American Journal of Pathology, Vol. 145, No. 3, September 1994
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Short Communication

15-Hydroxyeicosatetraenoic Acid Inhibits Neutrophil Migration across Cytokine-Activated Endothelium

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15-hydroxyeicosatetraenoic acid (15-HETE) is an eicosanoid, formed by the actions of 15-lipoxygenase, epoxygenases, and cyclooxygenases on arachidonic acid, whose tissue levels are often elevated during inflammation. The present study demonstrates that 15(S)-HETE is a potent inhibitor of polymorphonuclear neutrophil (PMN) migration across cytokine-activated endothelium* in vitro. *15(S)-HETE is rapidly esterified into PMN phospholipids, and we report that 15-(S)-HETE-remodeled PMN displayed blunted adhesion to, and migration across, human endothelial cells that had been activated with either interleukin-1β or tumor necrosis factor-α. Several lines of evidence suggested that 15(S)-HETE inhibited PMN transmigration by attenuating PMN responsiveness to endothelial cell-derived platelet-activating factor (PAF). The inhibitory action of 15(S)-HETE on transmigration was not restricted by the profile of adhesion molecules expressed by cytokine-activated endothelium. Interleukin-1β and tumor necrosis factor-α induce PAF production by endothelium, and PMN migration across cytokine-activated endothelium was inhibited by a PAF receptor antagonist. PMN migration across endothelium in response to exogenous PAF was dramatically inhibited following exposure of PMN to 15(S)-HETE. Furthermore, 15(S)-HETE-remodeled PMN displayed impaired cytoskeletal and adhesion responses when stimulated by exogenous PAF, two pivotal events in PMN migration across activated endothelium. 15(S)-HETE seemed to attenuate PMN responsiveness to PAF by inhibiting membrane-associated signal transduction events. In keeping with this interpretation, remodeling of PMN phospholipids with 15(S)-HETE was associated with a sixfold reduction in the affinity of specific high-affinity PAF receptors for their ligand and impaired PAF-triggered IP3 generation. In contrast, PMN adhesion responses stimulated by calcium ionophore or activators of protein kinase C remained intact. These results provide further evidence that 15(S)-HETE may be an important endogenous inhibitor of PMN-endothelial cell interaction that serves to limit or reverse neutrophil-mediated inflammation* in vivo. *(Am J Pathol 1994, 145:541–549)

Polymorphonuclear neutrophils (PMNs) are important effectors of tissue injury in inflammation.[1] Inflammatory diseases are frequently self-limiting, raising the possibility that endogenous substances are pro-

Supported, in part, by NIH grants DK44380 to HRB. MM and AP are recipients of Postdoctoral Fellowship Awards from the Juvenile Diabetes Foundation.

Accepted for publication June 6, 1994.

Portions of this work were published in abstract form in the Proceedings of the 26th Annual Meeting of the American Society of Nephrology in the J Am Soc Nephrol 4: 595, 1993, and in the Proceedings of the Meeting of the Federation of American Societies of Experimental Biology in the FASEB J 7: A871, 1993.

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duced at sites of inflammation that inhibit PMN trafficking and PMN-mediated tissue injury. 15-hydroxyeicosatetraenoic acid (15-HETE) is an attractive candidate in this regard. This eicosanoid is generated by the actions of 15-lipoxygenase, and in addition, cyclooxygenases and epoxygenases on arachidonic acid (reviewed in ref. 2). 15-HETE has been detected in nanomolar levels in human blood under basal conditions, and in higher concentrations following cellular activation *ex vivo*.[3] Although circulating concentrations of 15-HETE have not been reported in disease states, elevated levels of 15-HETE have been demonstrated in inflammatory exudates or tissues in human and/or experimental arthritis and dermatitis.[4,5] Furthermore, intradermal and intra-articular injections of exogenous 15-HETE reduce inflammation in human psoriasis[6] and carragheenan-induced experimental arthritis,[7] respectively.

PMN adhesion to endothelial cells is a pivotal event in their recruitment to sites of inflammation (reviewed in refs. 8,9). Chemoattractants, such as bacterial cell wall products, complement components, and leukotriene B_4, stimulate initial adhesion of PMNs and endothelial cells during host defense and inflammation, and we recently demonstrated that 15(S)-HETE is a potent modulator of chemoattractant-induced PMN-endothelial cell interactions.[10] Cytokines, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), play an important role in amplifying and sustaining this process by inducing *de novo* expression of adhesion molecules by endothelial cells and stimulating the biosynthesis of mediators by endothelial cells that increase the avidity of PMN adhesion molecules for cognate endothelial ligands. In the present study, we assessed the influence of 15(S)-HETE on the interactions of PMN and IL-1- and TNF-activated endothelial monolayers *in vitro*. Our results indicate that 15(S)-HETE inhibits PMN adhesion to, and migration across, activated endothelium probably by attenuating PMN responsiveness to endothelial cell-derived platelet-activating factor (PAF).

Materials and Methods

PMN Isolation

PMNs were isolated from venous blood from healthy volunteers by ficoll-hypaque centrifugation and dextran sedimentation, as described previously.[10] The final pellet was suspended in Dulbecco's phosphate-buffered saline (PBS, pH 7.4) and contained 96 ± 3% PMNs, as determined by light microscopy. Suspensions in which PMNs showed signs of activation (>10% of PMNs in clumps of two or more cells) or loss of membrane integrity (<97% of PMNs excluding trypan blue) were discarded. For studies of transmigration and adhesion, PMNs were radiolabelled with ^{111}In oxine (1 μCi/10^6 cells; Mediphysics, IL) for 30 minutes, washed three times in PBS to remove extracellular ^{111}In oxine and suspended in PBS/1% bovine calf serum (BCS).

Human Umbilical Vein Endothelial Cells (HUVECs)

HUVECs were isolated, as described previously,[11] by collagenase digestion (0.1% CLS 3; Worthington Biochem. Corp., NJ) and were propagated in RPMI 1640 cell culture medium (BioWhittaker, Walkersville, MD) supplemented with 15% BCS (Hyclone, Logan, UT), 15% NUserum (Collaborative Res. Inc., Lexington, MA), 50 μg/ml endothelial mitogen (Biomedical Technologies Inc., Stoughton, Massachusetts), 8 U/ml Heparin, 50 U/ml penicillin and 50 μg/ml streptomycin (HUVEC medium). HUVECs were characterized by morphological criteria and indirect immunofluorescence using an anti-serum to human factor VIII and studied at passage levels 2 and 3.

PMN Migration across HUVEC Monolayers

For studies of transmigration, HUVECs were grown to confluence in HUVEC medium on gelatin-coated (1%) Transwell polycarbonate filters containing 3-μ pores (Costar, Cambridge, MA), which were inserted into 24-well tissue culture plates (Costar). The HUVEC monolayer and filter divided each well into an upper "luminal" (100 μl) and lower "abluminal" compartment (600 μl). Confluence was determined by comparison to HUVEC monolayers plated at the same time and density on gelatin-coated, 24-well tissue culture plates. Migration was initiated by addition of ^{111}In-labeled-PMN (100 μl, 3 × 10^5 PMNs) to the luminal compartment. Assays were terminated by aspiration of nonadherent PMNs from the luminal compartment, removing Transwell inserts from the abluminal compartment, and washing PMNs that were loosely adherent to the undersurface of filters into the abluminal chamber with 1 ml of PBS (4 C) without Ca^{2+} and Mg^{2+}. The number of PMNs that migrated to the abluminal compartment was calculated by lysing cells with 0.5% Triton X-100, measuring the radioactivity with a γ-counter (LKB Clinigamma) and comparing counts to the specific activity of the original PMN suspension. Unless stated otherwise, results are expressed as percent of ^{111}In-labeled-PMN added to

the luminal compartment that migrated to the abluminal compartment.

PMN Adhesion to HUVECs

For studies of adhesion, HUVECs were grown to confluence in HUVEC medium on gelatin-coated (1%) 96-well plates (Costar) and then in RPMI 1640/10% BCS for 24 hours preceding study. Monolayers were washed once with 0.2 ml of PBS/1% BCS and coincubated with 0.2 ml of ^{111}In-labeled-PMN suspension (4×10^5 PMN/well) at 37 C.[10] Assays were terminated by removal of nonadherent cells by aspiration of medium and washing of monolayers with 0.2 ml of PBS/1% BCS. The contents of each well were solubilized with 0.1% sodium dodecyl sulfate/0.025 N NaOH, and the radioactivity was measured in a γ-counter (LKB Clinigamma). The number of adherent PMNs (PMN/mm^2 of HUVECs) was calculated from the specific activity of each PMN preparation.

Adhesion Molecule Expression by Cytokine-Activated HUVECs

Intercellular adhesion molecule-1 (ICAM-1) and E-selectin expression was assessed by incubation of confluent HUVEC monolayers with saturating concentrations of test or control monoclonal antibodies (MAb) in PBS/10% BCS for 1 hour. Monolayers were washed three times in PBS/10% BCS and then incubated with fluorescein isothiocyanate-labeled F(ab')2 goat anti-murine immunoglobulin G (Caltag Laboratories, San Francisco, CA) at 4 C for 1 hour. The contents of each well were washed three times and solubilized with 0.1% sodium dodecyl sulfate/2N NaOH, and the fluorescence was determined with an automated-fluorimeter microtiter plate reader (Pandex, Baxter Healthcare, Munsfield, MA).

F-Actin Content

The relative amounts of F-actin in PMNs before and after addition of agonist were determined by quantitating the amount of rhodamine-phalloidin bound to fixed extracted cells, by slight modification of a previously described technique.[12] Specifically, 5×10^6 cells/ml were incubated with stirring at room temperature. Two hundred-µl aliquots were removed and added to 200 µl of 7% formaldehyde, 0.1% TX-100, 0.33 mmol/L rhodamine-phalloidin (Sigma Chemical Co., St. Louis, MO) in PBS, pH 7.4. Cells were fixed overnight at 4 C in the dark, washed with PBS, and extracted with 1 ml MeOH. Extracted rhodamine-phallodin was quantitated with CytoFluor 2300TM Fluorescence Measurement System (Millipore, Bedford, MA) with excitation and emission wave-lengths of 530 and 590 nm, respectively.

Binding of [^{3}H]PAF to PMNs

For assessment of PAF-receptor function, PMN suspensions (2.5×10^6 cells/0.5 ml) were incubated with [^{3}H]PAF (1 nmol/L) at 4 C in the presence or absence of various concentrations of unlabeled PAF (0.1 to 1000 nmol/L). After the specified time, PMNs were centrifuged (~1 minute at 12,000 *g*) through silicon oil (0.4 ml), and the radioactivity associated with cell pellets was determined in a scintillation counter. The K_d and B_{max} of the high-affinity PAF-receptors were determined by Scatchard analysis.

Measurement of Inositol Trisphosphate (IP_3) Levels in PMN

For assessment of IP_3 levels in PMNs, incubations (10^7 PMN/1 ml PBS) were terminated by addition of 200 µl of 100% trichloroacetic acid and kept on ice for 15 minutes. Samples were centrifuged, and the supernatant collected and mixed vigorously with 2 ml of 1,1,2-trichloro-1,2,2-trifluoroethane-trictylamine mixture (3:1, V/V). Following separation (~3 minutes), the clear aqueous top layer was removed and analyzed for IP_3 content with an IP_3 radioreceptor assay kit (NEN, Boston, MA).

Reagents and MAbs

Phorbol myristate acetate and A-23,187 were obtained from Sigma Chemical Co. 15(S)-HETE was obtained from Biomol Research Laboratories Inc. (Plymouth Meeting, PA). The purity of eicosanoids was checked before incubation with cells by RP-HPLC and UV spectroscopy, and their concentrations were determined from their extinction coefficients. [^{3}H]-PAF was obtained from New England Nuclear (Boston, MA). MAbs Hu5/3 and H18/7 recognize functional epitopes on human ICAM-1 and E-selectin, respectively.[13] These MAbs were used to confirm the relative levels of ICAM-1 and E-selectin expression on HUVECs and the relative roles of these adhesion molecules in PMN migration across HUVECs after 4 and 24 hours of activation with cytokines

(vide infra). These MAbs and blocking anti-IL-8 antisera were gifts from Dr. F.W. Luscinskas, Harvard Medical School. Recombinant human IL-1β and TNF-α were purchased from R & D Systems (Minneapolis, MN).

Statistical Analysis

Data are expressed as mean ± SE. A two-tailed Student's *t*-test was used for statistical analysis. A *P* value of <0.05 was taken to represent a statistically significant difference between group means.

Results and Discussion

Remodelling of PMN Phospholipids with 15(S)-HETE Attenuates PMN Migration across Cytokine-Activated HUVECs

Activation of endothelium by cytokines promotes extravasation of circulating PMNs during inflammation, and PMN migration across monolayers of cytokine-treated endothelial cells seems to be a reasonable *in vitro* model of this process. In the present study, confluent monolayers of HUVECs excluded the passage of most PMNs under basal conditions (% of added PMNs migrating during 90-minute coincubation: 5.4 ± 0.3, $n = 4$). Transmigration was increased in a time- and concentration-dependent manner by prior exposure of HUVECs to IL-1β as described previously by other investigators (Figure 1)[13–17] (reviewed in ref. 8). Importantly, this action of IL-1β was observed in the absence of any deterioration in the integrity of HUVEC monolayers, as determined by assessment of their permeability to fluorescein-labeled anionic dextran (molecular weight 40,000; Molecular Probes, Eugene, OR) (% transmigration of dextran from luminal to abluminal chamber in 30 minutes: basal 1.2 ± 0.8%, IL-1β 1.7 ± 0.5%, $n = 3$). To determine the influence of 15(S)-HETE on the IL-1β induced PMN migration response, PMNs were incubated with 15(S)-HETE for 30 minutes, washed twice with PBS to remove extracellular 15(S)-HETE, and coincubated for 90 minutes with IL-1β-activated (1 ng/ml, 4 hours) HUVECs. PMNs rapidly esterify 15(S)-HETE into membrane phospholipids under these conditions, predominantly into the sn-2 position of inositol-containing lipids.[2,18] Exposure of PMNs to 15(S)-HETE did not affect their ^{111}indium content when compared to vehicle-treated controls (not

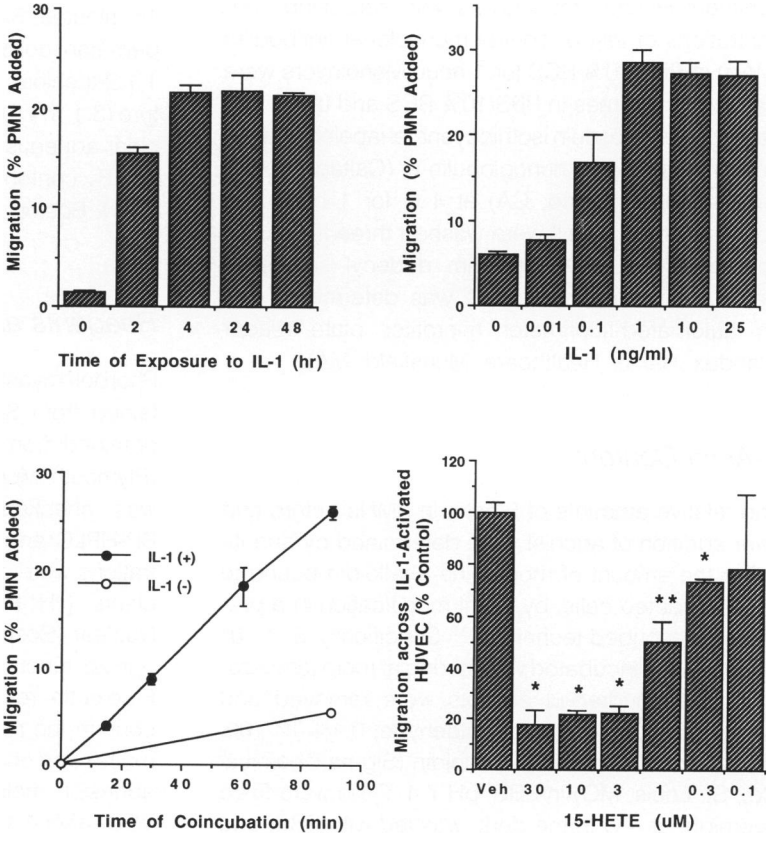

Figure 1. *PMN migration across IL-1-activated HUVECs: inhibition following remodeling of PMN phospholipids with 15(S)-HETE.* **Upper left:** *HUVECs were exposed to IL-1β (1 ng/ml) for different periods of time and washed twice, preceding coincubation (90 minutes) with PMNs* (n = 4). **Upper right:** *HUVECs were exposed to different concentrations of IL-1β for 24 hours and washed twice, before coincubation (90 minutes) with PMNs* (n = 4). **Lower left:** *Migration of PMNs across IL-1β-activated (1 ng/ml, 4 hours) HUVECs as a function of time of coincubation of PMNs and HUVECs* (n = 4). **Lower right:** *PMNs were exposed to 15(S)-HETE or vehicle for 30 minutes and washed twice before coincubation with IL-1β-activated (1 ng/ml, 4 hours) HUVECs for 90 minutes* (n = 3). *P < 0.01 and **P < 0.025 versus *vehicle-treated PMNs.*

shown). 15(S)-HETE-remodeled PMNs displayed dramatically impaired migration, and inhibition was observed with 15(S)-HETE concentrations as low as 0.3 μmol/L (Figure 1). It is noteworthy that these levels approximate those measured in activated blood *ex vivo*.[3] This action of 15(S)-HETE was not limited to PMN migration across IL-1β-activated HUVECs. Prior exposure of HUVECs to TNF-α also promoted PMN migration in a time- and concentration-dependent manner (not shown), as reported by other investigators,[8,9] and this response was also blunted following remodeling of PMNs with 15(S)-HETE (Table 1).

Approximately 90% of 15-HETE generated by 15-lipoxygenase is of the S configuration, whereas cyclooxygenases and epoxygenases usually generate the S and R enantiomers in at least equal amounts.[2] Transmigration was assessed following remodeling of PMNs with equimolar concentrations (30 μmol/L, 30 minutes) of either 15(S)-HETE or 15(R)-HETE to determine the chiral requirements for inhibition. Both isomers attenuated PMN migration; although greater inhibition was observed with the S isomer (76 ± 6% and 51 ± 5% inhibition for 15(S)-HETE and 15(R)-HETE, respectively; $n = 6$). Taken together, these results suggested that 15-HETE derived from multiple enzymatic sources may inhibit PMN migration across endothelium activated with two cytokines that have been implicated as stimuli for PMN recruitment *in vivo*.

The Inhibitory Action of 15(S)-HETE Is Not Dependent on the Profile of Adhesion Molecules Expressed by HUVECs

PMN adhesion to endothelial cells supported by interactions of leukocyte adhesion molecules is a pivotal event in PMN migration across endothelium *in vitro* and *in vivo*.[8] IL-1β and TNF-α induce *de novo* synthesis of ICAM-1 and E-selectin by HUVECs,[8,9] and most PMN adhesion to cytokine-activated HUVECs is blocked by MAbs against these molecules under static conditions.[8,13] The pattern of expression of ICAM-1 and E-selectin, and their relative roles in PMN adhesion and transmigration differs dramatically, however, depending on the duration of exposure of HUVECs to cytokines.[8,9] Both E-selectin and ICAM-1 are expressed in abundance and support adhesion and transmigration after 4 hours exposure.[8,9,13] In contrast, E-selectin is virtually undetectable after 24 hours and PMN adhesion is almost completely prevented by anti-ICAM-1 MAb in this setting.[8,9,13] We monitored PMN migration across HUVECs activated with IL-1β (1 ng/ml) or TNF-α (1 ng/ml) for 4 or 24 hours to determine if this action of 15(S)--HETE varied with the time of exposure to cytokines. In agreement with previous reports, ICAM-1 and E-selectin were expressed at equivalent levels following 4 hours exposure in the present study, whereas E-selectin was detected at low levels or undetectable after 24 hours ($n = 4$). 15(S)-HETE blunted transmigration under both temporal conditions (Table 1). Interestingly, 15(S)-HETE afforded significantly less blockade of transmigration following 24 hours exposure to IL-1β, when compared to other activation conditions; the reason(s) for this disparity is unclear. Nevertheless, these observations suggested that the inhibitory action of 15(S)-HETE on PMN transmigration was not restricted by the profile of adhesion molecules expressed by HUVECs.

Table 1. *15(S)-HETE Inhibits PMN Migration across TNF- and IL-1-Activated HUVECs*

Cytokine	Time of exposure	
	4 hours	24 hours
IL-1 (1 ng/ml)	56 ± 6%	25 ± 4%
TNF (1 ng/ml)	51 ± 6%	41 ± 7%

HUVEC monolayers were activated with TNF or IL-1 and washed three times with PBS, before coincubations with 15(S)-HETE-remodeled or diluent-treated PMNs. Results are expressed as % inhibition and were calculated by comparison of migration of 15(S)-HETE-remodeled PMNs with diluent-treated PMNs. Data are means ± SE of four experiments.

Remodeling of PMN Phospholipids Inhibits PMN Migration across IL-1-Activated HUVECs by Altering the Sensitivity of PMNs to Endothelial Cell-Derived PAF

Cytokines, as discussed above, also provoke PMN extravasation by inducing endothelial cell biosynthesis of several stimuli for chemotaxis and adhesion[15,16,19–21] (reviewed in ref. 8). PAF and IL-8, in particular, play pivotal roles in PMN migration across cytokine-activated endothelium in several model systems.[8,15,16,19,20,22] We assessed the influence of an anti-IL-8 anti-sera and a PAF-receptor antagonist on PMN migration across IL-1β-activated HUVEC (1 ng/ml, 4 hours) to define the relative contributions of these mediators to PMN trafficking in our model. Transmigration was not affected significantly by concentrations of anti-IL-8 antibody that abolished PMN migration in response to exogenous IL-8 ($n = 3$; data not shown). In contrast, this response was markedly reduced by 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phospho-(N,N,N-trimethyl) hexanolamine, a PAF-receptor antagonist[23] (Figure 2). These obser-

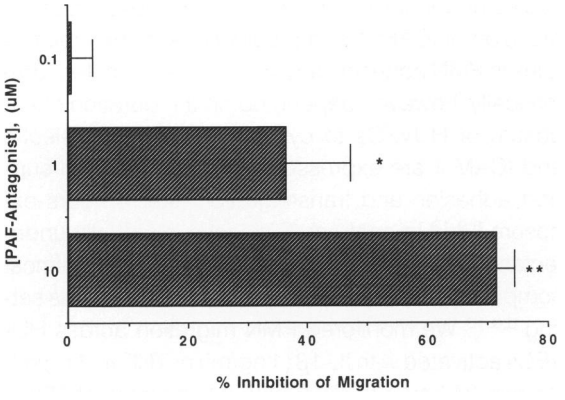

Figure 2. *PMN migration across IL-1-activated HUVECs is a PAF-dependent event. Migration of PMNs across IL-1β-activated (1 ng/ml, 4 hours) HUVECs during 90 minutes of coincubation was assessed following exposure of PMNs for 15 minutes to 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phospho-(N,N,N-trimethyl) hexanolamine (Biomol, PA), a PAF-receptor antagonist. Data are means ± SE of three to six experiments.* *P < 0.025; **P < 0.001.

vations suggested that 15(S)-HETE attenuates PMN migration across cytokine-activated HUVECs at least in part by blunting their responsiveness to endothelial cell-derived PAF. To test this hypothesis further, we assessed the influence of 15(S)-HETE on PMN migration induced by addition of exogenous PAF to the abluminal surface of resting HUVECs (Figure 3, upper panel). 15(S)HETE dramatically attenuated PAF-induced (10^{-7}M, 30 minutes) PMN migration under these conditions (Figure 3, upper panel), paralleling its effect on PMN migration across cytokine-activated HUVECs.

PMN adhesion to endothelium is a central step in PMN extravasation *in vivo* and is followed by dramatic changes in the PMN cytoskeleton and diapedesis (reviewed in refs. 8,24). PMN adhesion to HUVECs and F-actin content were assayed in the present study as indices of these events. PAF (10^{-7}M, 30 minutes) provoked markedly less adhesion and F-actin assembly with 15(S)-HETE-remodeled PMNs than with diluent-treated controls (Figure 3, middle and lower panels). Thus, 15(S)-HETE inhibited two pivotal events that subserve PAF-dependent PMN migration across endothelium.

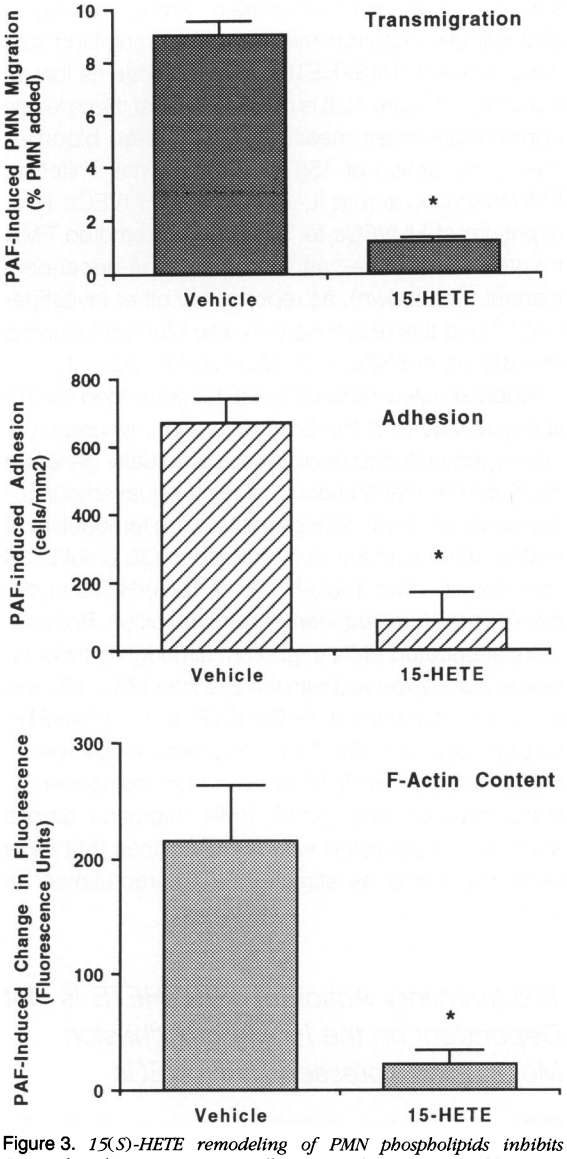

Figure 3. *15(S)-HETE remodeling of PMN phospholipids inhibits PAF-induced transmigration, adhesion, and F-actin assembly. PMNs were exposed to 15(S)-HETE (30 μmol/L) or vehicle for 30 minutes and washed twice, preceding exposure to PAF (10^{-7} mol/L, 30 minutes).* **Upper panel:** *PAF-induced (added to abluminal chamber) transmigration of PMNs across HUVEC monolayers* (n = 3, *P < 0.001). **Middle panel:** *PAF-induced adhesion of PMNs and HUVECs* (n = 6, *P < 0.001). **Lower panel:** *PAF-stimulated increase in F-actin content of PMNs* (n = 3, *P < 0.05). *Data are means ± SE.*

15(S)-HETE Remodeling of PMN Phospholipids Reduces the Affinity of PAF High-Affinity Receptors for Their Ligand and PAF-Induced IP_3 Generation

Because 15(S)-HETE blunted both PAF-induced adhesion and cytoskeletal rearrangements, it seemed likely that this eicosanoid reduced transmigration by inhibiting a PAF signal transduction event common to both processes. Indeed, we and others reported recently that 15(S)-HETE-remodeled PMN respond appropriately to A-23,187, a calcium ionophore, or phorbol myristate acetate, an activator of protein kinase C, agonists that bypass early signal transduction events.[10,25] Furthermore, in parallel experiments in the present study, neither A-23,187-induced (5

Table 2. *Remodeling of Neutrophils with 15(S)-HETE: Influence on PAF High-Affinity Cell-Surface Receptors and PAF-Stimulated IP_3 Formation*

Condition	PAF-receptor kinetics		PAF-stimulated IP_3 formation (pmol/10^6 PMNs)
	K_d (nmol/L)	B_{max} (fmol/4×10^6 PMNs)	
Vehicle-treated PMNs	5.26 ± 1.45	39.7 ± 11.2	3.82 ± 0.29
15-HETE-remodeled PMNs	31.7 ± 4.43*	32.0 ± 4.50	2.30 ± 0.20*

Specific binding of ^{3}H[PAF] (n = 3) and PAF-induced (10^{-7} mol/L, 30 minutes, n = 4) IP_3 generation were assessed with 15-HETE-remodeled (30 μmol/L, 30 minutes) and vehicle-treated neutrophils (PMNs) as in Materials and Methods. Data are means ± SE.
* $P < 0.01$ *versus* vehicle.

μmol/L, 30 minutes) nor phorbol myristate acetate-induced (10^{-7} mol/L, 30 minutes) PMN adhesion to HUVECs was blunted significantly by 15(S)-HETE, in contrast to the marked reduction in PAF-induced adhesion (% inhibition: A-23,187 30 ± 13%; phorbol myristate acetate 6 ± 20%; PAF 87 ± 13%; n = 6 to 8).

PAF provokes PMN chemotaxis and adhesion via interaction with specific high-affinity PMN cell-surface receptors and activation of a coordinated series of signal transduction events within PMN cell membranes that include activation of G-proteins and phospholipase C, hydrolysis of phosphatidylinositol bisphosphate, and generation of diacyl glycerol and IP_3.[26–28] We assessed the specific binding of ^{3}H-PAF to PMNs to explore the possibility that remodeling of PMN phospholipids with 15(S)-HETE attenuates PAF receptor-ligand interactions (Table 2). The density of high-affinity PAF receptors on PMNs was not altered significantly following exposure of PMNs to 15(S)-HETE. In contrast, their affinity was reduced by approximately sixfold. IP_3 generation was assessed as a distal event in PAF signaling within PMN cell membranes. PAF (10^{-7} mol/L) triggered a rapid elevation in IP_3 levels that was maximal after 5 seconds exposure, as reported previously by others,[27] and this response was significantly reduced following remodeling of PMNs with 15(S)-HETE (Table 2).

In aggregate, the results of our *in vitro* study indicate that 15(S)-HETE stereoselectively inhibits PMN migration across cytokine-activated endothelium probably by reducing PMN responsiveness to endothelial cell-derived PAF and that this action was mediated via a modulatory action of esterified 15(S)--HETE on ligand-receptor interactions and signal transduction within PMN cell membranes. The dramatic decrease in the affinity of PMN PAF-receptors for their ligand could reflect an action of 15(S)-HETE on PAF-receptors and/or their coupling to G-proteins. Along these lines, a recent report has implicated altered receptor-G-protein coupling as a mechanism for down-regulation of receptor-triggered superoxide anion generation in 15(S)-HETE-treated PMNs.[25] Given that 15(S)-HETE is rapidly esterified into inositol-containing phospholipids to levels that are comparable with arachidonic acid[2,18] and that the activity of membrane proteins may be exquisitively sensitive to the lipid composition of the surrounding cell membrane,[29,30] it is likely that 15(S)-HETE-remodeling may also influence other membrane-associated events. For example, changes in the arachidonic acid content of inositol-containing phospholipids have been shown to modulate phospholipase C activity in other model systems.[31] Furthermore, 15(S)-HETE-remodeled PMNs also generate 15(S)-HETE-remodeled diacyl glycerol (DAG) upon activation,[32] a biochemical change that may be associated with a change in DAG bioactivity. The relative contributions of these events to the inhibitory action of 15(S)-HETE on PAF-dependent PMN-endothelial cell interactions will be the subject of future studies.

The results of the present study support the contention that 15(S)-HETE may be an important endogenous inhibitor of PMN-endothelial cell interactions *in vivo* and suggest a mechanism for the anti-inflammatory actions of exogenous 15(S)-HETE in several diseases.[6,7] PMN extravasation to sites of inflammation is initiated by the generation of gradients of bacterial cell wall peptides, complement components (eg, C5a), and other chemoattractants (eg, LTB_4) within a local milieu. We demonstrated previously that 15(S)-HETE inhibits PMN transmigration induced by these mediators,[10] and the results of the present study indicate that 15(S)-HETE also inhibits PMN migration across cytokine-activated endothelium. Taken together, these observations indicate that 15(S)-HETE is a potent modulator of PMN-endothelial cell interactions that are important in the initiation, amplification, and maintenance of PMN trafficking to sites of inflammation. The multiple mechanisms that exist for 15-HETE formation and the diverse cell-types capable of generating 15-HETE[2] further suggest that this eicosanoid may be an important modulator of PMN trafficking *in vivo*. The further characterization of

the endogenous inhibitors of PMN-endothelial cell interaction, and of their mechanisms of action may suggest novel, specific, and relatively nontoxic therapeutic strategies for the treatment of PMN-mediated inflammation.

Acknowledgment

We thank Dr. Charles N. Serhan for his helpful discussions.

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