EVALUATION OF HEMORRHAGIC CYSTITIS INDUCED BY CYCLOPHOSPHAMIDE IN P2X<sub>7</sub> RECEPTOR KNOCKOUT MICE

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Introduction: Extracellular nucleotides are important signaling molecules that mediate many biological effects, through purinergic receptor activation (Ralevic et al., 1998). ATP is generated in response to cellular damage, and P2X<sub>7</sub> receptors have an essential role in the onset and maintenance of pathological changes (Chesselli et al., 2005). The hemorrhagic cystitis (HC) is a well-known adverse effect of therapy with cyclophosphamide (CYP) used in patients under treatment of many solid tumors (Mosque et al., 2007). These urotoxic effects are attributed to the CYP metabolite, named acrolein, which can be partially prevented by 2-mercaptoetanosulfonato of sodium (Mesna) (Katz et al., 1995). The present study aimed to determine the role of P2X<sub>7</sub> receptors in the model of hemorrhagic cystitis induced by CYP in mice.

Methods: Male C57/BL6 mice and mice of the same lineage, knockout for P2X<sub>7</sub> receptor (n= 4; 25-30 g) were used. HC was induced by a single administration of CYP (300 mg/kg, i.p.). Immediately after the i.p. injection of CYP, mice were housed in individual plastic cages to observe the spontaneous behavior for 4 h, for 2 min every half-hour. Three behavioral parameters were considered: (i) general activity (walking, rearing, climbing, grooming etc.); (ii) immobility time; and (iii) indicatives of visceral pain behavior (‘crises’). In addition, the spontaneous behavior of mice was also scored according to the following scale: 0 = normal; 1 = piloerection; 2 = strong piloerection; 3 = labored breathing; 4 = abdomen licking; and 5 = abdomen stretching and contractions (Olivar et al., 1999). We have also performed the gross examination of bladders at 6 h, in order to determine the presence of edema and hemorrhage. The wet weight of bladders (g per 100 g of body weight) was also...
registered at this time-point (Gray et al., 1986). Control animals received saline at the same intervals of time. Statistical analysis was performed using analysis of variance (ANOVA) followed by Bonferroni test. Values of P < 0.05 were considered as indicative of significance (GraphPad Prism 4.02). All the experimental procedures were approved by the Local Ethics Committee (08/00074, CEUA, PUCRS). **Results:** The results of the present study show that knockout mice for P2X<sub>7</sub> receptors display a reduced nociceptive behavior score induced by CYP (18 ± 6%). In addition, these animals had a marked reduction of edema formation (55 ± 25%) at the gross evaluation. The bladder wet weight was also significantly reduced in knockout mice (36 ± 7%). On the other hand, the hemorrhage index was not significantly altered in these animals. **Discussion:** In the recent years, the interest in the therapeutic potential of purinergic receptors has dramatically increased (Burnstock et al., 2006). Our study points out the importance of P2X<sub>7</sub> receptors in the HC induced by CYP. It is tempting to suggest that pharmacological inhibition of these receptors might represent a new therapeutical alternative for this pathological condition.

**Referências**


KANAT, O., Comparison of uroprotective efficacy of mesna and amifostine in Cyclophosphamide- induced hemorrhagic cystitis in rats. *Indian Journal of Cancer* 2006; 43, 1.
