Angiotensin II Receptor–Independent Antiinflammatory and Antiaggregatory Properties of Losartan
Role of the Active Metabolite EXP3179

Christine Krämer, Julia Sunkomat, Jana Witte, Maren Luchtefeld, Michael Walden, Boris Schmidt, Rainer H. Böger, Wolf-Georg Forssmann, Helmut Drexler, Bernhard Schieffer

Abstract—Angiotensin II (Ang II) type 1 receptor (AT1) antagonists such as losartan (LOS) are widely used for the treatment of hypertension and elicit antiinflammatory and antiaggregatory in vitro and in patients, although the underlying mechanism are unclear. Following computer-based molecule similarity, we proposed that on cytochrome-P450 degradation, the LOS metabolite EXP3179 is generated, which shows molecule homology to indomethacin, a cyclooxygenase inhibitor with antiinflammatory and antiaggregatory properties. Subsequently, serum-levels of EXP3179 were determined for 8 hours in patients receiving a single oral dose of 100 mg LOS. High-performance liquid chromatography followed by liquid chromatography–mass spectrometry (LC-MS) from serum samples revealed a maximum of 10⁻⁷ mol/L for EXP3179 peaking between 3 to 4 hours. The increase in serum-EXP3179 levels was associated with a significant reduction in platelet aggregation in vivo (−35±4%, P<0.001 versus control). EXP3179 generation was investigated in a chemical reaction mimicking the liver cytochrome-P450–dependent LOS-degradation and human endothelial cells were exposed to Ang II or lipopolysaccharides (LPS) in the presence of EXP3179 (10⁻⁷ mol/L). LPS- and Ang II–induced COX-2 transcription was abolished by EXP3179. Moreover, EXP3179 significantly reduced Ang II– and LPS-induced formation of prostaglandin F2α as determined by LC-MS. Thus, antiinflammatory properties of LOS are mediated via its EXP3179 metabolite by abolishing COX-2 mRNA upregulation and COX-dependent TXA2 and PGF2α generation. Serum levels of EXP3179 are detectable in patients in concentrations that exhibit antiinflammatory and antiaggregatory properties in vitro. (Circ Res. 2002;90:770-776.)

Key Words: cyclooxygenase ■ platelet aggregation ■ thromboxane A₂ ■ prostaglandin F₂α ■ atherosclerosis

Eicosanoids are critically involved in the modulation and perpetuation of acute and chronic inflammatory processes.1 Atherosclerosis is a chronic inflammatory disease characterized by enhanced thrombogenesis and increased serum levels of eicosanoids.3 The formation of arachidonic acid (AA) is responsible for eicosanoid formation in the vessel wall of acute coronary syndromes,3,4 which are rapidly transformed into prostaglandins, eg, thromboxane A2 (TXA2), a potent vasoconstricting and platelet-aggregating substance, and into leukotrienes known to perpetuate inflammatory processes.1–4 The rate-limiting enzymes for prostaglandin (PG) formation from AA are phospholipases (PLA), responsible for AA-generation and cyclooxygenases (COX) responsible for AA-processing.5 Whereas COX-1 is constitutively expressed in almost all cell types, COX-2 synthesis and release is induced by growth factors and cytokines, eg, interleukin (IL)-1 and platelet-derived growth factor (PDGF).4 COX-1 and COX-2 catalyze the formation of PGH₂ from AA, which is further metabolized to PGF₂α and to TXA₂.1,4 PGF₂α and TXA₂ are mediators of inflammation by stimulating platelet aggregation and local vasoconstriction1,3 and are elevated in patients with unstable angina.3,6 Nonsteroidal antiinflammatory drugs (NSAIDs), eg, acetylsalicylic acid, inhibit synthesis of prostaglandins and thromboxanes by blocking the cyclooxygenase activity of COX enzymes and thereby reducing morbidity and mortality of patients with coronary artery disease.7 Therefore, these NSAIDs are essential therapeutic components for the treatment of stable and unstable angina.6,7

COX-2 synthesis and release is induced by angiotensin II (Ang II), the effector molecule of the renin-angiotensin system (RAS) in vascular smooth muscle cells,8,9 and both are expressed at the coronary atherosclerotic plaque.10 These effects are mediated via the classic type 1 G protein–coupled receptor for Ang II named AT₁ receptor.8 Clinical results from the Heart Outcome Prevention Trial (HOPE) demon-
stratized that blockade of Ang II effects abolish vascular events such as unstable angina, myocardial infarction, or stroke in patients with severe atherosclerosis.11 While similar trials for AT1 antagonists are still pending, experimental data suggests that antagonists at the AT1 receptor level, eg, losartan (LOS) or irbesartan elicit antithrombotic properties by blocking inflammatory mediators.12–14 In mice and primate models of atherosclerosis,15,16 it was observed that LOS inhibits fatty-streak formation and reduces plasma lipids and surrogate markers of vascular injury, eg, monocyte chemoattractant protein (MCP)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin, independent from its effects on blood pressure.15–17 Moreover, recent observations indicated that LOS itself elicits antiaggregatory effects in vitro14,18 and in an animal model of arterial thrombosis. These effects are independent from changes in blood pressure.19 Together, these observations substantiate that LOS develops antiinflammatory and antiaggregatory effects independent from its antagonism at the AT1 receptor.

LOS is a prodrug and its main antihypertensive AT1-blocking metabolite EXP3174 is generated on liver first-pass. Therefore, we speculated that another LOS metabolite may be responsible for the antiinflammatory and antiaggregatory effects of losartan. To address this question, computer-based molecular comparison of known LOS metabolites20 with published antiinflammatory drugs was performed in search of a molecular homology. A candidate metabolite of LOS for this effect was isolated from serum-samples of patients receiving a single dose of 100 mg LOS orally. This candidate molecule was generated from LOS by mimicking the liver cytochrome-P450 pathway. To test the potential antiinflammatory and antiaggregatory properties of this candidate molecule, human umbilical vein endothelial cells (HUVECs) and isolated platelets were stimulated with Ang II and lipopolysaccharide (LPS) as AT1-independent stimulus of prostaglandin generation. Subsequently, COX-2 mRNA upregulation and downstream of the COX enzyme, PGF2α release, and TXA2-dependent platelet aggregation was determined. Finally, platelet aggregation was analyzed in patients after 100 mg LOS administration.

Here, we report that LOS elicits COX-2 inhibitory properties via its EXP3179 metabolite by blocking COX-2 mRNA upregulation, intracellular adhesion molecule (ICAM)-1 mRNA upregulation, and cyclooxygenase-dependent TXA2 and PGF2α generation in vitro. Moreover, EXP3179 abolishes AA-induced platelet aggregation in vitro, indicating an inhibitory effect on the cyclooxygenase activity of COX enzymes. EXP3179 is detectable in serum-samples of patients and promotes antiaggregatory effects in vivo. Thus, losartan has antiinflammatory properties independent of its blockade at the AT1 receptor.

Materials and Methods

Computer-Based Substructure Search

Beilstein database analysis (http://www.beilstein.com) was performed in search of a molecular similarity of LOS metabolites with known antiinflammatory drugs. Losartan metabolites recently suggested by Stearns et al20 were used as reference molecules.

In Vitro Synthesis of EXP3179

EXP3179 was synthesized from losartan in vitro in a chemical reaction mimicking the liver cytochrome-P450 metabolization. The reaction of LOS to EXP3179 was achieved by incubating LOS with RuCl3 and H2O2 in MeCN for 2 hours at 60°C followed by liquid-chromatography purification.

Northern Blot Analysis of COX-2 Expression

HUVECs (Oncogene Science, Inc) were maintained in serum-free conditions for 24 hours and stimulated with Ang II (10−7 mol/L) or LPS (100 µg/mL) in the presence of EXP3179 (10−7 mol/L). COX-2 mRNA expression was determined by Northern blot analysis.10,11 The 947-bp cDNA COX-2 probe was generated by PCR using oligonucleotide primers forward 5′-CAGATGAAAGCTTGGCCG-3′ and reverse 5′-ATGATTGCCCGACTCCC-3′. The blots were visualized by a PhosphorImager (Fuji Bas 1000) and exposed to autoradiography. Northern blots were analyzed using an image analysis system (Gel Doc 2000, Bio-Rad) and results were statistically processed (Sigma Plot).12

Reverse Transcription–Polymerase Chain Reaction

Total RNA was isolated and first strand synthesis was carried out with total cDNA using reverse-transcriptase and oligo d(T)primers.21 Semi-quantitative PCR was carried out by normalizing all cDNAs to GAPDH. Primer sequences for ICAM1: 5′-CAGAGTTGTAACCCACCAGT-3′ and reverse/antisense, 5′-CAGGGTAGGTTGGCCCCTTCAAG-3′. All cDNA was tested for equal amounts of GAPDH. PCR-fragments were densitometrically analyzed (GelDoc 2000, Bio-Rad) and blotted using Sigma Plot (Jandel Inc).

Prostaglandin Synthesis

PGF2α-concentration was determined by gas chromatography–tandem mass spectrometry (GC-MS/MS, model TQ 7000, ThermoQuest) in the supernatent media of HUVECs as described recently.21 Briefly, the internal standard [3,3-2H4]-PGF2α (MSD, Canada) was added to the supernatent aliquots at a final concentration of 2 ng/mL. Samples were acidified to pH 3 by the addition of 5 mol/L formic acid, and then extracted on octadecylsila solid phase extraction cartridges (Macherey-Nagel). Quantitation was performed by selected reaction monitoring of the product ions with mass-to-charge ratio (m/z) of 299 for PGF2α and 303 for the internal standard, which was produced by collision-activated dissociation from the parent ions at m/z 569 and 573, respectively. Concentrations of PGF2α were calculated from the respective ratio of the peaks of the ions m/z 299 and m/z 303 with the concentration of the internal standard, ie, 2 ng/mL. The peak area ratio for the internal standard was 0.003.21

Subject Characteristics

Twenty-eight patients with essential hypertension (see Table) were treated with a single oral dose of 100 mg LOS. Coronary artery disease had been ruled out angiographically in all patients and acetyl salicylic acid or other antiphlogistic drugs (NSAIDS) for the past 21 days was excluded. The baseline patient characteristics are summarized in the Table. The study was approved by the Hannover Medical School ethic committee. Before oral administration of 100 mg LOS, control blood samples were drawn and baseline hemodynamic parameters were obtained (Table). Measurements were performed for an 8-hour observation period. Blood samples were drawn from the antecubital vein. All samples were cooled on ice and serum was extracted at 4°C, 3000g. Serum samples were stored at −70°C until assays were performed.

Sample Preparation

For LOS-metabolite analysis, proteins from serum samples were precipitated by acetonitrile (40% vol/vol), and extracted on a C18-solid phase extraction columns (500 µg C18, Varian) with acetonitrile as eluent.
Patient Characteristics

<table>
<thead>
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<td>Age, years</td>
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<td>Male</td>
<td>16</td>
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<td>Essential hypertension</td>
<td>28</td>
</tr>
<tr>
<td>Smokers</td>
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<td>Potassium, mmol/L</td>
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<td>Sodium, mmol/L</td>
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<td>Quick, %</td>
<td>101.5±23.8</td>
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<tr>
<td>Creatinine, µmol/L</td>
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Liquid Chromatography–Mass Spectrometry (LC-MS)

Extracted samples were scanned for the presence of LOS metabolites using a double syringe micro-high-performance liquid chromatography (HPLC)-pump (ABI 140B, Applied Biosystems), a PE series 200 autosampler (Perkin-Elmer), and a ReproSil-Pur C18-AQ column (3 µm, 120 Å, 250×1 mm ID) (A. Maisch, Ammerbuch, Germany) coupled to an API 100 single quadrupole mass spectrometer controlled by the accompanying sample control 1.2 software (PE Sciec). The metabolites were eluted with an acetonitrile gradient (30% to 90% B within 30 minutes; eluent A: 0.06% [vol/vol] TFA in water; eluent B: 0.05% [vol/vol] TFA in acetonitrile, flow 20 µL/min, sample volume 20 µL) and scanned in selected ion mode adjusted to the expected molecular masses of LOS and its metabolites (420 Da, 422 Da, 437 Da, 439 Da, 453 Da, and 584 Da with an isolation width of 1.5 amu in steps of 0.1 amu). Counts for LOS and its metabolite to the COX inhibitor indomethacin. To test whether this latter is of clinical and/or pathophysiological relevance, serum concentrations of EXP3179 were determined. Blood samples were obtained from 28 patients with essential hypertension receiving a single oral dose of 100 mg LOS. The candidate molecule EXP3179 and the main AT1-blocking metabolite EXP3174 (used as control) were determined by LC-MS. While EXP3174 peaked at 4 hours as described recently,23,24 EXP3179 increased at 2 hours and peaked between 3 to 5 hours with a rapid decline at 6 hours (Figures 1A and 1B). Maximum serum concentrations were calculated for EXP3174 and EXP3179 using losartan as standard. Results demonstrated a maximum serum concentration of EXP3179 of 2.8×10^-7 mol/L, losartan 2.6×10^-7 mol/L, and EXP3174 of 3.7×10^-6 mol/L (Figure 1C). Subsequently, EXP3179 generation from LOS was investigated in vitro by establishing a proposed chemical reaction mimicking the liver cytochrome-P450 pathway of LOS degradation (Figure 1D). EXP3179 is catalyzed from LOS in a chemical reaction using ruthenium chloride and hydrogen peroxide for catalysis. 

To investigate whether EXP3179 exhibits antiinflammatory properties, human endothelial cells were stimulated with Ang II and bacterial LPS, as AT1 receptor-independent proinflammatory stimulus. Northern blot analysis revealed a significant COX-2 mRNA upregulation by 30 minutes induced by Ang II and LPS. Preincubation with EXP3179 blunted both Ang II- and LPS-induced COX-2 transcription completely (Figure 2A). In addition, ICAM-1 expression was investigated as an additional proinflammatory mediator. As shown in Figure 2B, LPS-induced ICAM-1 mRNA upregulation was blunted by EXP3179, whereas LOS had no impact on LPS-stimulated ICAM-1 mRNA upregulation. Next, Ang II– and LPS-mediated (and COX-dependent) PGF2α formation was determined by GC-MS in vitro. Both, LPS and Ang II induced a maximum PGF2α increase at 1 hour after stimulation (maximum LPS-increase: 619±32 pg/mL; Ang II increase: 524±72 pg/mL). This increase in PGF2α formation was significantly (P<0.001) reduced by EXP3179 (10^-7 mol/L) preincubation (Figure 3A).

Subsequently, the influence of EXP3179 on human platelet aggregation was determined. In platelet-rich plasma, EXP3179 inhibited dose-dependently AA-induced platelet aggregation, suggesting a blockade of cyclooxygenase activity of COX enzymes (Figure 3C). More specifically, EXP3179 also blunted dose-dependently U46619-dependent platelet aggregation (Figure 3B). Selectivity for this TXA2-dependent reaction was obtained by inducing platelet aggregation with thrombin (10 U/mL) in the presence of 10^-4 mol/L EXP3179 (Figure 3B).

To test whether LOS elicits antiaggregatory effects in vivo, platelet aggregation was tested in 5 patients after oral administration of 100 mg LOS over an 8-hour observation period. Results revealed a significant decline in platelet aggregation in all patients at 6 and 8 hours when receiving 100 mg LOS as compared with controls (Figure 4A). Similarly to the in vitro observations, we tested whether the LOS metabolite EXP3179 acts as a cyclooxygenase inhibitor in vivo, thereby abolishing COX-2-dependent PGF2α formation. Figure 4B demonstrates a significant reduction of PGF2α serum concentration of patients after an oral administration of 100 mg LOS. The synchronous increase in EXP3179 serum concen-
tration (Figure 1B) associated with a significant reduction in platelet aggregation and PGF2α synthesis in patients is consistent with the notion that LOS, independent of its antagonism at the AT1 receptor, elicits antiinflammatory and antiaggregatory effects via its EXP3179 metabolite by blocking COX-dependent prostaglandin formation.

Discussion

The present study demonstrates that the AT1-receptor antagonist LOS elicits antiinflammatory properties via its EXP3179 metabolite by blocking COX-2 mRNA upregulation and COX-2–dependent TXA2 and PGF2α generation in vitro. Moreover, EXP3179 abolishes dose-dependently arachidonic acid (AA)–induced platelet aggregation, suggesting
expansions are detectable in concentrations that develop antiaggregatory and antiinflammatory effects in vitro and platelet aggregation in vivo. These observations are consistent with the notion that EXP3179 develops typical characteristics of a classic antiinflammatory compound. Therefore, AT1-receptor antagonists such as losartan elicit antiinflammatory and antiaggregatory effects independent of blockade at the AT1 receptor in clinically relevant doses.

The pharmacological blockade of the renin-angiotensin system (RAS) by ACE-inhibitors and by AT1-receptor antagonists represents an established, beneficial, and successful treatment of patients with arterial hypertension, chronic renal

Figure 3. A, Human endothelial cells were stimulated with either Ang II (10^{-7} mol/L) or LPS (100 μg/mL). Prostaglandin F2α (PGF2α) was determined in the supernatant media using GC-MS. Data are given as mean±SEM, and each experiment was performed at least in triplicate. B, EXP3179 inhibits dose-dependently thromboxane A2 (TXA2)-induced platelet aggregation. Platelet-rich plasma (PRP) was generated from healthy volunteers as described recently. Platelets were stimulated with U46619 (10^{-7} mol/L), a TXA2 analog. Aggregation was determined turbimetrically. Thrombin (10 U/mL) was used as TXA2-independent stimulus of platelet aggregation. Data are given as mean±SEM, and each experiment was performed at least 5 times. C, Dose-dependent inhibition of arachidonic acid (AA)-induced platelet aggregation. Platelet-rich plasma was generated from healthy volunteers as described recently. Platelets were stimulated with AA (100 μmol/L). Aggregation was determined turbimetrically. EXP3179 abolished dose-dependently AA-induced platelet aggregation. Data are given as mean±SEM, and each experiment was performed at least 5 times.

Figure 4. A, Losartan-dependent inhibition of platelet aggregation in vivo. Platelet aggregation was determined turbimetrically in 5 patients after oral administration of 100 mg LOS. In all patients, acetyl salicylic acid or other nonsteroidal antiinflammatory drugs (NSAIDS) were excluded 3 weeks before the measurements. After the oral administration of LOS, blood samples were drawn for a 6-hour observation period. Blood samples were drawn from the antecubital vein, and platelet-rich plasma was generated as indicated above. Data are given as mean±SEM. This decrease in losartan-dependent platelet aggregation was accompanied with a losartan-dependent inhibition of prostaglandin F2α (PGF2α) formation determined by enzyme-linked immune absorbance in serum samples of 5 patients after oral administration of 100 mg LOS (B). In all patients, acetyl salicylic acid or other NSAIDS were excluded 3 weeks before the measurements. After the oral administration of LOS, blood samples were drawn for a 6-hour observation period and serum was isolated by centrifugation. PGF2α was determined by enzyme-linked immune absorbance, and each sample was measured in triplicate. Data are given as mean±SEM.

an inhibitory effect at the COX enzyme. EXP3179 serum levels are detectable in concentrations that develop antiaggregatory and antiinflammatory effects in vitro and platelet aggregation in vivo. These observations are consistent with the notion that EXP3179 develops typical characteristics of a classic antiinflammatory compound. Therefore, AT1-receptor antagonists such as losartan elicit antiinflammatory and antiaggregatory effects independent of blockade at the AT1 receptor in clinically relevant doses.
failure, and atherosclerosis. After the development of ACE inhibitors, benzyl-substituted imidazoles were developed in search for a more specific blockade of the RAS by blocking Ang II effects more selectively at the receptor level. First, the newly developed AT1 antagonist LOS was used to demonstrate the presence of individual classes of Ang II receptors and further expanded the knowledge about the cardiovascular effects of the RAS and its effector peptide Ang II. However, researchers involved in the development of LOS indicated that the antihypertensive effect of LOS cannot solely be related to its antagonism at the AT1 receptor. The authors induced a dose-dependent and transient decrease in blood pressure in conscious dogs with normal renin after administration of LOS, but not after administration of EXP3174, the active metabolite of LOS with a much higher affinity to the AT1 receptor than LOS itself. The pharmaceutical profile of all known LOS metabolites interacting with AT1 receptors was found to be significantly diminished in comparison to LOS or EXP3174. Additionally, a detailed analysis by a radioreceptor assay in combination with HPLC-monitoring indicates that LOS and EXP3174 are the only compounds responsible for the Ang II antagonism.

Because LOS as a prodrug is generated following cytochrome-P450 oxidation, we speculated that potential additional effects may be related to one of the oxidation products, namely additional LOS-metabolites. In this regard, Stearns and colleagues, using computer-based molecule modeling, suggested that EXP3179 may inhibit or deactivate cyclooxygenase directly. This latter may occur via a deactivation of cyclooxygenase activity of COX enzymes, eg, by deacetylation and thereby prevent COX-2/ICAM-1 mRNA upregulation and abolish PGF2α formation. The inhibition of TXA2 analog–induced platelet aggregation further substantiates this hypothesis of an antagonism at the TXA2-PG2 receptor level in platelets, but the rate-limiting enzymatic step still remains to be determined. Whether or not this process blocks the interaction of activated signaling intermediates, eg, racGTP and phospholipase A2 (PLA2) or lipoxigenases, with the phospholipid-cell membrane, thereby preventing the generation of arachidonic acid from the phospholipid membrane upstream of COX enzymes, remains to be determined.

Figure 5. Hypothetical Model of EXP3179 antiinflammatory and antiaggregatory properties. The presented observations that EXP3179 abolishes AT1-independent ICAM-1 and COX-2 mRNA upregulation and PGF2α synthesis downstream of COX-2 suggest that EXP3179 inhibits enzyme systems upstream of COX-2 or COX-2 activity directly. This latter may occur via a deactivation of cyclooxygenase activity of COX enzymes, eg, by deacetylation and thereby prevent COX-2/ICAM-1 mRNA upregulation and abolish PGF2α formation. The inhibition of TXA2 analog–induced platelet aggregation further substantiates this hypothesis of an antagonism at the TXA2-PG2 receptor level in platelets, but the rate-limiting enzymatic step still remains to be determined. Whether or not this process blocks the interaction of activated signaling intermediates, eg, racGTP and phospholipase A2 (PLA2) or lipoxigenases, with the phospholipid-cell membrane, thereby preventing the generation of arachidonic acid from the phospholipid membrane upstream of COX enzymes, remains to be determined.

Because the liver clearance is always the dominant path for elimination of enzyme systems upstream of COX-2, the rate-limiting enzymatic step remains to be determined. Whether or not this process blocks the interaction of activated signaling intermediates, eg, racGTP and phospholipase A2 (PLA2) or lipoxigenases, with the phospholipid-cell membrane, thereby preventing the generation of arachidonic acid from the phospholipid membrane upstream of COX enzymes, remains to be determined.

Isolated EXP3179 was used to perform further experiments in search for the antiinflammatory properties of EXP3179. Similarly to the known COX-inhibitor indomethacin, the influence of EXP3179 on COX-2 and ICAM-1 mRNA upregulation and the COX-dependent formation of prostaglandins PGF2α and thromboxane A2 was investigated. Therefore, endothelial cells were stimulated with Ang II or LPS as AT1-receptor–independent stimulus of COX-2 transcription. EXP3179 abolished COX-2 mRNA upregulation induced by both Ang II and LPS. This inhibition occurred in concentrations similar to those determined in serum samples of patients (10^-7 mol/L). In addition, prostaglandin synthesis downstream of COX-2 was also significantly reduced by EXP3179. These observations suggest that EXP3179 inhibits enzyme systems upstream of COX-2 or COX-2 activity directly (as suggested in Figure 5). Therefore, we speculate that EXP3179 may inhibit or deactivate cyclooxygenase activity of COX enzymes, eg, by deacetylation, similarly to acetyl salicylic acid, and additionally prevent COX-2 mRNA upregulation and abolish prostaglandin formation downstream of COX-2. The inhibition of U46619-induced platelet aggregation further substantiates this hypothesis of an antagonism at the TXA2-PG2 receptor level in platelets, but the rate-limiting enzymatic step still remains to be determined. Therefore, we tested whether EXP3179 acts as COX inhibitor by blocking AA-dependent TXA2 generation. The experiments summarized in Figure 3C demonstrate that EXP3179 abolishes dose-dependently AA-induced platelet aggregation as indirect marker for TXA2formation. Whether or not this process blocks the interaction of activated signaling intermediates, eg, lipoxigenases with the phospholipid-cell membrane, remains to be determined. A hypothetical model of potential EXP3179 inhibitory steps is summarized in Figure 5.

In this regard, Lin and coworkers indicated that the formation of AA-induced, PGH2-mediated constriction in
aortic rings of hypertensive rats is associated with a disruption in the coupling of PGH₂-synthesis to PGH₂ metabolism caused by lipoxynase products with the ability to inhibit COX enzymes. Therefore, we suggest that EXP3179 may function as an inhibitor of AA-formation at the phospholipid membrane upstream of COX-enzymes, thereby preventing COX-2 mRNA upregulation directly or blocking COX-2 activity (Figure 5).

In conclusion, LOS prevents, via its active antiinflammatory metabolite EXP3179, the development of COX-2–dependent eicosanoid formation. Whether or not this observation will have clinical impact on the inflammatory status of atherosclerotic lesions or the development of an unstable plaque by blocking enzyme pathways such as COX-2 will be addressed in future experiments. However, recent observations demonstrating that long-term AT₁-receptor blockade by LOS reduces significantly the formation of atherosclerotic lesions15–17 underline the potential clinical implication of the present observation.

Acknowledgments
This work was supported by the Deutsche Forschungsgemeinschaft Sonderforschungsbereich 566, Projekt B9, and Sonderforschungs- bereich TR02/B4. M.L. is supported by Lower-Saxony-Israel grant. The authors are in debt to Gerald A. FitzGerald, MD, for critical discussion.

References
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Circ Res. 2002;90:770-776; originally published online March 7, 2002;
doi: 10.1161/01.RES.0000014434.48463.35

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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In an article by Krämer et al (Circ Res. 2002;90:770–776), “Angiotensin II Receptor–Independent Antiinflammatory and Antiaggregatory Properties of Losartan: Role of the Active Metabolite EXP3179,” a collaborator who contributed significantly to the work was not listed in the authorship of the manuscript. In a petition to the journal, the authors stated their omission of Dimitrios Tsikas, Department of Clinical Pharmacology, Hannover Medical School, Hannover, Germany, as a coauthor of this work. The corrected authorship should read: Christine Krämer, Julia Sunkomat, Jana Witte, Maren Luchtefeld, Michael Walden, Boris Schmidt, Dimitrios Tsikas, Rainer H. Böger, Wolf-Georg Forssmann, Helmut Drexler, and Bernhard Schieffer. In addition, there is an error in the abstract of the work. Prostaglandin F2α was measured using GC-MS and not LC-MS, as stated incorrectly in the abstract.