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Sodium salicylate enhances the expression of cyclooxygenase-2 in endotoxin-stimulated human mononuclear cells

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Abstract

The effects of salicylate on the expression of cyclooxygenases, and on prostaglandin E_2 biosynthesis were examined in human peripheral blood mononuclear cells. Peripheral blood mononuclear cells were incubated in the presence of endotoxin, which induced expression of cyclooxygenase-2 protein, and caused a time-dependent increase of immunoreactive prostaglandin E_2 in the supernatant. The cycooxygenase-2 selective inhibitor *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS-398, 1 μM) suppressed the endotoxin-induced increase of prostaglandin E_2, without significantly affecting the expression of cyclooxygenase-1 or cyclooxygenase-2. In peripheral blood mononuclear cells exposed to endotoxin (18 h), 1.0 and 3.0 mM sodium salicylate reduced the prostaglandin E_2 concentration of the supernatant, and, at the same time, stimulated cyclooxygenase-2 expression. After a subsequent 2 h incubation of peripheral blood mononuclear cells in drug-free medium, prostaglandin E_2 concentrations in samples that had been exposed to endotoxin together with 1.0 or 3.0 mM salicylate were significantly higher than in samples exposed to endotoxin alone. These results show that salicylate can enhance the expression of cyclooxygenase-2 in endotoxin-exposed peripheral blood mononuclear cells and at the same time reduce prostaglandin E_2 formation. After washout and removal of salicylate-induced cyclooxygenase inhibition, increased cyclooxygenase-2 expression resulted in enhanced prostaglandin E_2 formation. It seems possible that under certain conditions salicylate-induced stimulation of cyclooxygenase-2 expression may contribute to its clinical pharmacological profile.

Keywords: Anti-inflammatory drug; Aspirin; Prostaglandin biosynthesis

1. Introduction

Aspirin (acetylsalicylic acid), which inhibits cyclooxygenases by acetylation of an essential serine at the active site of the enzyme (DeWitt et al., 1990), is rapidly deacetylated during and after absorption to form salicylate. Since the demonstration of cyclooxygenase inhibition as a mechanism of action of aspirin-like drugs (Vane, 1971), the pharmacology of salicylate has been a subject of discussion. Unlike aspirin, salicylate is only a weak inhibitor of cyclooxygenase in intact cells, being virtually ineffective in preparations of purified cyclooxygenase (Mitchell et al., 1993), thus raising questions as to the relevance of salicylate-induced cyclooxygenase inhibition in mediating its anti-inflammatory action (e.g., Higgs et al., 1987; Chiabrando et al., 1989).

More recent studies show that salicylate is a weak, competitive inhibitor with arachidonic acid of cyclooxygenase, therefore its activity being dependent on the amount of available arachidonic acid (Mitchell et al., 1997). These results help to explain lack of detectable cyclooxygenase inhibition by salicylate in preparations containing high amounts of endogenous arachidonic acid, and support therefore the original concept of cyclooxygenase inhibition as a factor underlying the anti-inflammatory action of salicylate (Higgs et al., 1987).

In addition to its direct effects on cyclooxygenase activity, salicylate has been reported to interfere with cell function at the transcriptional level (Amin et al., 1995; Chen et al., 1999; Kopp and Ghosh 1994; Schwenger et al., 1997, 1998; Pillinger et al., 1998; Yin et al., 1998; Vittimberga et al., 1999), suggesting that these effects can contribute to its therapeutic effects. With regard to salicylate's possible inter-

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ference with the induction of cyclooxygenase-2, there are conflicting results. Wu et al. (1991) and Xu et al. (1999) showed that salicylate can inhibit cyclooxygenase expression in human umbilical vein endothelial cells stimulated with interleukin-1 or phorbol ester. However, the majority of studies find no such inhibitory effects of salicylate on the expression of cyclooxygenase isoenzymes (O'Sullivan et al., 1993; Barrios-Rodiles et al., 1996; Fernandez de Arriba et al., 1999; Hinz et al., 2000).

We have now compared directly the effects of sodium salicylate on prostaglandin biosynthesis with its effect on the expression of cyclooxygenase isoenzymes using human peripheral blood mononuclear cells.

2. Materials and methods

2.1. Preparation of human peripheral blood mononuclear cells

Volunteer blood donors were healthy normal subjects, who were taking no systemic medication and showing normal values of C-reactive protein. Blood was prepared according to Haslett et al. (1985): Citrated blood (40 ml) was centrifuged at $300 \times g$ for 20 min at room temperature to remove platelet-rich plasma, after which erythrocytes were removed by addition of 6% Dextran T-500 (Sigma, Vienna, Austria) in 0.9% saline (50 ml). For separation of polymorphonuclear leukocytes (containing neutrophils and eosinophils) from peripheral blood monocytic cells (comprising monocytes and lymphocytes), the supernatant was layered on 15-ml Histopaque (1077 Density, Sigma). After centrifugation at $350 \times g$ for 20 min, the mononuclear cells of the interface were carefully removed, washed twice with phosphate buffered saline containing 5.5 mM glucose and 2.7 mM KCl, resuspended in SFM macrophage medium (Life Technologies, Lofer, Austria) containing penicillin (Sigma, 100 U/ml) and streptomycin (Sigma, 100 μg/ml), and counted using a Minos Vet cell counter (Roche, Vienna, Austria). Peripheral blood mononuclear cells were divided into aliquots containing 2×10^6 mononuclear cells/ml, and incubated at 37 °C. In order to assess the quality of the preparation, cytospins were performed and cells stained with Wright Giemsa (Diff-Quick, Dade, Düdingen, Switzerland) showing that monocytes represented between 10% and 15% of peripheral blood mononuclear cells.

2.2. Experimental protocol

Sodium salicylate, the cyclooxygenase-2 inhibitor *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS-398), dexamethasone or the respective vehicle were added to the samples 30 min before endotoxin (lipopolysaccharide *Escherichia coli* Serotype 055:B5; Sigma, 10 μg/ml) exposure. After an incubation period of 18 h (5 h in some experiments, see Results), samples were centrifuged and the supernatant collected for radioimmunological determination (radioimmunoassay (RIA)) of prostaglandin E_2. Pellets were used for determination of cyclooxygenase-1 and cyclooxygenase-2 immunoreactivity by Western blotting. In another group of experiments, cells were re-suspended in medium only (37 °C) and incubated for another 2 h; thereafter, the concentration of prostaglandin E_2 in the supernatants was determined by RIA. In each set of experiments, one aliquot was used to determine cell viability at the end of the experiment by Trypan blue staining.

2.3. Radioimmunoassay

Immunoreactive prostaglandin E_2 was determined as described previously (Jobke et al., 1973) using [5,6,8,11,12,14,15(*N*)-3*H*] prostaglandin E_2 (Amersham, Vienna, Austria) as tracer and synthetic prostaglandin E_2 (Sigma) as standard. The detection limit (defined as 10% inhibition of binding) was 4 pg corresponding to 147 pg/ml sample; the ED_{50} was 29 pg with an interassay variation (S.D.) of 3%.

2.4. Electrophoresis and blotting

Protein from cells was extracted using 50 mM Tris buffer containing 10 mM EDTA, 1% Triton X-100 and 1 mM phenylmethylsulphonyl fluoride. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 8% gels with electrophoresis at 150 V for 90 min in a BioRad Miniprotean Chamber (Biorad, Vienna, Austria). Samples for SDS-PAGE (90 μg protein/lane; protein was determined using Biorad Protein Assay) were treated with sample buffer (0.1 M Tris/HCl, pH 6.8, 4% SDS, 15% glycerol and 1% mercaptoethanol) at a ratio of 1:1 (v/v). For Western blot analysis, protein fractions were electrophoretically transferred to PVDF (hydrophobic polyvinylidene difluoride) membranes (Amersham) (150 mA; 4 °C, 60 min). Immunochemical detections of cyclooxygenase-1 and cyclooxygenase-2 were performed with sequence specific mouse anti-human cyclooxygenase-1 and cyclooxygenase-2 antibodies (dilution 1:500; Cayman, Ann Arbor, MI, USA). Immunoreactive bands were visualized with peroxidase-conjugated anti-mouse immunoglobulin (dilution 1:500) and subsequent ECL (enhanced chemiluminescence, Amersham) development. Quantitative evaluation of Western blots was carried out after scanning the blots by densitometric analysis using a computerized image analysis system (MCID M2, Imaging Research, St. Catherines, Ontario, Canada).

2.5. Drugs

N-(2-Cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS-398) was obtained from Cayman; stock solutions were prepared in dimethylsulfoxide and further diluted in 0.9% NaCl. Sodium salicylate was obtained from Sigma and

dexamethasone-21-dihydrogenphosphate disodium salt (Fortecortin®) from Merck (Darmstadt, Germany).

2.6. Data analysis

Within each experiment, samples obtained from one individual donor were assayed in triplicate. The resulting average was used to calculate means ± S.E.M. of n independent determinations. To determine the effect of drug treatment, data were calculated as percent of control (endotoxin stimulation alone) and expressed as means ± S.E.M. Statistical analysis was performed using Kruskal–Wallis One-Way Analysis of Variance, and Dunn's post test (SigmaStat, SPSS, Chicago, IL, USA). A value of $P < 0.05$ was considered significant.

3. Results

3.1. General observations

After incubation of peripheral blood mononuclear cells for 18 h in the absence of endotoxin, there was no detectable expression of cyclooxygenase-2 (Fig. 1), and the concentration of prostaglandin E_2 in the supernatants remained below the detection limit of the assay. Endotoxin (10 μg/ml) exposure induced the expression of cyclooxygenase-2 protein, without significantly affecting cyclooxygenase-1 (Fig. 1), and resulted in a time-dependent increase of the concentration of immunoreactive prostaglandin E_2 in incubation media (Fig. 2). There was no significant effect of endotoxin or endotoxin and sodium salicylate (3 mM) on cell viability (>90%) as determined by Trypan blue staining. Dexamethasone (1 μM) prevented endotoxin-induced cyclooxygenase-2 expression (Fig. 1) and reduced the endotoxin-induced rise in prostaglandin E_2 by 72.4 ± 1.9% ($n = 4$). Since these results pointed to a major contribution of cyclooxygenase-2 to endotoxin-induced prostaglandin E_2 formation, the effect of the selective cyclooxygenase-2 inhibitor NS-398 was investigated. NS-398 caused a concentration-dependent inhibition of endotoxin-induced prostaglandin E_2 formation showing an inhibitory potency (Fig. 3) that was expected for a cyclooxygenase-2 system (Panara et al., 1995). At the highest concentration (1 μM), NS-398 had no significant effect on cyclooxygenase-1 (122 ± 16% of control, $n = 4$) and cyclooxygenase-2 protein expression (95 ± 13% of control; $n = 4$) (Fig. 1) and caused a near-complete inhibition of prostaglandin E_2 formation (Fig. 3).

3.2. Effects of sodium salicylate on endotoxin-stimulated cyclooxygenase-2 expression and prostaglandin E_2 biosynthesis

Incubation of cells for 18 h with sodium salicylate (3 mM) in the absence of endotoxin did not significantly affect the expression of cyclooxygenase isoforms (cyclooxygenase-1: 95 ± 10% of control; $n = 2$; cyclooxygenase-2: non-detectable), and did not induce prostaglandin E_2 formation as indicated by the observation that prostaglandin E_2 in the supernatants remained below the detection limit of the assay ($n = 3$, data not shown).

In cells incubated for 18 h in the presence of endotoxin, 1 and 3 mM sodium salicylate increased the expression of cyclooxygenase-2 by 148 ± 50.6% ($n = 6$) and 268 ± 91.3% ($n = 6$), respectively, but did not significantly affect cyclooxygenase-1 expression (Fig. 1).

Although inducing cyclooxygenase-2 expression in peripheral blood mononuclear cells, sodium salicylate caused a concentration-dependent reduction of immunoreactive prostaglandin E_2 in the incubation media of endotoxin-exposed cells (Fig. 4). Only after cells had been transferred to salicylate/endotoxin-free medium for a further 2 h incubation period, there was a clear increase of prostaglandin E_2 detectable, depending on the concentration of sodium salicylate the cells had been exposed to (Fig. 4). In order to exclude a possible effect of prior salicylate exposure on the availability of arachidonic acid, a separate group

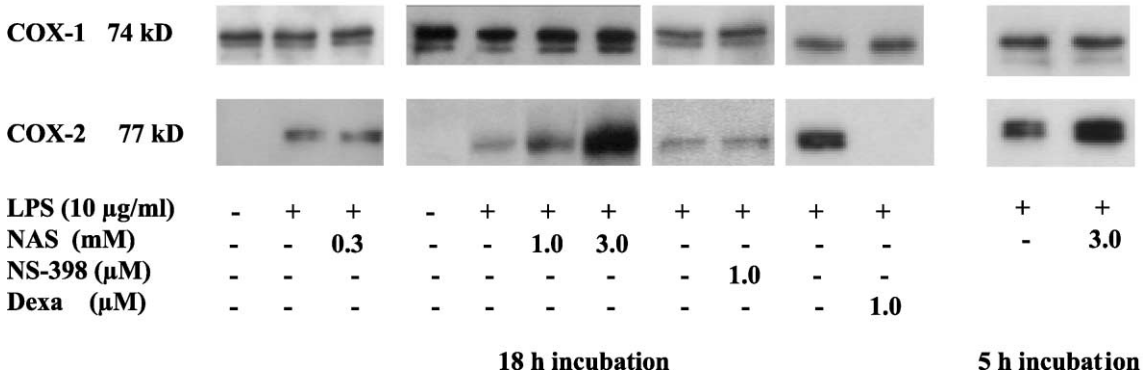

Fig. 1. Example of Western blot analysis of cyclooxygenase (COX)-1 and cyclooxygenase (COX)-2 expression in human peripheral blood mononuclear cells incubated for 18 h (5 h) in medium containing endotoxin (LPS), sodium salicylate (NAS), the cyclooxygenase-2 inhibitor NS-398, or dexamethasone (Dexa) at concentrations indicated. The endotoxin-induced rise in COX-2 was enhanced by salicylate (1–3 mM), but not affected by the COX-2 inhibitor NS-398.

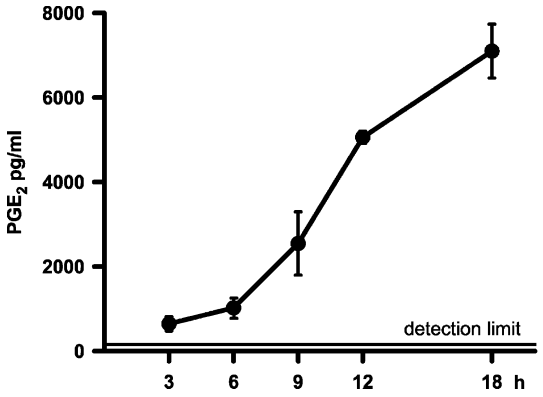

Fig. 2. Concentration of immunoreactive prostaglandin (PG) E_2 in incubation media of human peripheral blood mononuclear cells exposed to endotoxin (10 μg/ml) as determined at various intervals after the addition of endotoxin. Values are mean ± S.E.M.; $n = 4–6$.

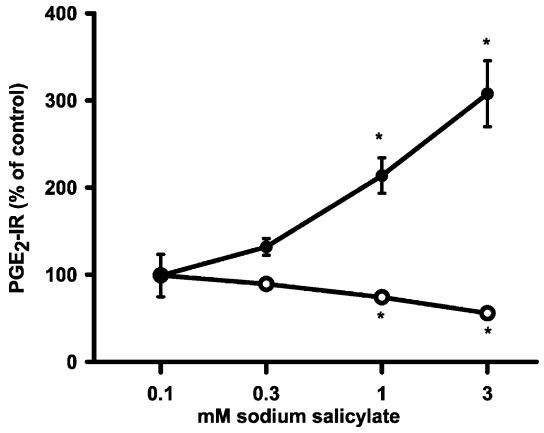

Fig. 4. Effect of sodium salicylate on the concentration of immunoreactive prostaglandin (PG)E_2 in incubation media of human peripheral blood mononuclear cells exposed to endotoxin (10 μg/ml) for 18 h (open circles); and on prostaglandin (PG)E_2 in incubation media obtained after transfer of peripheral blood mononuclear cells to drug-free medium and subsequent 2 h incubation (closed circles). Values were calculated as % of control and are expressed as mean ± S.E.M.; $n = 6–21$. $^*P < 0.05$ as compared to control.

of experiments was conducted, in which arachidonic acid (30 μM) was present in the medium during the final 2 h incubation period. Under these conditions too, prostaglandin E_2 formation was increased in samples that had been exposed to endotoxin and salicylate as compared to endotoxin alone (1 mM salicylate: + 51 ± 4%, $n = 5$; 3 mM salicylate: + 90 ± 44%; $n = 5$).

Additional experiments were conducted using a 5 h endotoxin/ ± salicylate exposure period showing very similar results to those observed with 18 h exposure. Sodium salicylate (3 mM) enhanced cyclooxygenase-2 expression (Fig. 1) and inhibited prostaglandin E_2 formation (− 66.2 ± 4.1%; $n = 6$) as determined in the 5 h incubation media. A subsequent 2 h incubation in drug-free medium showed that prostaglandin E_2 formation was increased (+ 52.3 ± 9%; $n = 3$) in samples that had been exposed to endotoxin together with 3 mM salicylate as compared to values obtained from samples exposed to endotoxin alone.

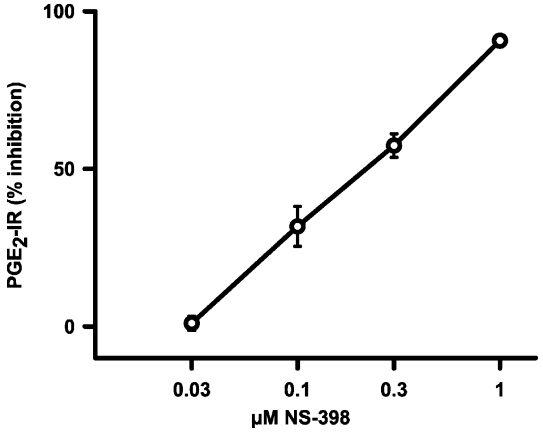

Fig. 3. Effect of NS-398 on the concentration of immunoreactive prostaglandin (PG) E_2 in incubation media of human peripheral blood mononuclear cells exposed to endotoxin (10 μg/ml) for 18 h. Values were calculated as % inhibition and expressed as mean ± S.E.M.; $n = 6–14$.

4. Discussion

In the present study, endotoxin-stimulated peripheral blood mononuclear cells were used to determine effects of salicylate on cyclooxygenase expression and activity. In this model, prostaglandin E_2 formation seemed largely dependent on cyclooxygenase-2 activity, because it was suppressed by the selective cyclooxygenase-2 inhibitor NS-398.

Sodium salicylate, used in concentrations similar to those shown to interfere with nuclear translocation of the transcription factor nuclear factor kappa B (Grilli et al., 1996; Kopp and Gosh, 1994; Yin et al., 1998) had no detectable effect on cyclooxygenase expression in non-stimulated cells. In contrast, it enhanced the expression of cyclooxygenase-2 protein in endotoxin-treated cells. At the same time, it reduced the concentration of prostaglandin E_2 in the medium indicating inhibition of cyclooxygenase-2 activity. It has to be assumed that under such conditions, effects of increased cyclooxygenase-2 expression and inhibition of its activity by salicylate overlap, i.e. that the inhibitory potency of salicylate is underestimated as soon as enhancement of enzyme expression becomes relevant. Furthermore, it has to be assumed that removal of salicylate-induced inhibition of cyclooxygenase-2 activity will unmask the consequences of increased cyclooxygenase-2 expression on prostaglandin E_2 formation. In fact, transfer of peripheral blood mononuclear cells to drug-free medium and a subsequent 2 h incubation

showed a pronounced increase of prostaglandin E_2 formation in samples that had been exposed to salicylate. Since a similar effect was observed in the presence of exogenous arachidonic acid, it seems reasonable to assume that increased prostaglandin E_2 biosynthesis was reflecting enhanced cyclooxygenase-2 expression rather than altered substrate availability in salicylate-exposed cells.

Both effects of salicylate, inhibition and subsequent enhancement of prostaglandin E_2 biosynthesis, were observed at similar concentrations. These results may indicate that inhibition of prostaglandin E_2 biosynthesis and stimulation of cyclooxygenase-2 expression are related phenomena. However, inhibition of prostaglandin E_2 formation by NS-398 did not cause a detectable increase of cyclooxygenase-2, suggesting that inhibition of prostaglandin E_2 formation alone is not sufficient to alter cyclooxygenase-2 expression.

The observation that sodium salicylate increased cyclooxygenase-2 expression in endotoxin-stimulated peripheral blood mononuclear cells was not expected in view of a number of studies finding no effect of salicylate on cyclooxygenase-2 expression (O'Sullivan et al., 1993; Barrios-Rodiles et al., 1996; Mitchell et al., 1997; Fernandez de Arriba et al., 1999; Hinz et al., 2000). We have no explanation for this apparent discrepancy, apart from pointing to differences in cell types and/or experimental protocols, and also to the fact that the experimental design of most studies aimed at detecting inhibition of cyclooxygenase-2 expression. Although it has been shown that several NSAIDs can enhance cyclooxygenase-2 in non-stimulated cells (Meade et al., 1999; Paik et al., 2000), these observations cannot serve to explain the present results: in these studies, cyclooxygenase-2 expression was enhanced presumably through activation of peroxisome proliferator-activated receptors (PPARs), which salicylate does not activate (Lehmann et al., 1997).

Other properties of salicylate may provide a better explanation for the observed enhancement of cyclooxygenase-2 expression in endotoxin-stimulated peripheral blood mononuclear cells. In particular, it has been shown that salicylate activates p38 mitogen-activated protein kinase (MAPK; Schwenger et al., 1997, 1998), which, in turn, is required for transcription and stabilization of cyclooxygenase-2 mRNA (Guan et al., 1998; Dean et al., 1999). Other possible implications of the activation of p38MAPK by salicylate have been discussed recently. Based on observations that p38 MAPK is essential for cytokine-induction of inducible nitric oxide synthase (Faure et al., 1999; Guan et al., 1999), Clark et al. (2001) have discussed a possible causal relationship between salicylate's ability to enhance cytokine-induced expression of inducible nitric oxide synthase (Nishio and Watanabe, 1998; Durak et al., 1999; Shimpo et al., 2000), and the occurrence of paradoxical pro-inflammatory effects of salicylate in infectious disease of children.

The present in vitro study did not address questions as to the effects of high-dose aspirin administration on cyclooxygenase-2 expression in peripheral blood mononuclear cells in patients. Preliminary data from our laboratories show, however, that in the in vitro model, aspirin has similar effects as sodium salicylate with regard to cyclooxygenase-2 expression in endotoxin-stimulated peripheral blood mononuclear cells. It is the topic of current work to further investigate mechanisms of action by which salicylate can modulate cyclooxygenase-2 expression and to obtain indications for possible occurrence and relevance of this effect in vivo. Our observations raise the possibility that in inflammatory disease, salicylate can enhance COX-2 expression leading to increased PG biosynthesis as soon as the concentration of salicylate falls below that required to oppose the consequences of increased COX-2 expression.

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References

Amin, A.R., Vyas, P., Attur, M., Leszczynska-Piziak, J., Patel, I.R., Weissmann, G., Abramson, S.B., 1995. The mode of action of aspirin-like drugs: effect on inducible nitric oxide synthase. Proc. Natl. Acad. Sci. U.S.A. 92, 7926–7930.

Barrios-Rodiles, M., Keller, K., Belley, A., Chadee, K., 1996. Nonsteroidal antiinflammatory drugs inhibit cyclooxygenase-2 enzyme activity but not mRNA expression in human macro-phages. Biochem. Biophys. Res. Commun. 225, 896–900.

Chen, L.C., Kepka-Lenhart, D., Wright, T.M., Morris Jr., S.M., 1999. Salicylate-enhanced activation of transcription factors induced by interferon-gamma. Biochem. J. 342, 503–507.

Chiabrando, C., Castelli, M.G., Cozzi, E., Fanelli, R., Campoleoni, A., Balotta, C., Latini, R., Garattini, S., 1989. Antiinflammatory action of salicylates: aspirin is not a prodrug for salicylate against rat carrageenin pleurisy. Eur. J. Pharmacol. 159, 257–264.

Clark, I., Whitten, R., Molyneux, M., Taylor, T., 2001. Salicylates, nitric oxide, malaria, and Reye's syndrome. Lancet 357, 625–627.

Dean, J.L., Brook, M., Clark, A.R., Saklatvala, J., 1999. p38 mitogen-activated protein kinase regulates cyclooxygenase-2 mRNA stability and transcription in lipopolysaccharide-treated human monocytes. J. Biol. Chem. 274, 264–269.

DeWitt, D.L., el-Harith, E.A., Kraemer, S.A., Andrews, M.J., Yao, E.F., Armstrong, R.L., Smith, W.L., 1990. The aspirin and heme-binding sites of ovine and murine prostaglandin endoperoxide synthases. J. Biol. Chem. 265, 5192–5198.

Durak, I., Karaayvaz, M., Ozturk, H.S., 1999. Aspirin enhances myocardial inducible nitric oxide synthase activity in guinea pigs. Clin. Chim. Acta 288, 169–170.

Faure, V., Hecquet, C., Courtois, Y., Goureau, O., 1999. Role of interferon regulatory factor-1 and mitogen-activated protein kinase pathways in the induction of nitric oxide synthase-2 in retinal pigmented epithelial cells. J. Biol. Chem. 274, 4794–4800.

Fernandez de Arriba, A., Cavalcanti, F., Miralles, A., Bayon, Y., Alonso, M., Merlos, M., Garcia-Rafanell, J., Forn, J., 1999. Inhibition of cyclooxygenase-2 expression by 4-trifluoromethyl derivatives of salicylate, triflusal, and its deacetylated metabolite, 2-hydroxy-4-trifluoromethylbenzoic acid. Mol. Pharmacol. 55, 753–760.

Grilli, M., Pizzi, M., Memo, M., Spano, P., 1996. Neuroprotection by aspirin and sodium salicylate through blockade of NF-kappaB activation. Science 274, 1383–1385.

Guan, Z., Buckman, S.Y., Miller, B.W., Springer, L.D., Morrison, A.R., 1998. Interleukin-1beta-induced cyclooxygenase-2 expression requires activation of both c-Jun NH_2-terminal kinase and p38 MAPK signal pathways in rat renal mesangial cells. J. Biol. Chem. 273, 28670–28676.

Guan, Z., Buckman, S.Y., Springer, L.D., Morrison, A.R., 1999. Both p38alpha(MAPK) and JNK/SAPK pathways are important for induction of nitric-oxide synthase by interleukin-1beta in rat glomerular mesangial cells. J. Biol. Chem. 274, 36200–36206.

Haslett, C., Guthrie, L.A., Kopaniak, M.M., Johnston Jr., R.B., Henson, P.M., 1985. Modulation of multiple neutrophil functions by preparative methods or trace concentrations of bacterial lipopolysaccharide. Am. J. Pathol. 119, 101–110.

Higgs, G.A., Salmon, J.A., Henderson, B., Vane, J.R., 1987. Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. Proc. Natl. Acad. Sci. U.S.A. 84, 1417–1420.

Hinz, B., Kraus, V., Pahl, A., Brune, K., 2000. Salicylate metabolites inhibit cyclooxygenase-2-dependent prostaglandin E(2) synthesis in murine macrophages. Biochem. Biophys. Res. Commun. 274, 197–202.

Jobke, A., Peskar, B.A., Peskar, B.M., 1973. On the specificity of antisera against prostaglandins A2 and E2. FEBS Lett. 37, 192–196.

Kopp, E., Ghosh, S., 1994. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 265, 956–959.

Lehmann, J.M., Lenhard, J.M., Oliver, B.B., Ringold, G.M., Kliewer, S.A., 1997. Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J. Biol. Chem. 272, 3406–3410.

Meade, E.A., McIntyre, T.M., Zimmerman, G.A., Prescott, S.M., 1999. Peroxisome proliferators enhance cyclooxygenase-2 expression in epithelial cells. J. Biol. Chem. 274, 8328–8334.

Mitchell, J.A., Akarasereenont, P., Thiemermann, C., Flower, R.J., Vane, J.R., 1993. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc. Natl. Acad. Sci. U.S.A. 90, 11693–11697.

Mitchell, J.A., Saunders, M., Barnes, P.J., Newton, R., Belvisi, M.G., 1997. Sodium salicylate inhibits cyclo-oxygenase-2 activity independently of transcription factor (nuclear factor kappaB) activation: role of arachidonic acid. Mol. Pharmacol. 51, 907–912.

Nishio, E., Watanabe, Y., 1998. Aspirin and salicylate enhances the induction of inducible nitric oxide synthase in cultured rat smooth muscle cells. Life Sci. 63, 429–439.

O'Sullivan, M.G., Huggins Jr., E.M., McCall, C.E., 1993. Lipopolysaccharide-induced expression of prostaglandin H synthase-2 in alveolar macrophages is inhibited by dexamethasone but not by aspirin. Biochem. Biophys. Res. Commun. 191, 1294–1300.

Paik, J.H., Ju, J.H., Lee, J.Y., Boudreau, M.D., Hwang, D.H., 2000. Two opposing effects of non-steroidal anti-inflammatory drugs on the expression of the inducible cyclooxygenase. Mediation through different signaling pathways. J. Biol. Chem. 275, 28173–28179.

Panara, M.R., Greco, A., Santini, G., Sciulli, M.G., Rotondo, M.T., Padovano, R., di Giamberardino, M., Cipollone, F., Cuccurullo, F., Patrono, C., Patrignani, P., 1995. Effects of the novel anti-inflammatory compounds, *N*-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulphonamide (NS-398) and 5-methanesulphonamido-6-(2,4-difluorothio-phenyl)-1-indanone (L-745,337), on the cyclo-oxygenase activity of human blood prostaglandin endoperoxide synthases. Br. J. Pharmacol. 116, 2429–2434.

Pillinger, M.H., Capodici, C., Rosenthal, P., Kheterpal, N., Hanft, S., Philips, M.R., Weissmann, G., 1998. Modes of action of aspirin-like drugs: salicylates inhibit erk activation and integrin-dependent neutrophil adhesion. Proc. Natl. Acad. Sci. U.S.A. 95, 14540–14545.

Schwenger, P., Bellosta, P., Vietor, I., Basilico, C., Skolnik, E.Y., Vilcek, J., 1997. Sodium salicylate induces apoptosis via p38 mitogen-activated protein kinase but inhibits tumor necrosis factor-induced c-Jun N-terminal kinase/stress-activated protein kinase activation. Proc. Natl. Acad. Sci. U.S.A. 94, 2869–2873.

Schwenger, P., Alpert, D., Skolnik, E.Y., Vilcek, J., 1998. Activation of p38 mitogen-activated protein kinase by sodium salicylate leads to inhibition of tumor necrosis factor-induced IkappaB alpha phosphorylation and degradation. Mol. Cell. Biol. 18, 78–84.

Shimpo, M., Ikeda, U., Maeda, Y., Ohya, K., Murakami, Y., Shimada, K., 2000. Effects of aspirin-like drugs on nitric oxide synthesis in rat vascular smooth muscle cells. Hypertension 35, 1085–1091.

Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for the aspirin-like drugs. Nature 231, 232–235.

Vittimberga Jr., F.J., McDade, T.P., Perugini, R.A., Callerey, M.P., 1999. Sodium salicylate inhibits macrophage TNF-alpha production and alters MAPK activation. J. Surg. Res. 84, 143–149.

Wu, K.K., Sanduja, R., Tsai, A.L., Ferhanoglu, B., Loose-Mitchell, D.S., 1991. Aspirin inhibits interleukin 1-induced prostaglandin H synthase expression in cultured endothelial cells. Proc. Natl. Acad. Sci. U.S.A. 88, 2384–2387.

Xu, X.M., Sansores-Garcia, L., Chen, X.M., Matijevic-Aleksic, N., Du, M., Wu, K.K., 1999. Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. Proc. Natl. Acad. Sci. U.S.A. 96, 5292–5297.

Yin, M.J., Yamamoto, Y., Gaynor, R.B., 1998. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 396, 77–80.