# The anti-inflammatory effect of nimesulide is associated to an increased 5'-nucleotidase/CD73 activity, in vivo

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Adenosine is emerging as a key regulatory molecule, mostly protective but in certain scenarios injurious, in the pathophysiology of inflammatory diseases. Adenosine mediates its anti-inflammatory activity primarily through the $A_{2A}$ receptor. The ecto-5'-nucleotidase (CD73) degrades AMP to adenosine and represents a key enzyme for adenosine accumulation at the site of injury (Zimmermann, 2000). Nimesulide is a Non Steroidal Anti-inflammatory Drug (NSAID) with selectivity toward COX- 2 enzyme. It has been proposed that, besides COX-2 inhibition, nimesulide pharmacological effects are due to other molecular mechanisms, still not well defined (Süleyman et al, 2008). Recently, it has been shown that nimesulide potentiates the anti-rheumatic profile of methotrexate in collagen – induced arthritis in mice. Authors hypothesize that both drugs might share a common mechanism involving adenosine release or adenosine receptor activation on immune cells (Al-Abd et al, 2010). Here we evaluated whether CD73/adenosine signalling was involved in the anti-inflammatory effect of nimesulide in carrageenan-induced rat paw edema. Edema was induced in male Wistar rats (200-250 g) by carrageenan (1% w/v) injection in the hind paw. Paw volume was measured at the time zero and each hour for 6 h by a hydropletismometer. Experiments were performed on animals treated with the $A_{2A}$ agonist, CGS21680 (2 mg/kg i.p.); with the $A_{2A}$ antagonist, ZM241385 (3 mg/kg i.p.), with CGS21680 (2 mg/kg i.p.) plus ZM241385 (3 mg/kg i.p.); with the vehicle (DMSO) and with nimesulide (5mg /kg i.p.) immediately before carrageenan injection. The effect of the CD73 inhibitor, adenosine 5'-(α,β-methylene) diphosphate, APCP (400 μg/paw) or of the $A_{2A}$ receptor antagonist, ZM241385 (3 mg/kg i.p.) was evaluated in controls and nimesulide treated rats. Prostaglandin $E_2$ ($PGE_2$) levels were evaluated by an Enzymatic Immune Assay (EIA) from different groups of animals, 3 h following edema induction, on plasma samples. AMP hydrolysis was assessed in samples of inflamed tissues and of plasma collected 3 h following edema induction, as a measure of ecto-5NT and soluble-5NT activity, evaluated as inorganic phosphate released following incubation with the substrate. CD73 expression on inflamed tissue each hour following edema induction was analysed by Western blotting. We found that treatment with nimesulide inhibited carrageenan-induced rat paw edema (area under the curve, AUC 1.66 ± 0.31 vs. 2.80 ± 0.34 ml x h; n=6, $p<0.05$); this effect was partially inhibited by the $A_{2A}$ antagonist, ZM241385 at a dose able of reverting the anti-inflammatory effect of CGS21680 on edema development. Plasma $PGE_2$ levels evaluated 3 h following edema induction were reduced following treatment with nimesulide but not by CGS21680 (nimesulide, 226±20.5, $p<0.01$; CGS21680, 517±116; control, 735±70 pg/ml). Western blot analysis showed that CD73 was expressed in inflamed tissues of control and nimesulide treated rats and that there was no difference between experimental groups. Furthermore, the anti-inflammatory effect of nimesulide was reverted by local administration of CD73 inhibitor, (APCP; 400 μg/paw). Moreover, at 3h following edema induction, AMP hydrolysis was increased both in paws and in plasma collected from animals treated systemically with nimesulide (nimesulide,0.155±0.034 vs 1.01± 0.26 nmol/μg protein n=4, $p<0.01$; nimesulide, 3.26±0.51 vs 1.46±0.13 nmol/μg protein n=5, $p<0.01$). Our results suggest that activation of 5'-nucleotidase/CD73 and adenosine signalling may is involved in the anti-inflammatory effect of nimesulide. Our findings contribute to delineate the biochemical mechanism underlying the pharmacological effect of nimesulide and further point at 5'-nucleotidase/CD73 as potential anti-inflammatory targets.

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Süleymanet al.(2008) Curr Med Chem 15:278-283.
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