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# Inhibition of Extracellular Release of Proinflammatory Secretory Phospholipase $A_2$ ($sPLA_2$) by Sulfasalazine

## A NOVEL MECHANISM OF ANTI-INFLAMMATORY ACTIVITY

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**ABSTRACT.** Sulfasalazine is widely used in rheumatoid arthritis and inflammatory bowel diseases. The mechanisms of its activity have not been elucidated. In leukocytes, sulfasalazine and its analogue, CL 42A, inhibited the formation of leukotrienes and possibly of the second messenger compounds at the level of phospholipase C. Partial inhibition of interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α) was also found. Since the synthesis of eicosanoids is induced by phospholipase $A_2$ and since secretory phospholipase $A_2$ ($sPLA_2$) is proinflammatory, we investigated the impact of sulfasalazine and related compounds on mRNA, protein synthesis, and release of $sPLA_2$ from osteoblasts. Sulfasalazine and CL 42A markedly inhibited extracellular release of $sPLA_2$. The impact of sulfasalazine was evident at 50 μM ($P < 0.001$) and maximal at 400 μM, and that of CL 42A at 10 μM ($P < 0.001$) and 200 μM, respectively. Split products of sulfasalazine, 5-aminosalicylic acid (400 μM) and sulfapyridine (400 μM), had no impact. The effect of sulfasalazine and CL 42A was evident regardless of whether the cells were stimulated with IL-1β/TNF-α, lipopolysaccharide/forskolin, or dibutyryl-cAMP. Sulfasalazine and CL 42A did not alter the level of $sPLA_2$ mRNA. Exposure of stimulated fetal rat calvaria osteoblasts (FRCO) to sulfasalazine did not show accumulation of the intracellular $sPLA_2$ protein as tested by western blot; however, enzymatic activity of $PLA_2$ in disrupted cells was definitely increased. Thus, the impact is on the post-transcriptional release of $sPLA_2$ rather than on the synthesis. There was also an increase in the extracellular release of prostaglandin $E_2$ from FRCO exposed to sulfasalazine or to CL 42A. In contrast, sulfasalazine had no effect on the extracellular release of gelatinase from the cells or on mRNA of cytosolic $PLA_2$ or cyclooxygenase 2. We conclude that the anti-inflammatory activity of sulfasalazine may be related, in part, to the selective inhibition of the extracellular release of proinflammatory $sPLA_2$. BIOCHEM PHARMACOL 53;12:1901–1907, 1997. © 1997 Elsevier Science Inc.

**KEY WORDS.** phospholipase $A_2$; sulfasalazine; inflammation

Sulfasalazine is widely used in the therapy of several inflammatory conditions, such as rheumatoid arthritis [1–5], spondyloarthropathies [6], and inflammatory bowel diseases [7, 8]. However, the mechanisms of its anti-inflammatory activity have not been elucidated completely. Studies using human granulocytes have implied that sulfasalazine inhibits the synthesis of phosphoinositide-derived second messenger compounds, probably at the level of phospholipase C or GTP-binding protein [9]. Inhibition of the synthesis of 5-lipoxygenase products and of eicosanoids has been reported [10]. Sulfasalazine was found to reduce IL-2§ secretion from cultured human T lymphocytes [11] and to inhibit IL-1β, IL-6, and TNF-α production by human peripheral blood mononuclear cells [12]. The sulfasalazine analogue CL 42A was generally more potent than sulfasalazine, whereas the split products, sulfapyridine and 5-ASA, were much less inhibitory than sulfasalazine.

The above studies showed inhibition of the synthesis of eicosanoids derived from both Cox and lipoxygenase paths. There was no *in vitro* inhibition of the activity of Cox-1 and -2 by sulfasalazine [13]. To our knowledge, there are no studies of the impact of sulfasalazine and related compounds on the synthesis or activity of the enzymes controlling the arachidonic acid cascade such as $sPLA_2$ or $cPLA_2$. Of special interest would be the study of $sPLA_2$ since this enzyme is strongly implicated as a pathogenetic agent in rheumatoid arthritis and inflammatory bowel diseases. Herein we report that sulfasalazine and CL 42A markedly inhibit extracellular release of proinflammatory $sPLA_2$.

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§ *Abbreviations:* IL, interleukin; FRCO, fetal rat calvaria osteoblasts; $PGE_2$, prostaglandin $E_2$; 5-ASA, 5-aminosalicylic acid; Cox, cyclooxygenase; TNF, tumor necrosis factor; LPS, lipopolysaccharide; $sPLA_2$, secretory phospholipase $A_2$; $cPLA_2$, cytosolic phospholipase $A_2$; and APMA, *p*-aminophenylmercuric acetate.

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## MATERIALS AND METHODS

### *Cell Cultures*

FRCO were cultured as described [14]. Single cell suspensions were prepared by digestion with type I bacterial collagenase (Sigma Chemical Co., St. Louis, MO, U.S.A.). FRCO were seeded onto 35-mm plastic dishes at $3 \times 10^4$ cells/cm$^2$ in Eagle's Minimum Essential Medium containing penicillin G (100 μg/mL), amphotericin B (0.3 μg/mL), gentamicin sulfate (50 μg/mL), and 15% fetal bovine serum. Medium was supplemented with β-glycerophosphate (10 μM) and ascorbic acid (50 μg/mL). Cells were grown to confluence and passaged as required. Cells up to the third passage were used in our experiments. Cell counts and viability, as tested by trypan blue exclusion, were determined throughout the duration of experiments. Cell viability at the initiation of cultures was invariably over 95% and at the termination of experiments over 90%.

Recombinant human (rh) IL-1β and rh TNF-α, were obtained from the Genzyme Corp., Cambridge, MA, U.S.A. Dibutyryl-cAMP (db-cAMP), LPS, and forskolin were obtained from Sigma. Sulfasalazine (mol. wt 398.4), the sulfasalazine analogue CL 42A (mol. wt 325), sulfapyridine (mol. wt 249), and 5-ASA (mol. wt 152) were obtained from Pharmacia Pharmaceuticals, Uppsala, Sweden.

Cytokines IL-1β (0.2 ng/mL) and TNF-α (25 ng/mL) were added to cell cultures, and the cells were cultured further for up to 48 hr. Sulfasalazine and sulfapyridine were dissolved in 0.1 N NaOH and then diluted with sterile PBS to a 40 mM concentration or as required. The pH was adjusted if needed with HCl. Further dilutions were done before each experiment. CL 42A was dissolved in ethanol to a 200 mM concentration and then diluted further as required. The final concentration of ethanol was 0.1%, and the same concentration was used in controls. 5-ASA was dissolved in medium, and pH was adjusted with 0.1 N NaOH. After dissolution all reagents were sterilized by filtration using 0.22 μm filters.

For kinetic experiments, the supernatant samples were withdrawn at various intervals and tested for $sPLA_2$ using both enzymatic assays and western blots (*vide infra*). FRCO were used for northern blot analysis as described in Results. Experiments were performed in triplicate and repeated at least twice. To exclude the possibility that lower $sPLA_2$ activity is related to the changes in the number of cultured cells, $sPLA_2$ activity was expressed both per milliliter of supernatant and per $10^6$ cells.

### *Phospholipase $A_2$ Assay*

Extracellular $PLA_2$ activity was assayed as described [15]. [$^{14}$C]Oleic acid-labelled *Escherichia coli* (strain K12C600) membrane phospholipids were used as the substrate. This substrate has been characterized with respect to phospholipid composition and distribution of radiolabel. Reaction mixtures contained 10 mg BSA, 2 mM $CaCl_2$, $2.8 \times 10^8$ radiolabelled *E. coli*, and 0.1 M Tris–HCl buffer, pH 7.5, in a total volume of 1.5 mL. Reaction mixtures were incubated at 37° for 30 min, and the reaction was terminated by filtration through a 0.45 μm Millipore filter, thereby retaining unhydrolysed *E. coli* membranes, and allowing the [$^{14}$C]oleic acid bound to the BSA carrier, released as a result of $PLA_2$ hydrolysis, to pass through the filter. Assays were performed in triplicate, and values shown represent the means of 3 determinations with the SD < 5% of the mean. Assays were performed in substrate excess, and enzyme activities were corrected for non-enzymatic hydrolysis. One unit of $PLA_2$ activity is defined as the hydrolysis of 56 pmol of phospholipid substrate (representing 1% of total *E. coli* phospholipid) in 30 min at 37°. None of the agents used in our experiments had a direct effect on the $PLA_2$ assay (data not shown). $PLA_2$ synthesized and secreted from FRCO has been identified previously as a group II non-pancreatic $PLA_2$. It was not recognized by anti-pancreatic $PLA_2$ antibody (data not shown) and did bind to monoclonal anti-rat $sPLA_2$ antibody known to recognize rat low molecular weight group II $PLA_2$ but not group I $PLA_2$.

### *Comparison of Extracellular and Intracellular Phospholipase $A_2$*

In separate experiments, cultured FRCO were washed with sterile PBS containing 50 U heparin sodium/mL (Organon Teknika, Toronto, Canada) in order to detach extracellular membrane-bound $sPLA_2$. Afterwards the cells were washed twice in PBS only and lysed by repeat (three times) freezing and thawing. Extracellular and intracellular $PLA_2$ were tested and expressed as units per milligram of protein of lysed cells. Each experiment was done in triplicate. Protein was estimated by the Lowry method using the protein assay kit (Sigma Diagnostics, St. Louis, MO, U.S.A.).

### *Gelatinase Assay*

Gelatinase assay was performed using the gelatin lysis assay as described [16, 17]. Briefly, 60 μL of FRCO supernatant was mixed with 10 μL collagenase buffer with or without 10 μL of APMA and 10 μL of soybean trypsin inhibitor, and incubated at room temperature for 1 hr. Then 10 μL of [$^3$H]gelatin was added, and the mixture was incubated further for 4 hr at 37°. After incubation, 50 μL of 2% unlabelled gelatin and 100 μL of 45% trichloroacetic acid were added and vortexed. The samples were kept for 15 min at 4° and centrifuged at 14,800 *g* for 10 min. The supernatant was collected and counted in a liquid scintillation spectrometer.

### *$PGE_2$ Assay*

Extracellular $PGE_2$ was examined using the $PGE_2$ Elisa Assay Biotrak Kit (Amersham, Oakville, Canada) and was expressed in picograms per milliliter.

### *Western Blot Analysis*

Cell culture supernatants (5–30 μL) were analyzed by SDS–PAGE using a 12% (w/v) gel at a constant voltage of 150 V. The proteins were transferred to a nitrocellulose membrane in 25 mM Tris–HCl, 192 mM glycine, 20% methanol, pH 8.3, buffer at 2 mA/cm. The membrane was incubated with a murine anti-rat $sPLA_2$ monoclonal antibody diluted 1:60 in Tris-buffered saline-Tween-20 buffer for 2 hr. After washing, the membrane was incubated with sheep anti-mouse IgG conjugated to horseradish peroxidase. Detection of immunoreactive bands was carried out using the ECL western blotting detection system (Amersham).

### *Northern Blot Analysis*

Total RNA was extracted with Trizol from FRCO (Trizol Reagent, Gibco BRL, Life Technologies, Burlington, Canada). The RNA (10 μg) was run on a 1% agarose, 2.2 M formaldehyde gel and transferred onto a nylon membrane (Gene Screen Plus, Du Pont, Missisauga, Canada) by capillary blotting. After immobilization of RNA at 80° for 2 hr, the blot was subjected to hybridization. After hybridization the blot was washed 3–4 times with 1× SSC, 0.5% SDS solution at 50° for 2 hr followed by one wash with 0.1× SSC for 10 min at room temperature (SSC = 0.15 M sodium chloride + 0.015 M sodium citrate). The probe used for hybridization was full-length cDNA. Rat $sPLA_2$, $cPLA_2$, and Cox-2 cDNAs and human G3PDH cDNA to monitor equal loading were labeled with [α-$^{32}$P]dCTP using a random primer labelling system (Pharmacia Biotech Inc., Piscataway, NJ, U.S.A.). The human G3PDH probe strongly hybridizes to rat G3PDH mRNA [18]. The blot was exposed to Kodak X-Omat AP film at −70° with an intensifying screen for periods ranging from 24 hr to 6 days as required. The signals on the autoradiographs were assessed by a computing densitometer (Molecular Dynamics and Image Quant, version 4.2, Packard, Canberra, CA, U.S.A.).

Statistical analysis was performed using Instat Statistics, version 2.0 (Graph Pad Software, San Diego, CA, U.S.A.).

## RESULTS

In concentrations up to 400 μM there was no direct *in vitro* impact of sulfasalazine or related compounds on enzymatic activity or immunoreactivity of $sPLA_2$. The viability and growth characteristics of FRCO were preserved.

FRCO were stimulated with IL-1β/TNF-α and were cultured with simultaneously added sulfasalazine for up to 48 hr (Fig. 1). Sulfasalazine at 10 μM inhibited extracellular release of $sPLA_2$ by 17% and at 400 μM by 70%. Incubation for 12 hr was sufficient to reduce markedly extracellular activity of $sPLA_2$.

When FRCO were stimulated with db-cAMP, sulfasalazine at 200 μM reduced the extracellular activity of $sPLA_2$ by 30% (Fig. 2). Stimulation of FRCO by LPS (100 ng/mL) and forskolin (10 μM) was also inhibited by sulfasalazine, 200 μM reducing the extracellular activity of $sPLA_2$ by 40%.

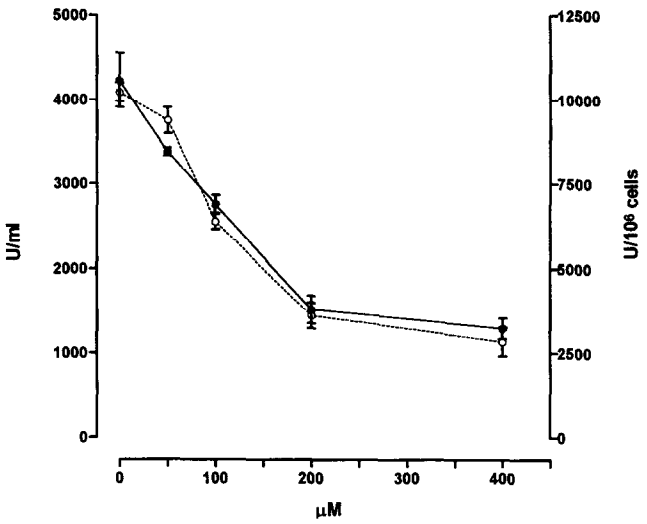


**FIG. 1. Activity of $sPLA_2$ in the medium of osteoblasts cultured in the presence of IL-1β (0.2 ng/mL) and TNF-α (25 ng/mL) and simultaneously exposed to increasing concentrations of sulfasalazine (0–400 μM). Solid line = $sPLA_2$, U/mL; dotted line = $sPLA_2$, U/10$^6$ cells. Values are means ± SD, N = 3.**

CL 42A markedly reduced the extracellular activity of $sPLA_2$ at much lower concentrations. In FRCO stimulated with IL-1β/TNF-α, 1 μM CL 42A reduced it by 22% and 200 μM by up to 71% (Fig. 3). Sulfapyridine and 5-ASA, in concentrations up to 400 μM, minimally reduced $sPLA_2$ activity, the former lowering it from 5542 ± 399 to 4524 ± 477 U/mL ($P < 0.05$) and the latter to 4784 ± 136 U/mL ($P < 0.05$).

To test whether sulfasalazine might have an impact on

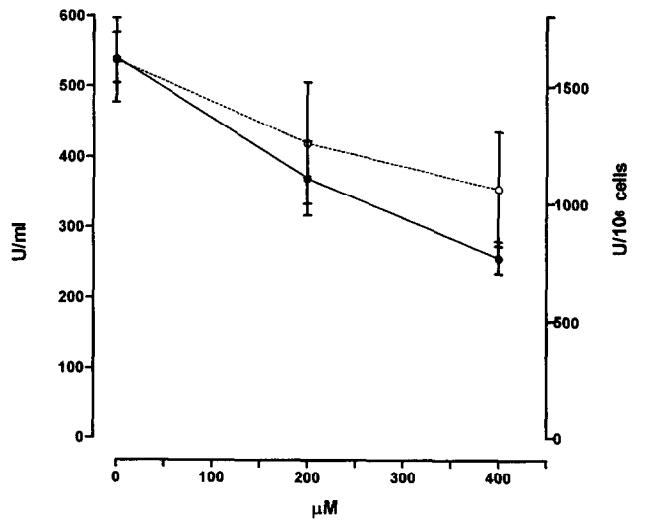


**FIG. 2. Activity of $sPLA_2$ in the medium of osteoblasts cultured in the presence of 4 mM db-cAMP and simultaneously exposed to increasing concentrations of sulfasalazine (0–400 μM). Symbols as in Fig. 1. Values are means ± SD, N = 3.**

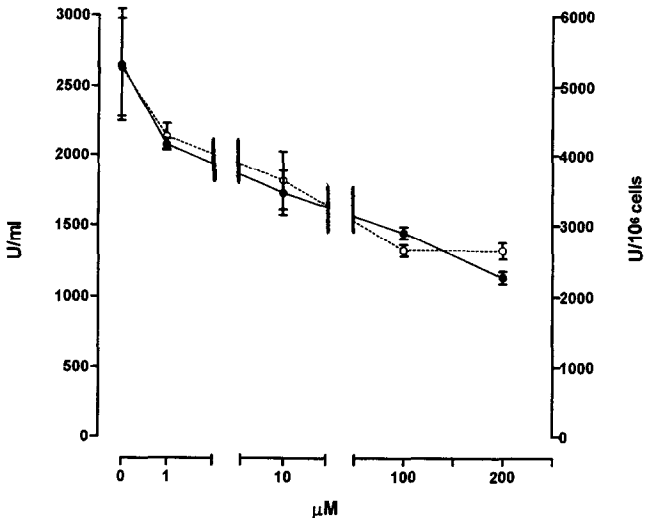


**FIG. 3. Activity of $sPLA_2$ in the medium of osteoblasts cultured in the presence of IL-1β (0.2 ng/mL) and TNF-α (25 ng/mL) and simultaneously exposed to increasing concentrations of CL 42A (1–200 μM). Symbols as in Fig. 1. Values are means ± SD, N = 3.**

the attachment of $sPLA_2$ to the outer cell membrane, in some experiments the cells after incubation with sulfasalazine were washed and incubated with medium containing 50 U heparin/mL for 1 hr. $sPLA_2$ activity was again estimated in the medium. There was no difference in the activity of $sPLA_2$ released from the cells exposed to sulfasalazine as compared with controls (data not shown).

Western blot analysis showed marked reduction in the extracellular protein content of $sPLA_2$, regardless of whether FRCO were stimulated with IL-1β/TNF-α (Fig. 4) or with LPS/forskolin (Fig. 5). There was, however, no visible reduction in the intracellular $sPLA_2$ protein content. Since in this model the western blot analysis may not detect visible changes in the intracellular content of $PLA_2$, this enzyme was tested by a different method relating $PLA_2$ enzymatic activity to the total protein content of disrupted cells (Table 1). Sulfasalazine significantly reduced extracellular $PLA_2$, whereas there was accumulation of intracellular $PLA_2$ content. Northern blot analysis showed no impact of sulfasalazine on mRNA of $sPLA_2$, $cPLA_2$, or Cox-2 (Fig. 6).

Addition of 400 μM sulfasalazine to FRCO stimulated with IL-1β/TNF-α increased the extracellular release of $PGE_2$ from 562 to 1096 pg/mL. A 5-fold increase in $PGE_2$ release was observed after incubation with 200 μM CL 42A.

To test whether the impact of sulfasalazine on $sPLA_2$ release was selective, in some experiments the supernatants were tested for the content of gelatinase (Table 2). There was no effect on the release of 72 kDa enzyme from the cells. There were only traces of the 92 kDa enzyme detected (data not shown).

## DISCUSSION

Sulfasalazine has been used successfully for therapy of several inflammatory diseases such as rheumatoid arthritis (RA) [1–5], Crohn's disease, and ulcerative colitis [7, 8]. Its clinical efficacy and tolerability have been confirmed in long-term follow-up studies of large groups of patients [1–5]. Both clinical and biochemical improvement have been reported in patients with RA [1–5].

In contrast to quite extensive clinical studies, the mechanisms of anti-inflammatory activity of sulfasalazine have not been entirely elucidated. It has been reported that sulfasalazine and its analogue, CL 42A, inhibit markedly the formation of 5-lipoxygenase products such as leukotriene $B_4$ ($LTB_4$), $LTC_4$, and 5-hydroxyeicosatetraenoic acid by human leukocytes [10]. Split products of sulfasalazine, 5-ASA and sulfapyridine, are much less potent [10]. It was suggested that sulfasalazine inhibits the synthesis of phosphoinositide-derived second messenger compounds at the level of phospholipase C or GTP-binding protein [9]. It was not clear how sulfasalazine inhibits synthesis of some prostaglandins [10], without inhibiting directly the activity of Cox-1 and Cox-2 [13]. It was suggested that the inhibitory effect of sulfasalazine may be related to the inhibition of other enzymes of the arachidonic acid cascade or to inhibition of the cellular signalling leading to the activation of Cox [13].

A different line of investigation showed reduction of circulating serum levels of IL-1 and TNF in RA patients treated with sulfasalazine [19]. *In vitro* study of normal human peripheral blood mononuclear cells stimulated with LPS has shown that sulfasalazine inhibited the synthesis of IL-1β, IL-6, and TNF-α. The authors suggested, but did not determine whether the above findings mean that there is inhibition of early steps of signal transduction by sulfasalazine [12]. It was also found that sulfasalazine inhibits, by not yet determined mechanisms, the secretion of IL-2 and IFN-γ from cultured human T lymphocytes [11]. Sulfapyridine and 5-ASA have no effect [11].

Although some of the above studies have shown that sulfasalazine has an inhibitory effect on the arachidonic

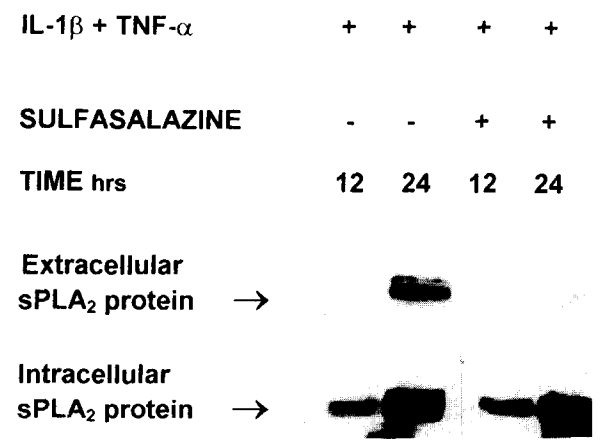


**FIG. 4. Western blot showing extracellular and intracellular $sPLA_2$ protein from osteoblasts cultured in the presence of IL-1β (0.2 ng/mL) and TNF-α (25 ng/mL) and simultaneously exposed to sulfasalazine (400 μM). Incubation times: 12 and 24 hr.**

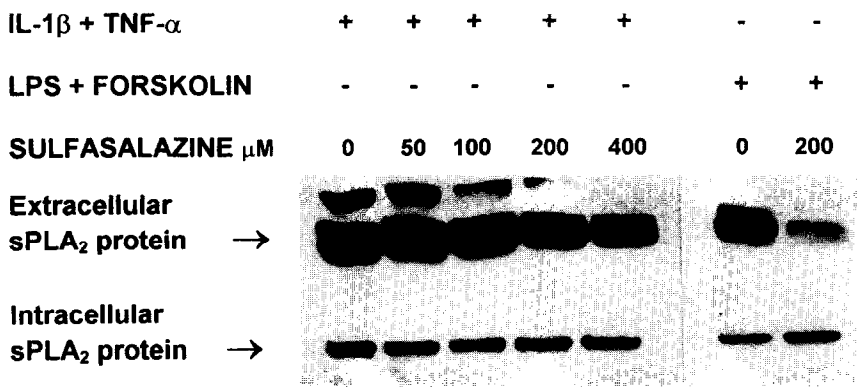


FIG. 5. Western blot showing extracellular and intracellular $sPLA_2$ protein from osteoblasts cultured in either the presence of IL-1β (0.2 ng/mL) and TNF-α (25 ng/mL) or lipopolysaccharide (100 ng/mL) and forskolin (10 μM), and simultaneously exposed to various concentrations of sulfasalazine.

acid cascade, reducing the synthesis of selected eicosanoids induced by either Cox or lipoxygenase [10], no studies of the impact of sulfasalazine on enzymes that proximally control the arachidonic acid cascade, such as $sPLA_2$ or $cPLA_2$, have been reported.

Non-pancreatic phospholipases $A_2$, such as low molecular weight $sPLA_2$ and high molecular weight $cPLA_2$ are distributed widely in mammalian cells [20]. $cPLA_2$ is primarily responsible for intracellular initiation of the arachidonic acid cascade, is not released extracellularly, and does not have direct proinflammatory activity [21]. To the contrary, $sPLA_2$, in addition to its participation in the arachidonic acid cascade, was found to initiate and/or propagate a variety of inflammatory reactions [20]. Several clinical and experimental lines of evidence support the above notion. Very high activity and immunoreactivity of $sPLA_2$ were found in the synovial fluids in inflammatory arthritides [22], and its serum level correlates with the activity of rheumatoid arthritis [23]. Very high enzymatic activity of circulating $sPLA_2$ was observed in systemic inflammatory reaction syndromes such as septic shock [24], malaria [25], or salicylate poisoning [26]. Experimentally, $sPLA_2$ induces dose- and time-dependent inflammatory reaction after injection into joints [27, 28], subcutaneous skin pouches [29], or the skin [30].

$sPLA_2$, identical to that discovered in synovial fluids [31], was implicated as a pathogenetic factor in Crohn's disease and ulcerative colitis [31–36]. It was suggested that mucosal $sPLA_2$ content may reflect the degree of inflammation [33, 35, 36] and that in Crohn's disease and ulcerative colitis circulating $sPLA_2$ may be useful in the evaluation of disease activity [32, 34]. In the ileal mucosa in Crohn's disease the content of $sPLA_2$ mRNA is very high, and it was suggested that $sPLA_2$ may be related to the recurrent ileitis [36]. Furthermore, in actively inflamed colonic mucosa in Crohn's disease and in ulcerative colitis, $sPLA_2$ protein and enzymatic activity and circulating $sPLA_2$ activity were found to be much higher than in inactive Crohn's patients or in controls [32, 34, 35]. Although the cells investigated by us (osteoblasts) are more relevant to articular diseases than to intestinal disorders, the fact that $sPLA_2$ in inflammatory bowel diseases is identical to that found in the articular milieu [31–36] may imply that the cells that synthesize and release $sPLA_2$ in the intestinal tract respond to sulfasalazine in the same way. Furthermore, it was reported that various cells that synthesize $sPLA_2$ share common regulatory pathways [37–39].

The above studies have shown that $sPLA_2$ activity correlates with disease activity in those conditions that respond to therapy with sulfasalazine. Therefore, it was important to investigate whether sulfasalazine is capable of inhibiting $sPLA_2$. Our study has shown that sulfasalazine and its analogue, CL 42A, markedly suppress the release of $sPLA_2$ from osteoblasts. The fact that sulfasalazine inhibited $sPLA_2$ release, regardless of whether the cells were stimulated with cytokines, cyclic AMP analogue, or LPS/forskolin, provides evidence that the pathway of stimulation was irrelevant to the inhibitory activity of sulfasalazine. Sulfasalazine had no effect on the content of mRNA of $sPLA_2$, $cPLA_2$, or Cox-2 and did not inhibit the synthesis of intracellular $sPLA_2$ protein.

To the contrary, accumulation of intracellular $PLA_2$

TABLE 1. Extracellular and intracellular phospholipase $A_2$ in FRCO exposed to sulfasalazine

| Group | Protein (mg/mL) | Phospholipase $A_2$ (U/mg protein) | |
|---|---|---|---|
| | | Extracellular | Intracellular |
| IL-1β, 0.2 ng/mL + TNF-α, 25 ng/mL | 0.29 ± 0.02 | 77,410 ± 6,495 | 4,655 ± 693 |
| IL-1β + TNF-α + sulfasalazine, 400 μM | 0.28 ± 0.02 | 55,529 ± 4,225* | 6,905 ± 458† |

Values are means ± SD, N = 3.
* $P = 0.001$.
† $P = 0.018$.

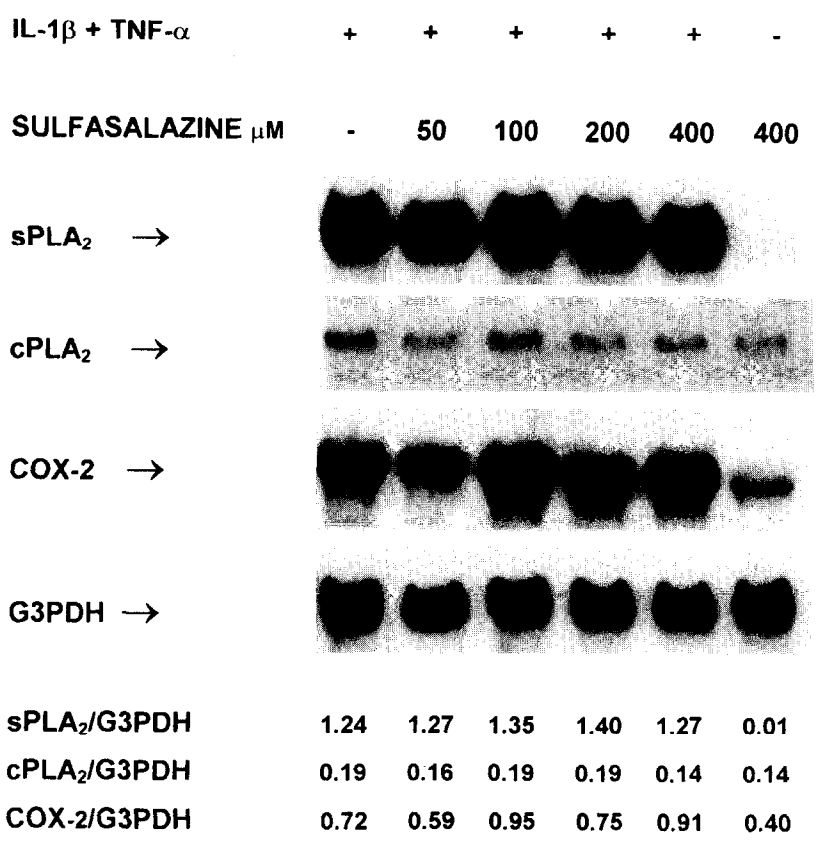


**FIG. 6. Northern blot showing mRNA content of $sPLA_2$, $cPLA_2$, Cox-2, and G3PDH in osteoblasts cultured in the presence of IL-1β (0.2 ng/mL) and TNF-α (25 ng/mL) and simultaneously exposed to increasing concentrations of sulfasalazine (0–400 μM).**

paralleling extracellular reduction of the enzyme was shown by testing disrupted cells. The western blot was found not to be sensitive enough to detect $PLA_2$ accumulation. Stimulated FRCO exposed to sulfasalazine or to CL 42A were found to release more $PGE_2$ than stimulated controls. This finding confirms the results of others who have shown an increase in $PGE_2$ release from human platelets stimulated with arachidonic acid and exposed to sulfasalazine [10]. Our findings suggest that intracellular accumulation of $PLA_2$ is activating the path of eicosanoid synthesis, at least as far as $PGE_2$ is concerned.

In summary, the effect of sulfasalazine on $sPLA_2$ release is post-translational. Since the mRNAs of $sPLA_2$, $cPLA_2$, and Cox-2 were not reduced by sulfasalazine, the impact seems also to be post-transcriptional. Sulfasalazine had no effect on the release of gelatinase from the cells. Thus, the impact on $sPLA_2$ release seems to be selective. The above findings add a new aspect to the elucidation of anti-inflammatory activity of sulfasalazine. It is quite possible that previously reported inhibition of secretion of IL-1β, IL-6, and TNF-α [12] and of IL-2 and IFN-γ [11] by sulfasalazine is analogous to our findings of post-transcriptional block of secretory mechanisms. Further studies are necessary to identify the inhibitory path of sulfasalazine.

**TABLE 2. Extracellular gelatinase released from FRCO exposed to sulfasalazine**

| Additives | [$^3$H]Gelatin (ng degraded/hr) Without APMA | With APMA |
|---|---|---|
| Sulfasalazine, 400 μM | 0 | 3.5 |
| IL-1β/TNF-α | 0 | 35 |
| IL-1β/TNF-α + sulfasalazine, 400 μM | 0 | 45 |

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