

## P2X7 purinergic receptor and its role in inflammatory and nociceptive alterations associated to cyclophosphamide-induced hemorrhagic cystitis in mice

<sup>1</sup><u>Rodrigo Braccini M. da Silva</u>, <sup>2</sup>Jerônimo Pietrobon Martins, <sup>4,5</sup>Maria Martha Campos, <sup>1,3</sup>Fernanda Bueno Morrone

Faculdade de <sup>1</sup>Farmácia and <sup>2</sup>Medicina; <sup>3</sup>Programa de Pós-Graduação em Biologia Celular e Molecular; <sup>4</sup>Instituto de Toxicologia; <sup>5</sup>Faculdade de Odontologia, PUCRS, Porto Alegre, RS.

## Resumo

**Introduction**: Extracellular nucleotides are important signaling molecules that mediate many biological effects, through purinergic receptors (Ralevic et al., 1998). ATP is generated in response to cellular damage, and P2X7 receptors have an essential role in pathological changes (Chesselli et al., 2005). This study evaluated the role of P2X7 receptors in the mouse model of hemorrhagic cystitis induced by CYP. Methods: Male Swiss, C57/BL6 and P2X7 receptor knockout (KO) mice (n= 5; 25-30 g) were used. HC was induced by a single administration of CYP (300 mg/kg, i.p.). The spontaneous behavior was scored for 2 min, every 30 min, in a total period of 4 h, according with Olivar & Laird (1999). We have also performed the gross examination of bladders at 6 h, to determine the edema and hemorrhage. A438079 (100 and 200 µmol/kg, i.p.) or Mesna (60 mg/kg) was administered in two doses; the first one was given 30 min prior CYP, and the following dose was administered 4 h after CYP. Control animals received saline solution. The relevance of P2X7 receptors was further assessed by using animals with genic deletion for this receptor, and C57/BL6 mice were used as the control group. Bladder myeloperoxidase (6 h) and IL-1 $\beta$  and TNF- $\alpha$  (4 h) levels were measured after CYP administration. Statistical analysis was performed using analysis of variance (ANOVA) followed by Bonferroni's test. P < 0.05 was considered as indicative of significance. The experimental procedures were approved by the Local Ethics Committee (08/00074). Results: The systemic administration of A438079 (100 and 200 µmol/kg, i.p.) produced a significant inhibition of the nociceptive behaviour evoked by CYP ( $43 \pm 1\%$  and  $45 \pm 9$  %, respectively). Additionally, P2X7 receptor KO mice displayed an inhibition of  $17 \pm$  3% of this response. Interestingly, A438079 (100  $\mu$ mol/kg and 200  $\mu$ mol/kg) was able to visibly inhibit the oedema and hemorrhage formation following the application of CYP. The oedema was also significantly reduced in P2X7 receptor KO mice (33 ± 14 %.) The increase of MPO activity was reduced by the treatment A438079 (100  $\mu$ mol/kg), (27 ± 8%). Interestingly, A430879 (100  $\mu$ mol/kg) was able to significantly reverse the increased production of IL-1 $\beta$  and TNF- $\alpha$  to the basal levels. **Discussion**: Our study shows the importance of P2X7 receptors in the HC induced by CYP. The pharmacological inhibition of these receptors might represent a new therapeutical alternative for this pathological condition.