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N-Acetylcysteine enhances the action of anti-inflammatory drugs as suppressors of prostaglandin production in monocytes

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THE anti-inflammatory effect of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with inhibition of cyclooxygenase (COX), the rate-limiting enzyme responsible for the synthesis of prostaglandins. Since oxygen free radicals can act as second cellular messengers, especially to modulate the metabolism of arachidonic acid and the prostaglandin tract, it seems plausible that antioxidants might affect the production of prostaglandin by activated cells. This research is focused on the effect of the antioxidant *N*-acetylcysteine (NAC) on the inhibition of prostaglandin E_2 formation in activated monocytes by specific and non-specific COX inhibitors. We found that lipopolysaccharide-induced prostaglandin E_2 formation was significantly reduced by rofecoxib and by diclofenac, two NSAIDs. Addition of NAC to each of these drugs enhanced the effect of the NSAIDs. These results suggest that one might expect either a potentiation of the anti-inflammatory effect of COX inhibitors by their simultaneous administration with NAC, or obtaining the same anti-inflammatory at lower drug levels.

Key words: Monocytes, Cyclooxygenase-2, Prostaglandin E_2, Non-steroidal anti-inflammatory drugs, *N*-acetylcysteine

Introduction

The biochemical basis of the process of inflammation is the formation of prostaglandins and leukotrienes by two chains of reactions stemming from a common precursor – the arachidonic acid. In the case of prostaglandins the cyclooxygenase (COX) enzyme system plays a pivotal role, while in the case of leukotrienes it is the lipoxygenase system.

Non-steroid anti-inflammatory drugs (NSAIDs) are the first line of choice in the treatment of inflammatory joint diseases. Inhibition of COX and therefore of prostaglandin production is the common mechanism of action of NSAIDs. It is known today that COX exists in two isoforms: COX-1 is present in the endothelium, stomach and kidney, and COX-2 is induced *in vivo* at inflammatory sites.[1] These findings have lead to the development of COX-2-specific drugs that alleviate the pain caused by inflammation of the joints but have a lower gastrointestinal toxicity, which it is usually associated with COX inhibition.

Antioxidants have already been investigated as candidates in the treatment of inflammatory joint diseases. Kroger *et al.*[2] showed that the inhibitory effect of nicotinamide upon collagen-induced arthritis in mice is enhanced by *N*-acetylcysteine (NAC). High doses of vitamin E were found effective when compared with diclofenac in chronic polyarthritis patients.[3] Also, in certain cells like mesangial[4] or colorectal cells,[5] antioxidants reduce COX-2 expression.

Abate *et al.*[6] addressed specifically the synergism between aspirin and vitamin E in reducing lipopolysaccharide (LPS)-induced prostaglandin E_2 (PGE_2) production in a macrophage cell line. They found that the combined inhibition of these two factors is larger than that of their sum. In the present paper, we consider two newer drugs; the non-specific COX inhibitor diclofenac and the specific COX-2 inhibitor rofecoxib, both in conjunction with NAC.

Methods

Materials

LPS from *Escherichia coli* (serotype type 026B6), NAC, diclofenac sodium salt and Histopaque-1077 were purchased from Sigma Chemical Co. (St Louis, MO, USA). Dulbecco's phosphate-buffered saline (PBS), RPMI medium 1640 and fetal calf serum (FCS) were purchased from Biological Industries (Bet Haemek, Israel). Rofecoxib (Vioxx) was a gift from Merck Sharp & Dohme (Petach Tikwa, Israel). The enzyme-linked immunosorbent assay (ELISA) kit for PGE_2 was purchased from R&D systems (Minneapolis, MN, USA).

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Isolation of human peripheral blood monocytes

Blood was collected by venipuncture from healthy volunteers who did not take any drugs. Twenty milliliters of heparinized blood (10 U of heparin/ml of blood) were layered carefully on an equal volume of Histopaque-1077 in a 50 ml conical centrifuge tube and the suspension was centrifuged for 30 min, 700 × *g* at room temperature. The mononuclear cell layer between the plasma and Histopaque layers was collected and washed three times with Dulbecco PBS (by centrifugation for 5 min at 4°C). The cells were resuspended in a known volume of RPMI 1640. The cells were counted and their viability was determined by Trypan blue exclusion. The purity of preparation was assessed to be 98–99% mononuclear cells, of which 10–12% were monocytes.

Cell incubation

The cells were placed (1 million/ml of mononuclear cells) in a 24-well dish, 1 ml of suspension in each well, and pre-incubated in a humidified incubator at 37°C and 5% CO_2 for 2 h in RPMI 1640 that also contained 5% FCS. The cells were washed three times with Dulbecco PBS pre-warmed to 37°C to discard unattached cells. Inspection of the nucleus morphology revealed that 98–99% of cells isolated in this manner were monocytes.

Measurement of PGE_2 accumulation

The plated cells were subsequently treated with LPS (10 μg/ml) and a combination of drugs with/without NAC in RPMI 1640 with 10% FCS for 18 h. The supernatant was then collected, centrifuged and PGE_2 was determined with an ELISA kit, according to the manufacturer's instructions.

Statistics

Parallel experiments with monocytes from the same donor were carried out with and without antioxidants. The results were compared by means of the Wilcoxon paired test.

Results

Effect of diclofenac and rofecoxib on LPS-induced formation of PGE_2

Since it was shown that the LPS-inducible cyclooxygenase in macrophages is COX-2,[7] we might assume that in the present experiments the measured PGE_2 production represents the COX-2 activity of these cells. Table 1 presents the effect of diclofenac at concentrations of 0.01 and 0.001 μM and that of rofecoxib at 0.01 and 0.001 μM on PGE_2 production by LPS-activated monocytes. The results are expressed as percent inhibition: % inhibition = [1 – PGE_2 (with NSAIDs)/PGE_2 (without NSAIDs)].

Table 1. Per cent inhibition by diclofenac and rofecoxib of LPS-induced PGE_2 formation in monocytes

Concentration (μM)	% inhibition	
	Diclofenac	Rofecoxib
0.001	39.0 ± 2.7	
0.01	79.4 ± 0.7	55.5 ± 1.86
0.1		70.5 ± 1.4

It is apparent that both NSAIDs inhibit the PGE_2 production, diclofenac being the more potent inhibitor. The effect of diclofenac only on PGE_2 production was investigated in mononuclear cells,[8] and an IC_{50} (the concentration required for 50% inhibition) value of 0.03 μM was obtained for COX-2 inhibition. In our system, which is different from that of Laufer *et al.*,[8] the IC_{50} values for diclofenac and for rofecoxib were 0.0035 and 0.0085 μM, respectively.

The effect of various NSAIDs on inhibition of PGE_2 production was carried out in a whole blood assay[9] where IC_{50} values of 0.038 and 0.84 μM for diclofenac and rofecoxib, respectively, were reported. To our knowledge, the present research is the first in which a comparison between a specific and a non-specific COX inhibitor with respect to PGE_2 production has been carried out in mononuclear cells.

Effect of NAC on diclofenac and rofecoxib-induced inhibition of PGE_2 formation

It is apparent from Fig. 1 that addition of 1 mM NAC to the incubation mixture containing either diclofenac or rofecoxib enhanced the inhibition of PGE_2 formation induced by these drugs. The effect is more prevalent when the NSAID-induced inhibition is low, for example in the case of 0.001 μM diclofenac. It is worthwhile to mention that 1 mM NAC alone depresses also prostaglandin production by 55.5%.

In the present experimental system, we found that vitamin E has no effect of on the inhibitory action of diclofenac and rofecoxib (results not reported).

Discussion

A synergistic effect of sulfosalicylic acid and vitamin E on stimulated PGE_2 release in a macrophage cell line (J774.1A) was recently reported by Abate *et al.*[6] In that work, a relative high concentration of sulfosalicylic acid (1 μM) inhibited the LPS-induced PGE_2 release by 70%; addition of 300 μM vitamin E enhanced this inhibition to 90%. This trend could be

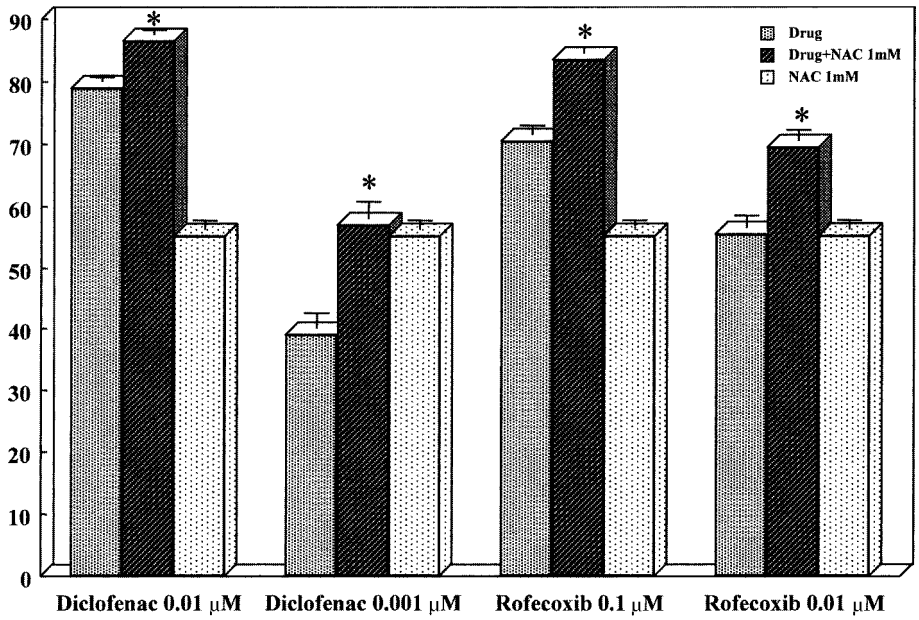

FIG. 1. The effect of 1 mM NAC on the diclofenac-induced and rofecoxib-induced inhibition of PGE_2 formation in activated monocytes. * $p<0.05$ for % inhibition (NSAID + NAC) versus % inhibition (NSAID), Wilcoxon paired test. All data shown are mean ± SEM of number of observations (*n*). Number of observations: 0.01 μM diclofenac, $n = 9$; 0.001 μM diclofenac, $n = 6$; 0.1 μM rofecoxib and 0.01 μM rofecoxib, $n = 8$.

explained by COX-2 expression experiments only when the sulfosalicylic acid and the vitamin E concentrations were both 300 μM. Since in the present experimental system no enhanced inhibition of PGE_2 formation was observed when NAC was replaced by vitamin E, we conclude that the inhibitory effect of NAC on PGE_2 formation is not related to its antioxidant properties. Abate *et al.*,[6] who could not observe a synergistic effect on the effect of sulfosalicylic acid when vitamin E was replaced by another antioxidant, namely vitamin C, confirm this thesis.

We demonstrated in previous research that NAC exerts its inhibitory action on leukotriene production by alveolar macrophages by suppressing arachidonic acid formation (the PLA2 enzyme system).[10] Since it was reported[11] that monocytes release arachidonic acid upon stimulation with a variety of soluble or particulate agents, our results could be explained by an effect of NAC on arachidonic acid formation in these cells.

As we have shown that the NSAID-induced inhibition of prostaglandin formation is enhanced by NAC, one might expect that *in vivo* experiments with this combination of agents will exhibit either a potentiation of the anti-inflammatory effect of NSAIDs or an anti-inflammatory effect at lower NSAID drug levels.

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