Adenosine signalling mediates the anti-inflammatory effect of nimesulide, in vitro

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Adenosine is an endogenous nucleoside that accumulates in inflamed tissues following the breakdown of adenosine triphosphate (ATP). The ecto-5'nucleotidase/CD73 by converting adenosine monophosphate (AMP) to adenosine represents the 'peacemaker' enzyme for extracellular adenosine generation (1). It has been well established the protective role of adenosine in inflammation primarily through the activation of the A_{2A} receptor (2).
Nimesulide is a Non-Steroidal Anti-inflammatory Drug (NSAID), selective cyclooxygenase 2 (COX-2) inhibitor. On the inflammatory process, nimesulide has shown a variety of effects that are not due to the sole COX-2 inhibition but to a multifactorial biochemical mechanism that is still unclear (3). Recently, it has been hypothesized a mechanism involving adenosine (4). In the present study we investigated whether CD73/adenosine/A_{2A} signalling pathway was involved in the anti-inflammatory effect of nimesulide, in vitro.
J774A.1 cultured macrophages were pre-incubated with: the A_{2A} agonist, CGS 21684 (1 μM) alone or in combination with the A_{2A} antagonist, ZM 241385 (10 μM); nimesulide (100 μM) alone or in combination with the A_{2A} antagonist, ZM 241385 (10 μM) or with the CD73 inhibitor, APCP (5 μM). All incubations were performed 1 h before cell activation with lipopolysaccharide from Escherichia coli (LPS, 1 μg/ml) except for ZM 241385 that in some experiments was added 6 hours following cell incubation with nimesulide. All controls were performed by cell incubation with DMSO at a final concentration < 0.01 %.
Nitric oxide (NO) release was measured in the culture medium by Griess reaction. Levels of Prostaglandin E_2 (PGE_2) were assayed in the cell culture medium by an Enzymatic Immune Assay (EIA). CD73 activity was assessed by quantifying the conversion of etheno adenosine monophosphate (ε-AMP), a bioactive, fluorescent analog of AMP to etheno adenosine, using Ultra-Performance Liquid Chromatography (UPLC) analysis. CD73 mRNA was silenced by small interfering RNA (siRNA). All the biochemical analysis were performed 24 h following LPS challenge.
We found that the adenosine A_{2A} receptor agonist, CGS 21680, reduced nitrite production following cell activation with LPS and this effect was reversed by the concomitant addition of ZM 241385 (control, 22.7 ±1.4 μM; CGS 21680, 16.9 ± 0.79 μM; CGS 21680 *plus* ZM 241385, 29.4 ± 0.59 μM; n=6, $p<0.001$). Nimesulide inhibited nitrite production from LPS–activated J774 and its effect was reversed by the A_{2A} antagonist, ZM 241385 (control, 23.2 ± 0.89 μM; nimesulide, 15.6 ± 0.53 μM; nimesulide *plus* ZM 241385, 25.3 ± 0.41 μM; n=6, $p<0.001$) and by the CD73 inhibitor, APCP (control, 21.3 ± 1.15; nimesulide, 14.7 ± 0.77 μM; nimesulide *plus* APCP, 22.4 ± 1.29 μM; n=6, $p<0.001$). Nimesulide increased CD73 activity in J774 evaluated as etheno adenosine accumulation (348.5 ± 25.6 vs 212.4 ± 34.7 pmol/min; n=6, $p<0.05$). Nimesulide also inhibited PGE_2 production from LPS activated J774 (102.7 ± 33.3 vs 643.7 ± 24.8 pg/ml; n=6, $p<0.001$). Nimesulide did not inhibit nitrite accumulation by lipopolysaccharide-activated SiRNA CD73 silenced J774 macrophages.
Our data demonstrate that the anti-inflammatory effect of nimesulide, in vitro, in part is mediated by CD73-derived adenosine acting on A_{2A} receptors. Our study may open a path to re-evaluate the mechanism of action of nimesulide and to project innovative anti-inflammatory drugs that have anti-inflammatory effect by targeting an endogenous anti-inflammatory pathway.

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