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Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions

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ABSTRACT **Aspirin [acetylsalicylic acid (ASA)], along with its analgesic–antipyretic uses, is now also being considered for cardiovascular protection and treatments in cancer and human immunodeficiency virus infection. Although many of ASA's pharmacological actions are related to its ability to inhibit prostaglandin and thromboxane biosynthesis, some of its beneficial therapeutic effects are not completely understood. Here, ASA triggered transcellular biosynthesis of a previously unrecognized class of eicosanoids during coincubations of human umbilical vein endothelial cells (HUVEC) and neutrophils [polymorphonuclear leukocytes (PMN)]. These eicosanoids were generated with ASA but not by indomethacin, salicylate, or dexamethasone. Formation was enhanced by cytokines (interleukin 1β) that induced the appearance of prostaglandin G/H synthase 2 (PGHS-2) but not 15-lipoxygenase, which initiates their biosynthesis from arachidonic acid in HUVEC. Costimulation of HUVEC/PMN by either thrombin plus the chemotactic peptide fMet-Leu-Phe or phorbol 12-myristate 13-acetate or ionophore A_{23187} leads to the production of these eicosanoids from endogenous sources. Four of these eicosanoids were also produced when PMN were exposed to 15*R*-HETE [(15*R*)-15-hydroxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid] and an agonist. Physical methods showed that the class consists of four tetraene-containing products from arachidonic acid that proved to be 15*R*-epimers of lipoxins. Two of these compounds (III and IV) were potent inhibitors of leukotriene B_4-mediated PMN adhesion to HUVEC, with compound IV [(5*S*,6*R*,15*R*)-5,6,15-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid; 15-epi-lipoxin A_4] active in the nanomolar range. These results demonstrate that ASA evokes a unique class of eicosanoids formed by acetylated PGHS-2 and 5-lipoxygenase interactions, which may contribute to the therapeutic impact of this drug. Moreover, they provide an example of a drug's ability to pirate endogenous biosynthetic mechanisms to trigger new mediators.**

Aspirin [acetylsalicylic acid (ASA)] is the lead nonsteroidal antiinflammatory drug and is widely used to relieve inflammation (1, 2). Recent results indicate that its use in low doses is also associated with previously unappreciated beneficial effects, including reduced risk of heart disease (3) and decreased incidence of lung, colon, and breast cancer (4), and *in vitro* it can inhibit nuclear factor NF-κB transcription, which may be relevant in treatment of patients with human immunodeficiency virus (5). ASA's target is the cyclooxygenase activity of prostaglandin G/H synthase (PGHS), a key enzyme in the biosynthesis of prostaglandins (PGs) and thromboxanes (TXs). It does not inhibit the lipoxygenases (LOs) that are involved in production of proinflammatory mediators such as the leukotrienes (LTs) (6–8). Although blockage of PGs and TXs can account for many of ASA's pharmacologic properties, the mechanism of action of this important drug in several clinical settings is still a subject of interest and debate (1, 2).

Eicosanoid biosynthesis by transcellular and cell–cell interactions is now recognized as an important means of both amplifying and generating new mediators, particularly those produced by LOs (9). In humans, lipoxin (LX) biosynthesis is an example of LO–LO interactions via transcellular routes (reviewed in ref. 10). LXs show counterregulatory actions in several tissues. They inhibit polymorphonuclear leukocyte (PMN) transmigration (11), modulate adhesion to endothelial cells (EC) (12), and inhibit chemotaxis of PMN and eosinophils (13, 14). In the microcirculation, LXs are vasodilatory and inhibit leukotriene B_4 (LTB_4)-induced inflammatory responses (15). EC–leukocyte interactions (i.e., rolling, adhesion, etc.) are central to host defense (16), and transcellular metabolism between PMN and EC (17) can promote generation of LTs by EC (18). EC do not produce LTs without leukocyte-derived substrates (18). Earlier results showed that ASA inhibits prostacyclin production by EC, which is an important mechanism in understanding its vascular impact (6–8). Recently, we observed that ASA therapy before angioplasty elevates LO-derived eicosanoids [i.e., LTs and (5*S*,6*R*,15*S*)-5,6,15-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid (LXA_4)] during atherosclerotic plaque rupture in patients (19). Thus, potent vasoconstrictors [e.g., leukotriene C_4 and D_4 (LTC_4 and LTD_4)] and vasodilatory LXA_4 are still generated in vessel lumen when ASA is taken. Here, we report that human EC/PMN coincubations in the presence of ASA generate a previously unreported class of eicosanoids that, in turn, regulate EC–PMN interactions.†

MATERIALS AND METHODS

Synthetic (5*S*,6*R*,15*R*)-5,6,15-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid (15-epi-LXA_4) methyl ester was prepared by total organic synthesis (21) by N. A. Petasis (Department of Chemistry, University of Southern California). Human umbilical vein EC (HUVEC) and PMN were obtained as described (12, 18). Cell viabilities of HUVEC and PMN, as determined by their ability to exclude trypan blue, were 93 ±

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Abbreviations: aspirin, acetylsalicylic acid (ASA); 15*R*-HETE, (15*R*)-15-hydroxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid; EC, endothelial cells; HUVEC, human umbilical vein EC; IL-1β, interleukin 1β; LO, lipoxygenase; LX, lipoxin; LXA_4, (5*S*,6*R*,15*S*)-5,6,15-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid; 15-epi-LXA_4, (5*S*,6*R*,15*R*)-5,6,15-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid; LXB_4, (5*S*,14*R*,15*S*)-5,14,15-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid; PGHS, prostaglandin G/H synthase (cyclooxygenase); PMN, polymorphonuclear leukocytes; LTs, leukotrienes; BCECF, carboxyfluorescein pentaacetoxymethyl ester; PG, prostaglandin; RP-HPLC, reversed-phase HPLC; RT-PCR, reverse transcription and PCR amplification.

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2% and 97 ± 1%, respectively. These values were not significantly altered during incubations.

HPLC analyses were performed with a reversed-phase HPLC (RP-HPLC) system, which consisted of a dual pump gradient (LKB) and a diode array detector (Hewlett–Packard 1040M series II). Post-HPLC analyses were performed with HPLC^{3D} CHEMSTATION software. GC/MS was performed with a Hewlett–Packard 5890 gas chromatograph series II and a 5971A mass-selective detector quadrapole as in ref. 22.

LXA_4 ELISA was from ELISA Technologies (Lexington, KY). Reverse transcription (RT) and PCR amplification (RT-PCR) of PGHS-1, PGHS-2, and 15-LO mRNA was carried out as described (23, 24). PMN adhesion to HUVEC was performed as described (12) with carboxyfluorescein pentaacetoxymethyl ester (BCECF)-labeled PMN.

RESULTS AND DISCUSSION

Coincubations of HUVEC and PMN in the presence of ASA resulted in the formation of several previously undescribed eicosanoids. Fig. 1A shows a RP-HPLC analysis of fractions obtained from cytokine [interleukin 1_β (IL-1_β)]-treated HUVEC coincubated with PMN. The chromatographic profiles from stimulated cells exposed to ASA revealed the presence of four major products, which gave strong UV absorbance (Fig. 1*B*). On-line spectral analysis of materials eluting beneath peaks labeled I–IV showed that they each displayed a triplet of absorbing bands characteristic of conjugated tetraene-containing chromophores indicative of the LX basic structure (maxima at 301 nm and shoulders at 288 and 316 ± 2 nm; see ref. 10). When [^{3}H]arachidonic acid was added to ASA-treated HUVEC/PMN suspensions (80×10^6 cells per ml; HUVEC/PMN cell ratio of 1:10), materials beneath the peaks labeled I–IV (Fig. 1*A*) carried both the tritiated label and UV chromophores (n = 2, where n = number of separate donors). Without ASA these products were not detected in coincubations (Fig. 1), nor were they generated by ASA treatment of either PMN or HUVEC incubated separately (data not shown). Also, HUVEC treated with IL-1_β (155 ± 18 ng; n = 4) and incubated with ASA generated 5–6 times higher levels than those without IL-1_β treatment (27 ± 6 ng; n = 4). Since neither HUVEC nor PMN significantly further metabolizes exogenous LXs (10), these results indicate that these compounds originated from transcellular routes.

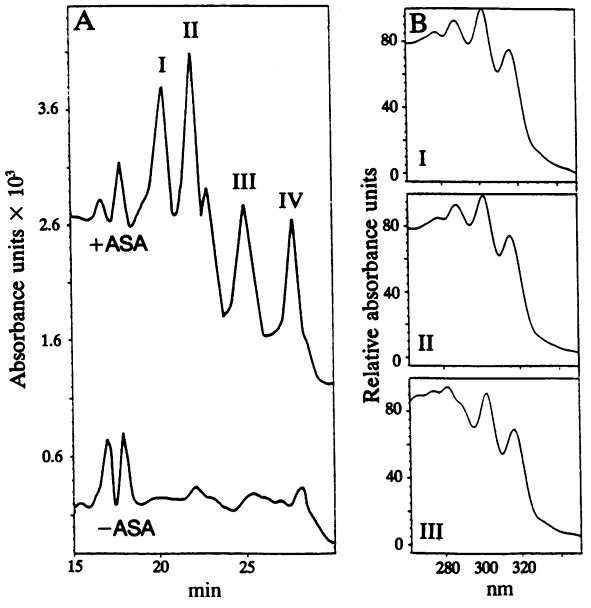

FIG. 1. (*A*) Profile of products from HUVEC and human PMN. Confluent HUVEC were exposed to IL-1_β at 1 ng/ml for 24 hr, washed in Hanks' balanced salt solution (HBSS), and treated with 500 μM ASA or vehicle (0.1% EtOH) for 20 min at 37°C and 20 μM arachidonic acid for 60 s. Next, each was incubated with fresh isolated PMN (HUVEC/PMN cell ratio of 1:50) followed by costimulation with 5 μM ionophore A_{23187} in 4 ml of HBSS for 30 min at 37°C. The HPLC column, Waters μBondapak C_{18} (3.9 × 300 mm), was eluted with MeOH/H_2O/acetic acid, 60:40:0.01 (vol/vol), at 0.6 ml/min, and the detector was set to record UV signals from 220 to 400 nm. Representative chromatograms with (upper trace) and without (lower trace) ASA were plotted at 300 nm (n = 4). (*B*) On-line UV spectra. Similar spectra, each showing absorbance consistent with tetraene chromophores, were obtained from peaks I–IV.

To characterize parameters leading to formation of these products during HUVEC/PMN coincubations, immunoreactive LX was monitored by ELISA (25). ASA dramatically increased formation, while cells incubated for 20 min at 37°C with either indomethacin or salicylate or exposed to dexamethasone for 24 hr did not result in their appearance (Fig. 2*A*). Formation of the new products was also observed at lower ASA concentrations (100 and 250 μM) (145 ± 14 and 166 ± 31 ng per plate, respectively; n = 2, with triplicate determinations). Indomethacin added together with ASA prevented this increase. Production from endogenous sources of arachi-

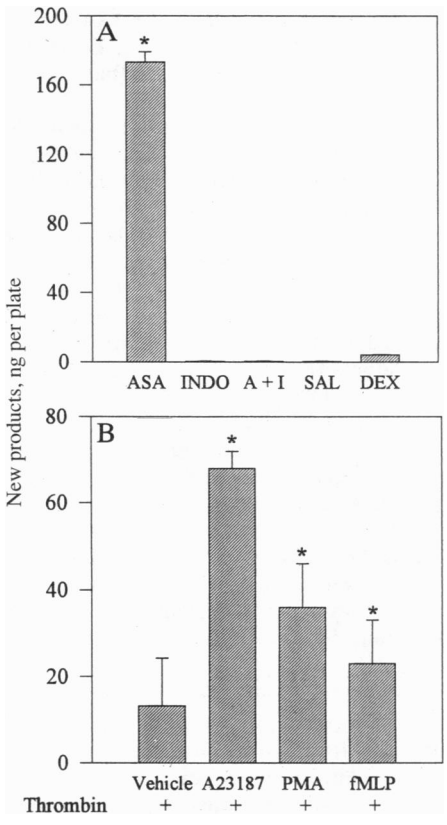

FIG. 2. (*A*) Selective generation by ASA. Confluent HUVEC were exposed for 24 hr at 37°C to 1 ng of IL-1_β per ml and treated for 20 min at 37°C with 500 μM ASA, 500 μM indomethacin (INDO), 500 μM ASA plus 500 μM INDO (A+I), 500 μM salicylate (SAL), or 2 μM dexamethasone (DEX). HUVEC/PMN coincubations were for 30 min at 37°C as in Fig. 1*A*. HUVEC were preincubated with 2 μM DEX for 24 hr. Quantitation of immunoreactive LXA_4 was determined by ELISA (25). Values are expressed in ng per plate (mean ± SEM; n = 3). *, $P < 0.01$, treatment with vs. without ASA. (*B*) Generation from endogenous sources. IL-1_β-treated HUVEC were exposed to 500 μM ASA or vehicle (0.1% EtOH) for 20 min at 37°C and to 1 unit of thrombin per ml for 2 min and were coincubated with PMN, followed by addition of 5 μM A_{23187} or 100 nM fMet-Leu-Phe or 100 nM PMA for 30 min at 37°C. Quantitation was as in Fig. 2*A*. Values are expressed in ng per plate (mean ± SEM; n = 2, with six determinations). *, $P <$ 0.05 vs. control values without ASA.

donate was obtained when HUVEC treated with IL-1_β were exposed to ASA and coincubations were challenged with thrombin (1 unit/ml) plus 100 nM fMet-Leu-Phe or 5 μM ionophore A_{23187} or 100 nM phorbol 12-myristate 13-acetate (PMA) (Fig. 2*B*). Formation of the new products from endogenous sources was also observed when cells were stimulated with either 5 μM A_{23187} (45 ± 10 ng per plate) or 100 nM PMA (28 ± 8 ng per plate) alone but was not statistically significant with either 1 unit of thrombin per ml or 100 nM fMLP added separately with these cell numbers (Fig. 2*B* and data not shown). These results indicate that these unique arachidonic acid-derived products are formed following receptor–ligand activation and that activation of both cell types was required for their biosynthesis. Since these tetraene-containing products crossreacted in the LXA_4 ELISA and given the assay's stereospecific profile (cf. ref. 25), the results indicated that the structure from carbon-1 (C1) through C12 of LXA_4 was recognized in the HUVEC/PMN-derived compounds. However, they clearly did not coelute with LXA_4 in three different RP-HPLC systems (n = 4), suggesting that the C12 through C20 segments of the HUVEC/PMN-derived compounds were different (*vide infra*). Together, these results indicate that the formation of unique eicosanoids from the LX series generated by HUVEC/PMN interactions is a selective feature of ASA treatment.

Is 15-LO Involved in the Biosynthesis of These Eicosanoids? During cell–cell interactions, transcellular biosynthesis of tetraene-containing eicosanoids (i.e., LX) is originated by initial oxygenation of arachidonic acid by either 15-LO [the (15*S*)-15-hydroperoxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid (15*S*-HPETE) route] or 5-LO (LTA_4 route) with subsequent reactions (10). To assess whether endothelial PGHS and/or LO pathways were involved in formation of the ASA-triggered products, mRNA levels of PGHS-1, PGHS-2, and 15-LO were monitored by RT-PCR. Consistent with recent reports (26, 27), the cytokine markedly stimulated PGHS-2 expression that was maximal at 24 hr (Fig. 3). In contrast, PGHS-1 was not induced in the same magnitude, and no evidence of 15-LO was detected with either HUVEC alone or IL-1_β-activated HUVEC, which clearly ruled out 15-LO involvement in the biosynthesis of the ASA-triggered products. Recent findings indicate that, when ASA inhibits PG formation, the resulting ASA-acetylated PGHS-2 enzymatically converts arachidonic acid to (15*R*)-15-hydroxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid (15*R*-HETE), which carries its C15 hydroxyl group in an *R* configuration (28–32). Hence, agents such as ASA that acetylate PGHS-2 (but not other nonsteroidal antiinflammatory drugs tested to date) switch the enzyme active site from PGs to 15*R*-HETE formation (28–32). In support of PGHS-2

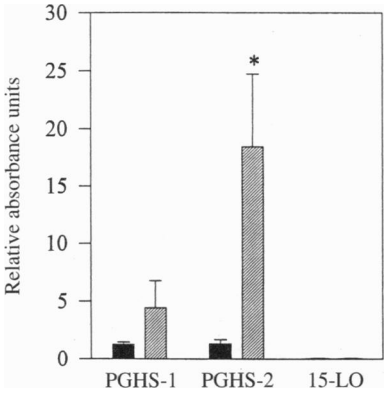

FIG. 3. RT-PCR of total cellular RNA from HUVEC. Total RNA was extracted after 24 hr in the absence (■) or presence (□) of IL-1_β at 1 ng/ml. Results are means ± SEM of three separate experiments. *, $P < 0.05$ IL-1_β vs. no cytokine treatment.

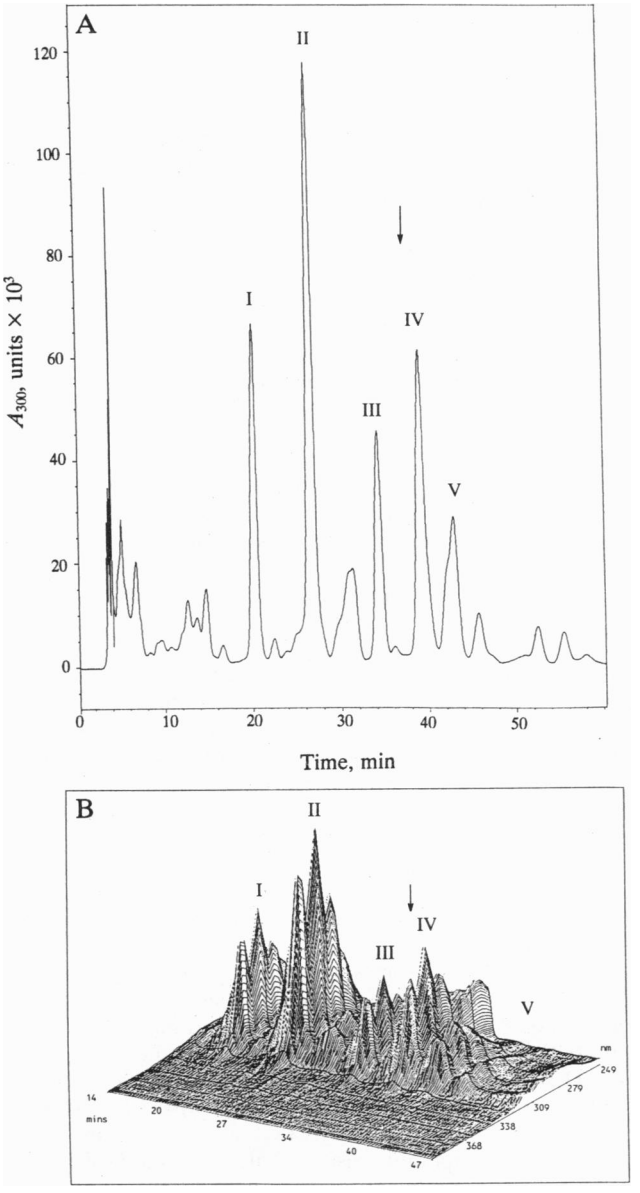

FIG. 4. PMN-derived products: RP-HPLC chromatogram (*A*) and three-dimensional plot (*B*). PMN (50 × 10^6 cells per ml) were incubated with 10 μM 15*R*-HETE and 5 μM A_{23187} for 30 min at 37°C. The column, Waters Nova-pak C_{18} (3.9 × 300 mm), was eluted as in Fig. 1. This is representative of 14 donors. The arrow denotes the retention time of LXA_4, which is not present.

in the biosynthesis of the new products (I–IV), HUVEC exposed to both ASA and indomethacin did not generate the new products (Fig. 2). Also, these results with indomethacin plus ASA are consistent with inhibition of 15*R*-HETE generation observed in human recombinant PGHS-2 (31) and tracheal epithelial cells (32), where indomethacin prevents ASA-acetylation of the enzyme. Together these results provide evidence that acetylated PGHS-2 enhanced by IL-1_β, rather than 15-LO activity, is required in the formation of these eicosanoids.

In view of LX biosynthetic routes in humans (10), it was possible that, during coincubations, PMN could have transformed ASA-triggered endothelial-derived 15*R*-HETE to the new compounds (Fig. 1). To test this, stimulated PMN were incubated with 15*R*-HETE. Analysis of resulting materials revealed production of five major products that again did not coelute with any of the known LXs (n = 14) (Fig. 4). They carried tetraene chromophores (Fig. 4*B*), and compounds I

through IV each matched those labeled I–IV from ASA-treated cells shown in Fig. 1*A* (n = 4). They each (I–IV) coeluted as free acids as well as methyl esters in three RP-HPLC systems (data not shown). Compound V from PMN (Fig. 4) was not generated in appreciable quantities from arachidonic acid by HUVEC/PMN coincubations (n = 4). When compared as substrates, 15*R*-HETE and (15*S*)-15-hydroxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid (15*S*-HETE) (10 μM) proved to be essentially equally converted by stimulated PMN (5 μM ionophore A_{23187} for 20 min at 37°C) into two separate classes of tetraene products. Conversion of 15*R*-HETE to compounds I–V (Fig. 4) ranged from 4.7% to 36.1% with PMN from 14 donors, which was not statistically different from conversion of 15*S*-HETE to LXs and also blocks LT formation (see ref. 10). Also, incubations with a 5-LO inhibitor (5 μM Rev-5901 isomer for 20 min at 37°C; n = 3) completely blocked the conversion of 15*R*-HETE, implicating leukocyte 5-LO.

To further evaluate the materials beneath peaks I–V, they were individually collected and their methyl ester trimethylsilyl derivatives were prepared and taken for GC/MS. Derivatives from compounds I–V each displayed prominent ions at m/e 171, 173, and 203 with ions of lower intensity at 289, 379, 482, and 582 (molecular ion, M^+) (Table 1)—ions that were indicative of the trihydroxytetraene–LX backbone (10). Since each derivative gave retention times in HPLC and GC different from those of native LXA_4, the presence of these ions in their mass spectra and UV spectra provided further evidence that compounds I–V were previously undescribed members of the LX series. The methyl ester trimethylsilyl derivative of compound I displayed a base peak at m/e 173, indicating a 5,14,15-trihydroxyeicosatetraenoate methyl ester, and compounds II through V each gave base peak ions (100%) at m/e 203, supporting the presence of hydroxyl groups at C5, C6, and C15, or 5,6,15-trihydroxy eicosatetraenoate methyl ester isomers of LX (Table 1).

Since the new eicosanoids were generated by EC–PMN interactions, we assessed their activities in LTB_4-induced adhesion of PMN to endothelium. LXA_4 at nanomolar levels inhibits chemotaxis (13, 14), transmigration (11), and adherence (12) of PMN to HUVEC and was included for the purpose of direct comparison. Exposure of PMN to nanomolar levels of the combined fractions I–V, as a mixture of biologically derived compounds, also inhibited PMN adhesion (n = 2, data not shown). When tested individually, two compounds, III and IV (see Figs. 1 and 4), each markedly inhibited adhesion (Fig. 5*A*). Neither of compounds I and II nor their precursor, 15*R*-HETE (10 nM), significantly altered PMN adhesion. Compound IV was a potent inhibitor (Fig. 5*B*) and matched the properties (Table 1) of synthetic 15-epi-LXA_4 in UV-HPLC and GC/MS. 15-epi-LXA_4 did not stimulate either PMN adhesion to HUVEC or homotypic aggregation between isolated PMN in these concentrations (n = 3; data not shown). These results indicate that both compounds III and IV are potent inhibitors of PMN adhesion and that compound IV is 15-epi-LXA_4.

When 15-epi-LXA_4 was added to PMN suspensions and after subsequent extraction and RP-HPLC, ≈50% was recovered as its corresponding 11-*trans* isomer. Similar results were obtained when the compound was extracted from buffer or methanol-inactivated cells. Thus, it was concluded that appearance of compound II reflects workup-induced isomerization of the native product, as is the case for LXA_4. This all-*trans* isomer—namely, 15-epi-11-*trans*-LXA_4—coeluted with compound II (see Figs. 1 and 4) and was inactive in the bioassay (Fig. 5*A*). In view of their behavior in GC and the ions present in mass spectra—namely, a base peak at m/e 203—compounds III and V are also isomers of 15-epi-LXA_4. Compound III possessed marked bioactivity (Fig. 5*A*). It had different double-bond geometry and chirality at C5 and C6 from those of 15-epi-LXA_4 (since they did not coelute) and was not derived from 15-epi-LXA_4. Its complete stereochemistry remains to be determined. Compound I gave a base peak at m/e 173 consistent with a (5*S*,14*R*,15*S*)-5,14,15-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid (LXB_4)-related product but was biologically inactive. These findings, together with the known biosynthetic routes for LXs (10), identified compound I as 15-epi-8-*trans*-LXB_4. It is of interest to note that UV-HPLC and GC/MS analyses of material eluting beneath the

Table 1. Physical properties of compounds I–V

Compound	GC,‡	MS diagnostic ions‡
LXA_4	24.1	171, 173, 203 (100%), 289, 379, 482, 582
I	23.9	171, 173 (100%), 203, 289, 379, 482, 582
II	23.9	171, 173, 203 (100%), 289, 379, 482, 582
III	23.8	171, 173, 203 (100%), 289, 379, 482, 582
IV§	23.9	171, 173, 203 (100%), 289, 379, 482, 582
V	24.7	171, 173, 203 (100%), 289, 379, 482, 582

Compounds I–V were obtained from human PMN incubated with 10 μM 15*R*-HETE and 5 μM A_{23187} for 30 min at 37°C.
‡C values (equivalent chain length) and fragmentation patterns were obtained for the methyl esters of the trimethylsilyl derivatives of each parent compound.
§Compound IV matched synthetic 15-epi-LXA_4.

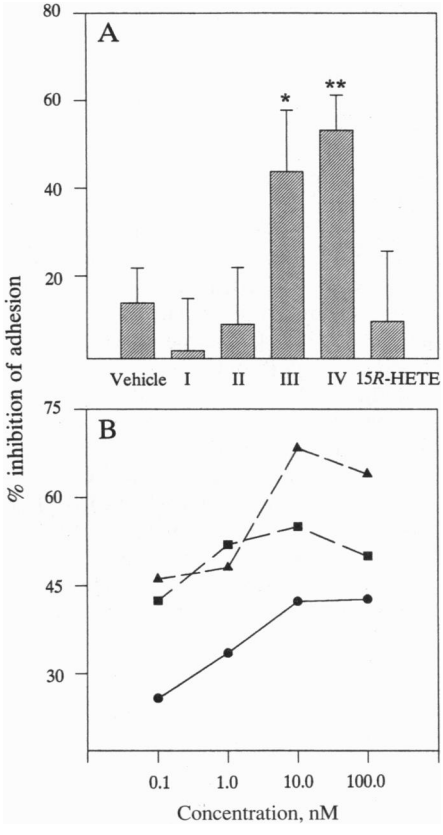

FIG. 5. Inhibition of PMN adhesion to HUVEC. (*A*) Activity of compounds I–IV. BCECF-labeled PMN were treated with vehicle or equimolar (10 nM) concentrations of compounds I–IV or 15*R*-HETE for 15 min at 37°C and then washed once before coincubation with HUVEC and addition of 100 nM LTB_4. Data are means ± SEM of 3–9 experiments. Results are expressed as percent inhibition of LTB_4-induced adhesion. *, $P < 0.05$; **, $P < 0.001$ for compounds vs. vehicle. (*B*) Concentration dependence. BCECF-labeled PMN were exposed to LXA_4 (●), 15-epi-LXA_4 (▲), or compound IV (■) for 15 min and treated as in Fig. 5*A*. Representative curves are from 7–10 experiments performed in duplicate. Results are expressed as the percent inhibition of PMN adhesion in the presence of 100 nM LTB_4. All values were significantly ($P < 0.05$) different from control values.

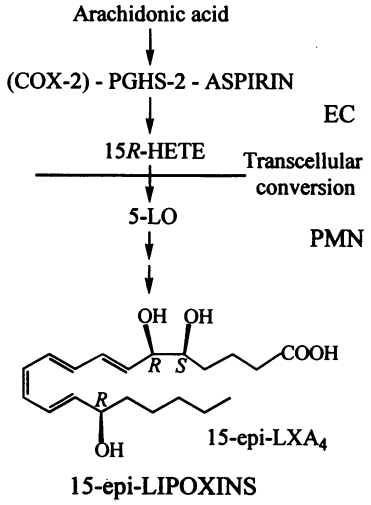

FIG. 6. Proposed scheme: a novel mechanism of ASA action. Acetylation of PGHS-2 (cyclooxygenase 2) results in 15*R*-HETE generation by HUVEC and transcellular conversion by activated PMN to 15-epi-LXs. 15*R*-HETE can also be generated by P450. Biosynthesis is likely to proceed via a 15-epi-5(6)-epoxytetraene intermediate and subsequent steps blocking LT formation. See text for details.

peak at 31.2 min (Fig. 4*A*) were consistent with 15-epi-LXB_4 (data not shown). Although this compound was not observed in all chromatograms, its presence suggests that 15-epi-LXB_4 can also be formed by ASA treatment. Together, the physical properties of compounds I–V point to the formation of a 15-epi-5(6)-epoxytetraene intermediate [i.e., (15*R*)-15-hydroxy-LTA_4] produced from EC-derived 15*R*-HETE.

Our results provide evidence that ASA triggers formation of another class of eicosanoids during cell–cell interactions exemplified here by interactions between EC and PMN and identified as the 15-epimer class of LXs. Generation of 15-epi-LXs involves conversion of arachidonic acid by acetylated PGHS-2 in EC and 5-LO in leukocytes (Fig. 6). Although the results obtained clearly rule out the involvement of 15-LO in 15-epi-LX formation, they don't preclude a possible contribution of 15*R*-HETE that can be generated by cytochrome P450 in other cells (33). The amounts produced by coincubations of EC/PMN from endogenous sources were within the potency range of these compounds, since 15-epi-LXA_4 was active at subnanomolar concentrations (Fig. 5). PGHS-2 is expressed in tissues where ASA has beneficial effects [i.e., lung epithelial cells (32), colorectal mucosa (34), and macrophages (35)]. These are additional sites where exposure to leukocyte traffic [e.g., lymphocytes (36) and neutrophils], cells which carry 5-LO, can be high. Also, changes in chirality of alcohol groups in eicosanoids, as in the present findings (Figs. 1 and 4) or native LXs (10) dramatically alter their biologic activities, in some cases converting active to inactive compounds or vice versa. The biosynthetic switch from 15*S*-hydroxyl-carrying LXs to 15*R*-hydroxyl-carrying LXs triggered by ASA enhanced (≈2 times) activities [i.e., 15-epi-LXA_4 (compound IV) > LXA_4 >>>> 15-epi-11-*trans*-LXA_4 (compound II) (Fig. 5)].

Cytokine regulation of eicosanoid-generating enzymes can enhance or "turn on" the formation of LXs at "later stages" of inflammation or tissue injury during cell–cell interactions (10, 37). The present results establish a previously unrecognized biosynthetic circuit involving PGHS-2 interactions with the 5-LO. Thus, these enzymes are likely to be in place before ASA therapy is administered. The finding that ASA triggers biosynthesis of a previously unrecognized class of products from cell–cell interactions *in vitro* suggests that drugs such as ASA may influence inflammation, differentiation, or proliferation by pirating endogenous biosynthetic mechanisms to produce different mediators. This does not exclude potential "shunting" of arachidonic acid to LO pathways but rather opens a previously unrecognized biosynthetic route. The ability of ASA to stimulate 15-epi-LX formation provides new avenues for investigating mechanisms underlying important actions of ASA in humans.

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