

## Inhibition of CD73 exacerbates carrageenin – induced pleurisy in rat

E. Caiazzo<sup>1</sup>, F. Maione<sup>1</sup>, S. Morello<sup>2</sup>, N. Mascolo<sup>1</sup>, T. Iuvone<sup>1</sup>, C. Cicala<sup>1</sup>

<sup>1</sup>Dept. of Pharmacy, University of Naples 'Federico II', Naples, Italy

<sup>2</sup>Dept. of Pharmaceutical and Biomedical Sciences, University of Salerno, Italy

Adenosine has been recognized to be a molecule with autocrine/paracrine functions, acting as a signal molecule to preserve host defence and tissue integrity during inflammation and trauma. The ecto - 5'- nucleotidase (CD73), working in concert with the nucleoside triphosphate phosphohydrolases (NTPDase1; CD39) degrades AMP to adenosine and represents a key enzyme for adenosine accumulation at the site of injury (1). There is evidence that CD73 on endothelial cells plays an important role in the control of vascular leakage and neutrophil adhesion and migration and its endothelial expression is increased under hypoxia and correlates to extracellular adenosine accumulation (2). A study performed on mice knockout for CD39 and CD73 have shown that these enzymes represent an innate protective pathway from airway damage caused by mechanical ventilation, protecting from increased vascular leakage and cell accumulation into the lung (3). The objective of our study was to investigate on the role of CD73 in carrageenin – induced pleurisy in rats. Briefly, male Wistar rats (220 – 260 g) were anaesthetized with enflurane; following a small skin incision, pleurisy was induced by injecting 200 µl of carrageenin solution (1% w /v) into the pleural cavity through the sixth intercostal space. Groups of rats were treated locally with the CD73 inhibitor, adenosine 5'-( $\alpha,\beta$ -methylene) diphosphate (APCP, 400 µg/site) or with the respective vehicle (distilled water) immediately before carrageenin injection. 4 and 72 hours following pleurisy induction, rats were sacrificed, the chest was opened and washed with 2 ml of heparinised (10 U/ml) saline. Exudates were collected and then were analyzed for their content in cells and cytokines. Rat treatment with APCP significantly increased cell accumulation ( $20.55 \pm 3.85 \times 10^6$ /ml vs.  $0.4 \pm 0.090 \times 10^6$  / ml; n= 4 p<0.01) and cytokine content (TNF $\alpha$ ,  $103.8 \pm 28.28$  pg/ml vs.  $15.49 \pm 6.34$  pg/ml; n= 3, p < 0.05; IL-1 beta,  $27.60 \pm 0.58$  pg/ml vs.  $0.82 \pm 0.82$  pg/ml; n= 3 , p<0.0001; IL-6,  $122.8 \pm 24.04$  pg /ml vs.  $51.25 \pm 8.72$  pg / ml; n= 3 p< 0.05) into the thoracic cavity 4 hours following pleurisy induction. Our results demonstrate that inhibition of CD73 activity exacerbates carrageenin – induced pleurisy in rat and further suggest that adenosine plays an important role as endogenous modulator of inflammation.

1. Antonioli L, Pacher P, Vizi ES, Haskó G. CD39 and CD73 in immunity and inflammation. Trends Mol Med. 2013 Apr 16. doi:pii: S1471-4914(13)00054-3.3
2. Thompson LF, Eltzschig HK, Ibla JC, Van De Wiele CJ, Resta R. et al., Crucial role for ecto-5'-nucleotidase (CD73) in vascular leakage during hypoxia. J Exp Med. 2004; 200 (11):1395-405.
3. Eckle T, Fullbier L, Wehrmann M, Khoury J, Mittelbronn M et al, Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. J Immunol. 2007 Jun 15;178(12):8127-37.