis we performed the adoptive



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transference of WT neutrophils from immunized mice to CCR2^{-/-} immunized mice. This transference restored arthritis phenotype (neutrophil infiltration and articular hypernociception) to CCR2^{-/-} mice after mBSA challenge. **Conclusion:** Our findings suggest that CCL2-CCR2 chemotaxis pathway is involved directly in the detrimental infiltration of neutrophils to the joints in experimental arthritis. Therefore, we envisage that CCR2 blockage is a potential strategy for human rheumatoid arthritis treatment.

Menção Honrosa

Juliana Akinaga

01.004 Agonist driven α 1A-adrenoceptor phosphorylation, desensitization and internalization: Differential recruitment of PKC α and GRK2. Akinaga J¹, Alcántara-Hernández R², García-Sáinz JA², Pupo AS¹ ¹Unesp-Botucatu – Farmacologia, ²UNAM – Fisiologia Celular

Karin Scheschowitsch

04.028 Nitric oxide and peroxynitrite as signaling agents for NOS-2 expression in vascular smooth muscle cells. Scheschowitsch K¹, Sordi R¹, Moraes JA², Barja-Fidalgo TC², Assreuy J¹ ¹UFSC – Pharmacology, ²UERJ – Pharmacology

Isabel D. Machado

01.029 Participation of cytosolic glucocorticoid receptor and Annexin-A1 on neutrophil traffic from bone marrow into blood: Adhesion molecule expression and SDF-1 α /CXCR4 axis. Machado ID¹, Santin JR¹, Ferraz-de-Paula V¹, Perretti M², Farsky SHP¹ ¹USP – Pharmaceutics Science, ²William Harvey Institute – Immunopharmacology

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