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Anti-inflammatory potential of 2-styrylchromones regarding their interference with arachidonic acid metabolic pathways

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Anti-inflammatory potential of 2-styrylchromones regarding their interference with arachidonic acid metabolic pathways

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Cyclooxygenases (COXs) are the key enzymes in the biosynthesis of prostanoïds. In mammalian cells, COXs exist in at least two isoforms (COX-1 and COX-2). COX-1 is a constitutive enzyme, expressed in almost every cell type, responsible for the physiological production of prostaglandins (PGs) i.e., PGI₂, PGE₂, PGF₂α, and PGD₂, in diverse organs and thromboxane A₂ (TXA₂) in platelets. The expression COX-2 is highly stimulated in the inflammation-related cell types including macrophages and mast cells when challenged by LPS, phorbol esters, cytokines, or growth factors. This effect leads to the production of large amounts of PGs, in particular PGE₂ and PGD₂, which are pro-inflammatory mediators that increase vascular permeability and promote edema at the sites of inflammation.

Lipoxygenases (LOXs) are enzymes that produce hydroxy acids and leukotrienes (LTs). From the LOXs existent in the mammalian tissues, 5-LOX, which is mainly found in cells of myeloid origin, i.e., polymorphonuclear leukocytes (PMN), mast cells, or macrophages, is the most implicated in inflammatory and allergic disorders. 5-LOX metabolizes arachidonic acid to yield, among other products, LTB₄, a potent chemoattractant mediator of inflammation.

2-Styrylchromones (2-SC) are a chemical family of oxygen heterocyclic compounds, vinyllogues of flavones (2-phenylchromones), whose occurrence in nature has been reported. Considering the effective anti-inflammatory effect already demonstrated for several flavones, the aim of the present study was to evaluate the anti-inflammatory potential of 2-SC, for the first time, by studying its COX-1 and COX-2 inhibitory capacity. Different compounds from this group were studied (Fig. 1). The effect of the same compounds on the LTB₄ production by stimulated human neutrophils was also evaluated.

Several of the tested 2-SC were able to inhibit both COX-1 activity and LTB₄ production which makes them dual inhibitors of the COX and 5-LOX pathways. This type of compounds may exhibit anti-inflammatory activity with a wider spectrum than that of classical non-steroidal anti-inflammatory drugs (NSAIDs) by inhibiting 5-LOX product-mediated inflammatory reactions towards which NSAIDs are ineffective. Although the inhibition of COX-2 could not be achieved by any of tested compounds, it cannot be excluded the hypotheses of these compounds being inhibitors at higher concentrations (≥250 μM).

The most effective compounds in this study were those having structural moieties with proved antioxidant activity (3,4′-catechol and 4′-phenol substituted B-rings). It is conceivable that 2-SC inhibit the LTB₄ production by acting as 5-LOX inhibitors, in conformity with flavonoids and other phenolic compounds. On the other hand, the mechanism through which 2-SC inhibit COXs is likely to consist in the scavenging of the radical intermediates involved in COX enzyme catalysis especially the phenoxyl radical formed on a tyrosine residue. **Fig. 1. General structure of the tested 2-styrylchromones**

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Cyclooxygenases (COXs) are key enzymes in the biosynthesis of prostanoids. COXs exist in at least two isoforms (COX-1 and COX-2). COX-1 is a constitutive enzyme while the expression of COX-2 is highly stimulated in the event of inflammatory processes, leading to the production of large amounts of prostaglandins (PGs), in particular PGE2 and PGL2, which are pro-inflammatory mediators.

Lipoxygenases (LOxs) are enzymes that produce hydroxy acids and leukotrienes (LTs). 5-LOX metabolizes arachidonic acid to yield, among other products, LTB4, a potent chemoattractant mediator of inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used remedy in inflammatory disorders. However, they cause several adverse effects, the most important being gastric injury up to gastric ulceration, renal failure and asthma1. On the other hand, the COX-2 selective drugs, generically known as COXIBs, have recently been a cause of controversy due to the enhanced cardiovascular risk they carry. Thus, alternative therapeutic solutions, with high anti-inflammatory potency but with fewer side effects, are needed, especially for the control of chronic inflammatory diseases, which implicate longer therapies.

The aim of the present work was to evaluate the anti-inflammatory potential of 2-styrylchromones (2-SC), a chemical family of oxygen heterocyclic compounds, vinyllogues of flavones (2-phenylchromones), by studying their COX-1 and COX-2 inhibitory capacity as well as their effects on the LTB4 production by stimulated human polymorphonuclear leukocytes (PMNL).

Determination of LTB4 production by human PMNL

Human PMNL were isolated from peripheral blood of healthy volunteers as previously described2. Neutrophil suspensions (5 x 106 cells/ml) in HBSS were pre-incubated at 37°C for 10 min with the 2-styrylchromones (25 and 10 μM) or with the lipoxygenase inhibitor, NDGA (1 μM).

The cells were subsequently incubated with A23187 (5 μM) and arachidonic acid (10 μg/mL) for 8 min. The reactions were stopped by the addition of cold methanol, the samples were subsequently centrifuged at 13,000 x g for 1 min, and the supernatants were stored at -70°C until analysis. The amount of LTB4 in the samples was measured using a commercial EIA kit (Cayman Chemical Co.), according to the manufacturer’s instructions.

COX-1 and COX-2 inhibition assays

The inhibition of COX-1 (ovine) and COX-2 (human recombinant) by 2-SC was determined by quantifying the levels of PGF2α using a specific EIA kit according to the manufacturer’s instructions (Cayman Chemical Co.). The COX inhibitors indomethacin and celecoxib were used as positive controls.

The most effective compounds in this study were those having structural moieties with proved antioxidant activity (3’,4’-catechol and 4’-phenol substituted B-ring). It is conceivable that 2-SC inhibit the LTB4 production by acting as redox inhibitors of 5-LOX, in conformity with flavonoids and other phenolic compounds. On the other hand, the mechanism through which 2-SC inhibit COX-1 is likely to consist in the scavenging of the radical intermediates involved in COX enzyme catalysis especially the phenoxy radical formed on a tyrosine residue.

Some of the tested 2-SC showed to inhibit both COX-1 and 5-LOX pathways. This type of compounds may exhibit anti-inflammatory activity with a wider spectrum than that of classical NSAIDs by further inhibiting 5-LOX product-mediated inflammatory reactions towards which NSAIDs are ineffective.

Fig 1. Chemical structures of the tested 2-SC

Fig 2. Inhibition of LTB4 production by 2-SC (25 μM) and NDGA (1 μM) determined by EIA. ***P<0.001, **P<0.01, *P<0.05, significantly different from control.

Fig 3. Inhibition of COX-1 activity determined by EIA. ***P<0.001, **P<0.01, *P<0.05, significantly different from control.

References

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