

Uncoupled regulation of leukotriene C₄ synthase in platelets from aspirin-intolerant asthmatics and healthy volunteers after aspirin treatment

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Summary

Background We have reported that thromboxane A₂ induces suppression of leukotriene (LT) C₄ synthase activity in human platelets.

Aim In the present study, we describe a mechanism whereby aspirin treatment can lead to increased formation of LTC₄, which is a potent bronchoconstrictor and inflammatory mediator. This mechanism is also demonstrated to be present in platelets from aspirin-intolerant asthmatics (AIA).

Methods The effect of arachidonic acid or platelet agonists on LTC₄ synthase activity was investigated in platelets obtained from healthy volunteers, aspirin-intolerant asthmatics or aspirin-tolerant asthmatics after *in vivo* treatment or *in vitro* pre-incubation with aspirin.

Results Incubation of normal platelets with arachidonic acid or collagen provoked approximately 50% reduction of platelet LTC₄ synthase activity, as determined by the conversion of LTA₄ to LTC₄. However, the inhibitory effect of arachidonic acid or collagen was not observed after oral administration of aspirin prior to collection of the platelets. Arachidonic acid-induced inhibition of LTC₄ synthase activity was totally abolished in platelets collected from peripheral blood already 30 min after aspirin ingestion but was fully restored in platelets collected 3 to 7 days after the administration of aspirin. Treatment of platelet suspensions with aspirin *in vitro* dose-dependently counteracted the suppressive effect of arachidonic acid on LTC₄ formation, with total reversal at approximately 40 µM. In contrast, the major aspirin metabolite, salicylic acid did not alter arachidonic acid-induced reduction of LTC₄ synthase activity. Similarly, LTC₄ synthase activity in platelets from AIA and aspirin-tolerant asthmatics (ATA) was reduced by approximately 50% after pre-treatment with arachidonic acid *in vitro*. Again the inhibitory effect was abolished when platelets were pre-incubated in the presence of aspirin.

Conclusion The results indicate that oral aspirin administration can lead to uncoupling of thromboxane A₂-dependent negative feedback mechanisms, which may normally restrict the production of cysteinyl leukotrienes. This mechanism can be of potential interest in aspirin-induced asthma.

Keywords acetylsalicylic acid, aspirin-sensitive, asthma, cyclooxygenase, leukotriene C₄ synthase, leukotriene C₄, platelets, thromboxane A₂

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Introduction

The cysteinyl leukotrienes (cys-LT), 5(S)-hydroxy-6(R)-S-glutathionyl-7, 9-*trans*-11, 14-*cis*-eicosatetraenoic acid (LTC₄), 5(S)-hydroxy-6(R)-S-cysteinylglycyl-7, 9-*trans*-11, 14-*cis*-eicosatetraenoic acid (LTD₄) and 5(S)-hydroxy-6(R)-S-cysteinyl-7, 9-*trans*-11, 14-*cis*-eicosatetraenoic acid (LTE₄), induce bronchoconstriction, oedema formation and mucus secretion in the human airways, and have been demonstrated to be important mediators of bronchial asthma. Accordingly, anti-leukotriene drugs, which inhibit the biosynthesis or action of these leukotrienes, have recently been introduced as a novel

pharmacological principle for clinical treatment of asthma [1]. The first step in LT biosynthesis involves release of arachidonic acid (AA) from membrane phospholipids, a reaction that can be catalysed by various phospholipases. The subsequent metabolism of liberated AA to leukotrienes is initiated by 5-lipoxygenase (LO), which in co-operation with the 5-lipoxygenase-activating protein (FLAP), catalyses transformation of the substrate to an unstable epoxide, 5(6)epoxy-7, 9-*trans*-11, 14-*cis*-eicosatetraenoic acid (LTA₄). This intermediate can either be converted to LTB₄ by a cytosolic LTA₄ hydrolyase or transformed to LTC₄ by a membrane-bound LTC₄ synthase, which specifically conjugates LTA₄ with glutathione. Once formed, LTC₄ is actively transported to the extracellular space, where it is metabolized to LTD₄ and LTE₄ by successive elimination of a γ-glutamyl residue and glycine, respectively (for review, see [1]).

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The ability to produce leukotrienes from endogenous substrate is essentially restricted to various cell types of myeloid origin. Among those, in particular eosinophils, basophils and mast cells express LTC₄ synthase [2–5]. In addition, platelets have been demonstrated to contain this enzyme [6], allowing these cells to efficiently transform LTA₄ to LTC₄ [7]. This suggests that platelets, although devoid of 5-LO activity, can be involved in *in vivo* biosynthesis of cys-LT via transcellular mechanisms [8], as, for example, activated neutrophils have been demonstrated to release major amounts of LTA₄ [9]. The presence of LTC₄ synthase in platelets is of interest, because these cells are originally derived from the same type of stem cell as the myeloid LTC₄-synthase expressing cells [10]. Furthermore, platelets are not only of importance for the maintenance of haemostasis, but have also been suggested to play a role in allergic and inflammatory conditions [11, 12], although their precise role in these processes has never been convincingly clarified. Interestingly, platelets found in bronchoalveolar lavage fluid from allergic asthmatic patients after local antigen challenge were frequently attached to alveolar macrophages or neutrophils, i.e. LTA₄-producing cells [13]. This provides further support for the concept that platelets may participate in the synthesis of cysteinyl leukotrienes in asthma via transcellular mechanisms [8].

A subpopulation of asthma patients possesses a specific sensitivity to aspirin and other cyclooxygenase inhibitors, which induce severe acute asthma in these individuals. The detailed mechanism behind aspirin-intolerant asthma (AIA) is unknown, although strong evidence indicates that the acute bronchial spasm is provoked by increased formation of cys-LT [14, 15]. In agreement, the urinary excretion of LTE₄ (the major urinary metabolite of LTC₄ in humans) in AIA patients was elevated after oral or bronchial provocation with aspirin, and correlated with the severity of the provoked airway symptoms [16, 17]. Furthermore, antileukotriene drugs have been demonstrated to markedly reduce the airway symptoms induced by aspirin challenge [18, 19]. In addition, a chronic over-production of cys-LT in the airways of AIA patients is indicated by findings of significantly higher basal urinary excretion of LTE₄ in patients with AIA, as compared with aspirin-tolerant asthma patients and normal healthy subjects [16, 17]. The over-production of cys-LT in AIA has been correlated to the presence of markedly elevated numbers of LTC₄ synthase-expressing cells in bronchial biopsies from aspirin-intolerant asthmatics, as compared with tissue from aspirin-tolerant asthmatics and normal individuals [20]. Interestingly, the over-production has also been speculated to be related to increased representation of a genetic polymorphism of the LTC₄ synthase promoter gene in AIA patients [21–23].

Most data indicate that suppression of cyclooxygenase activity is the common trigger event in aspirin-induced asthma. Increased airway prostanoid formation has been documented both in AIA and aspirin-tolerant asthma (ATA) patients [24]. In particular, a specific role for prostaglandin (PG) E₂ in AIA has been postulated [25]. This compound, which has been demonstrated to attenuate leukotriene synthesis in macrophages and eosinophils *in vitro* [26, 27], has been found in increased concentrations in bronchoalveolar lavage fluid in AIA [24]. Furthermore, inhaled PGE₂ has been demonstrated to suppress urinary LTE₄ excretion and protect against aspirin-induced bronchoconstriction in these patients [28, 29]. Accordingly, it

has been postulated that aspirin-induced attenuation of PGE₂ formation may provoke AIA.

The mechanisms regulating LTC₄ synthase activity are poorly understood, although it has been demonstrated that the production of LTC₄ in myeloid cells may be controlled via phosphoregulatory mechanisms [30–33]. In human platelets, ligand binding to the receptors for collagen, thromboxane (TX) A₂ or thrombin or stimulation with the phorbol ester PMA provoked attenuation of LTC₄ synthase activity via phosphoregulatory mechanisms [34]. Furthermore, the findings indicated that LTC₄-synthase activity could be regulated both via protein kinase C- and tyrosine kinase-dependent mechanisms [34]. The finding that TXA₂ can be a negative regulator of LTC₄ biosynthesis is of interest, as it is well documented that increased LTC₄ production is paralleled by elevated synthesis of platelet-derived TXA₂ in asthmatics after allergen challenge [16, 35]. The present study indicates that *in vivo* ingestion of aspirin can uncouple the down-regulation of LTC₄ synthase activity via inhibition of TXA₂ formation. This effect was observed in normal platelets as well as in platelets from AIA and ATA patients. It is suggested that this mechanism may contribute to the increased formation of cysteinyl leukotrienes observed in AIA patients after aspirin challenge.

Materials and methods

Materials

Leukotriene C₄, AA and PGB₂ were obtained from Biomol Research Laboratories (Plymouth Meeting, PA, USA). Leukotriene A₄-methyl ester was a generous gift from Robert Zipkin, Biomol Research Laboratories. The methyl ester was saponified as described [34]. Thrombin, collagen, U-46619 (9, 11-dideoxy-9 α , 11 α -methano-epoxy-prostaglandin F_{2 α}), fatty acid-free human serum albumin, salicylic acid and aspirin were purchased from Sigma (St Louis, MO, USA), and Magnecyl® 500 mg from Pharmacia & Upjohn, Stockholm, Sweden.

Patients and healthy subjects

Peripheral blood was collected from healthy volunteers, mainly laboratory staff. No one had taken non-steroidal anti-inflammatory drugs within 10 days before blood sampling. To investigate the effect of *in vivo* treatment, healthy volunteers were given 2 $\times$ 500 mg aspirin (Magnecyl®, Pharmacia) orally. Blood was collected immediately before and 0.5, 2, 5 and 24 h, as well as 2, 3, 4 and 7 days, after drug administration.

In separate experiments three groups of six subjects each were studied. The first group consisted of three male and three female AIA patients, age ranging from 32 to 62 years, with a history strongly suggesting aspirin-induced asthma with nasal polyposis and a positive bronchial challenge with lysine-acetyl salicylic acid [36]. The second group (ATA) consisted of four male and two female patients, age range 40–55 years, with chronic asthma and nasal polyposis but no history of aspirin intolerance. All six patients except one underwent bronchial challenge tests with lysine-acetyl salicylic acid, and these were all negative. The third group consisted of healthy volunteers; three men and three women, age range 25–57, with no history of allergy, asthma or airway disease. Baseline blood samples were collected from all subjects. In addition a second blood sample was withdrawn,

from four AIA and three ATA patients, approximately 1 h after completed bronchial lysine–acetylsalicylic acid challenge. All blood samples from the three groups of individuals were blindly treated as described.

All participants gave their informed consent and the study was approved by the Ethics Committee of the Karolinska Institute.

Preparation of platelet suspensions

Peripheral blood was collected using 7.5% (v/v) 77 mM EDTA as anticoagulant. After centrifugation of the blood samples at 200 *g* for 15 min, the platelet-rich plasma was removed and further centrifuged at 650 *g* for 20 min. The platelet-containing pellet was suspended and washed twice in Tris-buffered saline containing 1.5 mM EDTA and resuspended in calcium-free Krebs buffer to a final concentration of 400×10^6 platelets/mL. The platelet suspensions contained less than 1 leucocyte/ 2×10^4 platelets as judged by light microscopy.

Incubation procedures

Platelet suspensions (0.5 mL) were equilibrated at 37 °C for 2 min in the presence of calcium chloride (0.9 mM) and human serum albumin (0.3 mg/mL). In the *in vitro* experiments platelets were pre-incubated for 10 min with or without aspirin or salicylic acid. Thereafter the suspensions were incubated for 5 min with or without AA (8 μ M), collagen (200 μ g/mL), thrombin (0.2 U/mL) or U-46619 (1 μ M), prior to assay of LTC₄ synthase activity.

Determination of LTC₄ synthase activity

Leukotriene C₄ synthase activity in platelet suspensions was assayed by incubation with LTA₄ (10 μ M) for 5 min at 37 °C. The production of LTC₄ was terminated by addition of 5 vol. ethanol, containing prostaglandin B₂ as internal standard. Leukotriene analyses were performed by reversed phase-high performance liquid chromatograph (RP-HPLC), using a Nova-Pak C₁₈ column (3.9 $\times$ 150 mm, Waters Ass., Milford, MA, USA), eluted with acetonitrile/methanol/water/acetic acid (27 : 18 : 54 : 0.8, v/v, apparent pH 5.6) 1 mL/min. For identification and quantification of the products, a computerized diode array spectrophotometer (Waters 991) or a variable wavelength UV-detector (IDC Spectromonitor III) connected to an integrator (LDC/Milton Roy CI-4000) was used. Detection of leukotrienes was performed by measurement of UV-absorbance at 280 nm. Products were identified using cochromatography with synthetic standards and on-line UV-spectroscopy.

Statistical analysis

Results are expressed as mean $\pm$ SD. Statistical analyses were performed using Student's *t*-test for paired samples comparing LTC₄ formation in unstimulated platelets and platelets exposed to either of the agonists. A *P*-value < 0.05 was considered statistically significant (**P* < 0.05, ***P* < 0.01 and ****P* < 0.001).

Results

Effect of aspirin and salicylic acid on arachidonic acid-induced inhibition of LTC₄ synthase activity

Approximately 50% decrease in the conversion of exogenous LTA₄ to LTC₄ was observed after incubation of the platelets

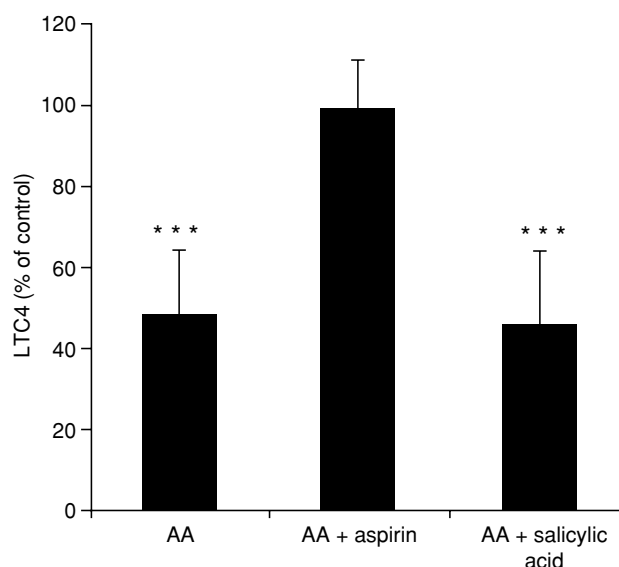


Fig. 1. Effect of aspirin and salicylic acid on arachidonic acid-induced attenuation of platelet LTC₄ synthase activity *in vitro*. Platelet suspensions (400×10^6 /mL) from healthy volunteers were pre-incubated in the absence or presence of aspirin (100 μ M) or salicylic acid (100 μ M) for 10 min at 37 °C prior to incubation with AA (8 μ M) for another 5 min. Thereafter LTC₄ synthase activity was assayed by incubation with 10 μ M LTA₄ for 5 min. Each value represents the mean of six to seven experiments performed in duplicate and is expressed as percentage of basal LTC₄ synthase activity in control incubations without any additions. Error bars indicate standard deviation. Statistical analysis was performed using Student's *t*-test. ****P* < 0.001. AA = arachidonic acid.

with exogenous AA (8 μ M) (Fig. 1), confirming our earlier findings [1]. Basal LTC₄ synthase activity was reflected by the production of 698 ± 364 pmol LTC₄/mL (mean $\pm$ SD; *n* = 7) in unstimulated control suspensions, while 335 ± 112 pmol LTC₄/mL was produced after treatment of the platelets with AA. Pre-incubation of the platelets with 100 μ M aspirin for 10 min totally counteracted the inhibitory effect of AA on the LTC₄ synthase activity (Fig. 1). In contrast, salicylic acid (100 μ M; 10 min) did not affect AA-induced inhibition on LTC₄ formation.

The effect of aspirin on AA-induced inhibition of LTC₄ synthase was dose-dependent (Fig. 2), with 50% and 100% reversal of the inhibitory effect at 20 and 40 μ M aspirin, respectively (Fig. 2).

Effect of aspirin on arachidonic acid-induced inhibition of LTC₄ synthase in AIA and ATA patients

To investigate whether aspirin can uncouple AA-induced inhibition of LTC₄ synthase activity and increase LTC₄ formation also in AIA, the LTC₄ synthase activity was determined in platelets from AIA and ATA patients as well as healthy volunteers. Notably, there was no difference between the three groups concerning basal LTC₄ synthase activity. Thus, in platelet suspensions from AIA patients, ATA patients and healthy volunteers, 353 ± 88 , 369 ± 180 and 466 ± 175 pmol LTC₄/mL (*n* = 7) was formed, respectively, and basal LTC₄ production was not significantly influenced by aspirin. After incubation with arachidonic acid the LTC₄ formation was attenuated to 46 ± 6 , 48 ± 11 and $42 \pm 14\%$ (*n* = 6) of basal activity in the three groups, respectively (Fig. 3). Aspirin (100 μ M) totally

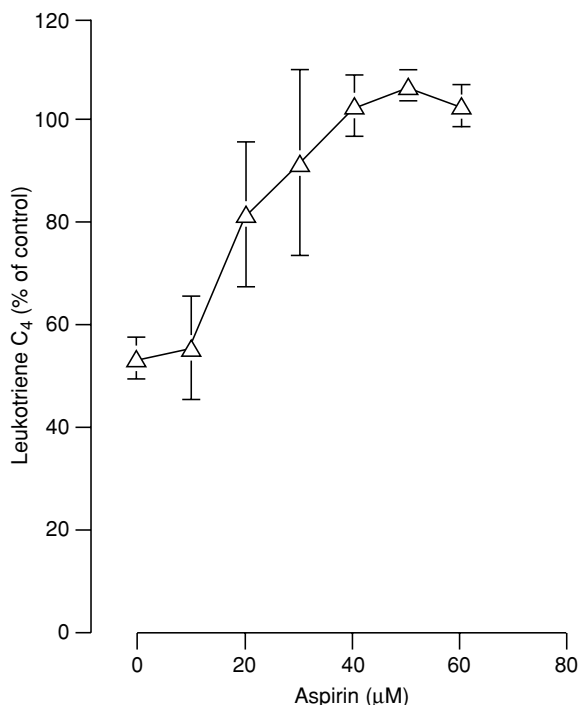


Fig. 2. Dose–response curve of the effect of aspirin on arachidonic acid-induced attenuation of platelet LTC₄ synthase activity *in vitro*. Platelet suspensions ($400 \times 10^6/\text{mL}$) from healthy volunteers were pre-incubated in the absence or presence of aspirin (10–60 μM) for 10 min at 37 °C prior to incubation with arachidonic acid (8 μM) for another 5 min. Thereafter LTC₄ synthase activity was assayed by incubation with 10 μM LTA₄ for 5 min. Each value represent the mean of three experiments performed in duplicate and is expressed as percentage of basal LTC₄ synthase activity in control incubations without aspirin and arachidonic acid. Error bars indicate standard deviation.

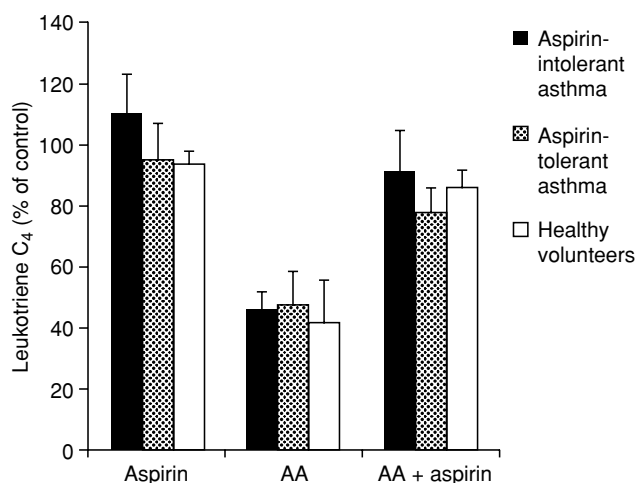


Fig. 3. Effect of *in vitro* aspirin on platelet LTC₄ synthase activity in aspirin-intolerant asthma and aspirin-tolerant asthma patients. Platelet suspensions ($400 \times 10^6/\text{mL}$) from AIA patients, ATA patients and healthy volunteers were pre-incubated in the absence or presence of aspirin (100 μM) for 10 min at 37 °C prior to incubation with arachidonic acid (8 μM) for another 5 min. Thereafter LTC₄ synthase activity was assayed by incubation with 10 μM LTA₄ for 5 min. Each value represents the mean of six experiments performed in duplicate and is expressed as percentage of basal LTC₄ synthase activity in control incubations without any additions. Error bars indicate standard deviation. Statistical analysis was performed using Student's *t*-test. AA = arachidonic acid.

counteracted the inhibitory effect on the LTC₄-synthase activity in platelets from AIA patients as well as in platelets from the two other groups. After *in vivo* lysine-acetylsalicylic acid provocation platelet suspensions from AIA and ATA patients produced 370 ± 85 ($n=4$) and 351 ± 56 ($n=3$) pmol LTC₄/mL, respectively. The unchanged basal LTC₄ formation in peripheral platelets from AIA after lysine-acetylsalicylic acid bronchial provocation indicates that this challenge did not have systemic effects. Again, AA attenuated the LTC₄ formation to 50 ± 9 and $51 \pm 10\%$ of basal activity in the two groups and the inhibition was totally counteracted after pre-treatment of the platelets with aspirin.

Effect of oral aspirin administration on the regulation of platelet LTC₄ synthase activity

Platelets were isolated from peripheral blood collected from healthy volunteers immediately before and 2 h after ingestion of aspirin (1 g) and LTC₄ synthase activity was assayed in resting platelets as well as in platelets exposed to various agonists. In resting platelets, no significant difference in basal LTC₄ synthase activity was observed before and after aspirin administration: 970 ± 390 and 1065 ± 496 pmol LTC₄/mL (mean $\pm$ SD, $n=5$), respectively (Fig. 4). In platelets collected before aspirin administration, AA, collagen, thrombin and the thromboxane receptor agonist U-46619 all significantly suppressed the LTC₄ synthase activity to about 35–45% of control levels (Fig. 4, left panel). However, in platelets collected 2 h after ingestion of aspirin neither AA nor collagen induced attenuation of LTC₄ formation (Fig. 4, right panel). In contrast, the inhibitory effects of thrombin and U-46619 on the conversion of LTA₄ to LTC₄ were unaffected by aspirin administration (Fig. 4).

Time course studies of the effect of *in vivo* aspirin treatment on arachidonic acid-induced regulation of platelet LTC₄ synthase activity

The effect of *in vivo* aspirin on the regulation of platelet LTC₄ synthase activity was studied in platelets collected from healthy volunteers at various times after oral administration of the drug (30 min to 7 days). The inhibitory effect of AA on LTC₄ synthase activity was totally blocked already 30 min after aspirin ingestion and was fully restored 3 to 7 days after drug administration (Fig. 5). Neither basal platelet LTC₄ synthase activity nor the inhibitory effect on enzyme activity induced by U-46619 was changed with time in these experiments (Fig. 5).

Discussion

We have earlier demonstrated that TXA₂ and various platelet agonists can induce attenuation of LTC₄-synthase in human platelets [34]. In this paper we extend these findings by describing a mechanism whereby aspirin treatment can cause elevated synthesis of cysteinyl leukotrienes via altered LTC₄ synthase activity. Thus, oral administration of aspirin to healthy subjects abolished AA- or collagen-induced suppression of *ex vivo* platelet LTC₄ synthase activity. In contrast, these agonists attenuated the conversion of LTA₄ to LTC₄ in platelets obtained from the same subjects immediately before aspirin ingestion. The findings suggest that cyclooxygenase inhibition leading to

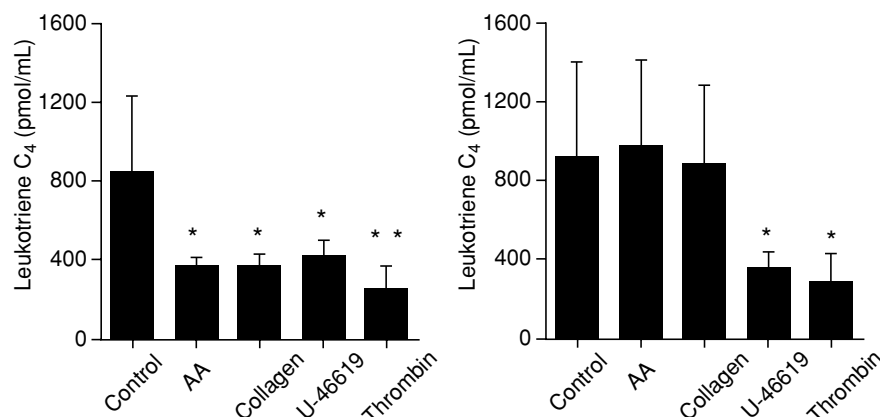


Fig. 4. Effect of oral aspirin administration on agonist-induced inhibition of platelet LTC₄ synthase activity *ex vivo*. Platelet suspensions (400×10^6 /mL) were prepared from peripheral blood collected from healthy volunteers immediately before (left panel) or 2 h after oral administration of 2×500 mg aspirin to the donors (right panel). Thereafter the platelets were pre-incubated for 5 min at 37 °C prior to incubation with arachidonic acid (8 μ M), collagen (200 μ g/mL), U-46619 (1 μ M) or thrombin (0.2 U/mL) for another 5 min. Leukotriene C₄ synthase activity was assayed by incubation with 10 μ M LTA₄ for 5 min. Each value represents the mean of five to seven experiments performed in duplicate. Error bars indicate standard deviation. Statistical analysis was performed using Student's *t*-test for paired samples comparing LTC₄ formation in unstimulated platelets and platelets exposed to either of the agonists, respectively. **P* < 0.05, ***P* < 0.01.

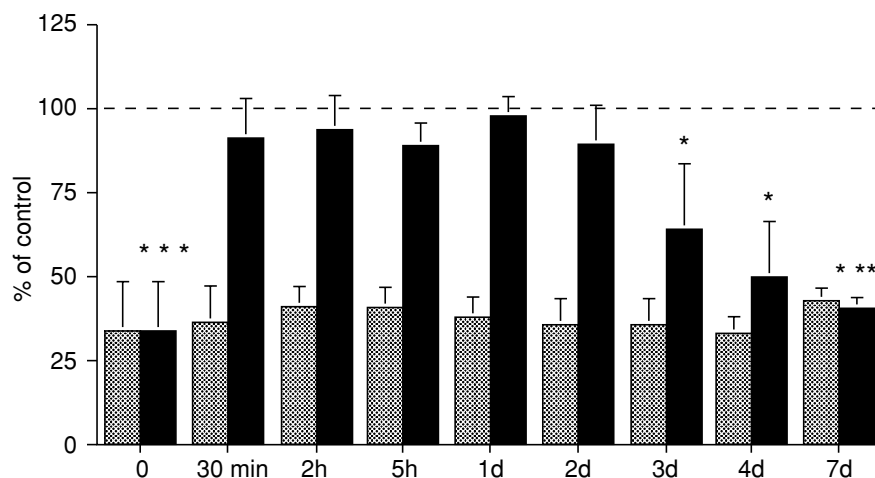


Fig. 5. Time-course of the effect of oral aspirin administration on arachidonic acid- and U-46619-induced inhibition of platelet LTC₄ synthase activity *ex vivo*. Platelet suspensions (400×10^6 /mL) were prepared from peripheral blood collected from healthy volunteers immediately before and various times after oral administration of 2×500 mg aspirin to the donors. Thereafter the platelets were pre-incubated for 5 min at 37 °C prior to incubation without (control) or with arachidonic acid (8 μ M, black bars) or U-46619 (1 μ M, grey bars) and the LTC₄ synthase activity was assayed by incubation with 10 μ M LTA₄ for 5 min. Each value represents the mean of four experiments performed in duplicate. Error bars indicate standard deviation. Statistical analysis was performed using Student's *t*-test for paired samples comparing LTC₄ formation in unstimulated platelets and platelets exposed to either of the agonists, respectively. **P* < 0.05, ****P* < 0.001.

attenuated thromboxane biosynthesis can provoke increased formation of cysteinyl leukotrienes via uncoupling of LTC₄ synthase down-regulation. This is the first described mechanism connecting aspirin ingestion with an effect on LTC₄ synthase. Suppression of LTC₄ synthase activity provoked by the thromboxane receptor agonist U-46619 or thrombin was not affected by aspirin treatment, excluding interference of this drug with ligand receptor binding or the subsequent signal events mediating the inhibition of LTC₄ synthase activity. In agreement, *in vitro* aspirin treatment dose-dependently prevented AA-induced inhibition of platelet LTC₄ synthase activity, while the major aspirin metabolite salicylic acid was without effect on the production of LTC₄. Neither *in vitro* nor *in vivo* aspirin treatment altered basal LTC₄ synthase activity in platelets from healthy volunteers.

Leukotriene C₄ synthase activity was inhibited by pre-treatment with arachidonic acid also in platelets from patients with AIA and ATA and the inhibitory effect was abolished by aspirin also in platelets from these patients. This opens up the possibility that aspirin treatment can lead to elevated biosynthesis of cysteinyl leukotrienes under certain conditions, such as asthma, with ongoing formation of leukotrienes and thromboxane. Therefore it is tempting to speculate that ongoing thromboxane production in the respiratory tissue of AIA patients may constitute an intrinsic negative feedback loop, which limits the over-production of cys-LT under basal conditions. When thromboxane formation is prevented by aspirin or other cyclooxygenase inhibitors, LTC₄ synthase activity is further elevated, leading to increased biosynthesis of cys-LT and provocation of airway symptoms. In agreement with this concept, the

elevated excretion of cysteinyl leukotrienes and aggravated airway symptoms after oral or bronchial aspirin provocation of AIA was reported to be paralleled by suppressed levels of urinary thromboxane metabolites [17, 37, 38]. It should be observed that TXA₂-induced regulation of cysteinyl leukotriene formation may not only be restricted to suppression of LTC₄ synthase activity in platelets. Thus, other LTC₄-producing cell types, such as eosinophils and smooth muscle cells, have been demonstrated to possess TXA₂ receptors [39, 40], and might thus respond to elevated TXA₂ concentrations with down-regulated LTC₄ synthase activity. Furthermore, although platelets have been indicated as the principal source of TXA₂ in asthma [35], this compound may also be produced by other relevant cell types, such as mast cells [41].

Two lines of evidence suggest the involvement of platelet-derived TXA₂ in AIA. First, bronchial asthma is characterized by an on-going inflammation in the airways, with release of pro-inflammatory cytokines, such as IL-1 and TNF- α [42, 43]. Thus, it may be postulated that formation of prostaglandins, e.g. PGE₂, in the inflamed respiratory tissue is mainly dependent on the inducible form of the cyclooxygenase isoenzymes, cyclooxygenase-2 (COX-2) [44, 45]. In agreement, this enzyme has been demonstrated to be induced during inflammatory conditions in human respiratory epithelial cells [46, 47], bronchial smooth muscle cells [48, 49] and lung fibroblasts [50], as well as in macrophages and mast cells [45, 51]. Accordingly, enhanced expression of COX-2 in the airways of AIA and ATA patients has been reported [45]. In contrast, platelets only possess the constitutive COX-1 [52, 44]. Interestingly, pharmacological studies have demonstrated that aspirin and many other conventional non-steroidal anti-inflammatory drugs hold a relative selectivity against COX-1 [53, 46]. In the light of this, and taking into account the low doses of orally administered aspirin needed to provoke severe bronchoconstriction in many AIA patients [37, 38, 54], it may be assumed that AIA is provoked via inhibition of COX-1-dependent prostanoid formation. In agreement, the two selective COX-2 inhibitors rofecoxib and celecoxib have been reported to be well tolerated by aspirin-intolerant patients [55, 56, 57].

Secondly, in the present study the capacity of AA to reduce platelet LTC₄ synthase activity was not fully restored until 3 to 7 days after aspirin administration. As irreversible inhibition of platelet cyclooxygenase with aspirin blocks the production of TXA₂ throughout the life-span of these cells (7 to 10 days), these findings most probably reflect the time needed to generate a partially new population of platelets with intact cyclooxygenase activity. In contrast, aspirin only inhibits prostaglandin production for a few hours in nucleated cells, capable of *de novo* synthesis of the enzyme [58]. In this context it is of interest that the adverse reaction to aspirin in AIA is followed by a refractory period, during which symptoms do not develop if re-challenge is performed [59, 60]. The refractory period usually lasts for 3 to 4 days, i.e. for the same period of time as uncoupled cyclooxygenase-dependent regulation of the platelet LTC₄ synthase activity could be demonstrated in this study. Although the mechanisms behind the desensitization are unknown, it may be assumed that these two phenomena are related.

A role for TXA₂ in AIA has earlier been considered. However, this hypothesis was contradicted by the findings that inhibition of TXA₂ synthase, resulting in markedly decreased levels of

serum TXB₂, did not provoke bronchoconstriction in aspirin-sensitive asthmatics [61, 62]. However, in these studies a concomitant almost 10-fold increase in the levels of 6-keto-PGF_{1 α} (a stable PGI₂ metabolite) was observed [61], indicating shunting of the substrate towards production of PGI₂ and probably also PGE₂, which may have counteracted the emergence of asthma symptoms. In addition, the utilized thromboxane synthase inhibitor, imidazole salicylate, has been shown to be a scavenger and reduce the content of reactive free radicals in inflamed tissue and leucocytes [63, 64], a common mechanism for many lipoxygenase inhibitors. Thus, a concomitant inhibition of leukotriene synthesis can not be excluded and may have contributed to the tolerability to this drug.

In summary, the present findings suggest a mechanism whereby aspirin can induce increased production of cysteinyl-leukotrienes in AIA patients via inhibition of ongoing formation of platelet-derived TXA₂. It is important to note that the data do not explain the underlying mechanisms leading to intrinsic over-production of cysteinyl-leukotrienes in the airways of AIA patients, an issue that was not addressed in this study. However, it may be assumed that this over-production is due to a higher degree of ongoing inflammation, leading to elevated liberation of AA and stimulation of leukotriene biosynthesis. Although the LTC₄ formation is elevated in AIA, the present findings indicate that the LTC₄ synthase activity may still be partially inhibited due to the increased production of TXA₂. In the present paper we have mimicked this situation by treating platelets with AA before assay of LTC₄ synthase activity. If the platelets were pre-treated with aspirin before the addition of AA, TXA₂ biosynthesis, and thus the suppression of LTC₄ synthase, were abolished. The effect was equally obvious in platelets from AIA patients, as well as from patients with ATA and healthy controls. This suggests that TXA₂-dependent regulation of LTC₄ synthase activity is a physiological mechanism, not specifically related to AIA. However, elevated LTC₄ synthase activity after aspirin treatment may be of clinical relevance only in a situation with (a higher degree of) ongoing LTC₄ formation in the airways such as in AIA patients. In accordance, aspirin-induced elevation of LTC₄ synthase activity will not lead to increased LTC₄ formation in normal lung tissue, in which the leukotriene pathway is not activated. In AIA patients, however, aspirin-induced uncoupling of LTC₄ synthase may provoke a further increase in LTC₄ biosynthesis leading to clinical symptoms.

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