Differential Effects of Arachidonic and Eicosapentaenoic Acid-Derived Eicosanoids on Polymorphonuclear Transmigration Across Endothelial Cell Cultures

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ABSTRACT

The beneficial effects of fish oil on inflammation have been attributed to the content of eicosapentaenoic (EPA)/docosahexaenoic acid. EPA is also a substrate for arachidonic acid (AA) cascade enzymes, but it induces the production of alternative eicosanoids such as 3-series prostanoids and 5-series leukotrienes, which are considered to be less proinflammatory than AA metabolites. However, the molecular basis of this action is poorly understood. In this study, we compared the effects of prostaglandin (PG) E2 and PGE3 on endothelium permeability, and the effects of leukotriene (LT) B4 and LTB5 on endothelium permeability and mononuclear adhesion and migration. In our study, both prostaglandins increased trans-endothelial Evans blue-albumin (EBA) permeability in a concentration-dependent manner. It is interesting that the effect of PGE3 was significantly more pronounced than the effect of PGE2, and both were antagonized by EP1 and EP2 antagonists. LTB4 and LTB5 had a slight effect on EBA extravasation. However, we observed the enhancement of endothelial permeability in the presence of polymorphonuclear (PMN) cells, probably a consequence of an interplay between leukotriene and prostanois effects. LTB4 caused significant increases in the number of PMN cells adhering to endothelial cells, whereas LTB5 did not induce a significant effect. This effect of LTB4 appears BLT1 receptor-dependent and was mediated through the enhancement of lymphocyte function-associated antigen-1, membrane attack complex-1, E-selectin, and intercellular adhesion molecule-1 expression. Finally, we observed that, unlike LTB5, which had a weak effect, LTB4 was a highly potent chemoattractant. An understanding of the differences in the effects of LTB4/LTB5 on PMN cell adhesion and migration may help to explain the beneficial impact of ω-3 fatty acids in inflammatory processes.

There is extensive documentation on the beneficial effects of fish oil, such as the reduction of joint pain and inflammation from rheumatoid and osteoarthritis (Belch et al., 1988), improved anticarcinogenic action (Jho et al., 2004), and major positive effects on lipid profile, plaque formation, and the reduction of infarction from coronary atherosclerosis (Kristensen et al., 2003), which is now considered an inflammatory disease. These benefits have been attributed to the content of eicosapentaenoic (EPA)/docosahexaenoic (DHA) acid in fish oil. However, the molecular basis for the health benefits of dietary fish oil is poorly understood. The most immediate effect is increased incorporation of EPA and DHA into the sn-2 position of plasma membrane phospholipids, which is where arachidonic acid (AA) is usually esterified. Thus, EPA and DHA replace linoleic acid and AA, the main substrates to produce eicosanoids by cyclooxygenase, lipoxygenase, and cytochrome P-450 pathways (Smith and Murphy, 2002).

Experimental studies have shown that diets rich in fish oil significantly reduce the amount of AA present in membrane phospholipids (Mitjavila et al., 1996). Research has also shown that such diets disrupt the release of AA from membrane phospholipids and consequently the synthesis of AA metabolites such as PGE2 (Moreno et al., 2001), whereas the presence of EPA/DHA in membrane phospholipids is increased.

ABBREVIATIONS: EPA, eicosapentaenoic acid; AA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; EBA, Evans blue-albumin; LFA-1, lymphocyte function-associated antigen-1; LT, leukotriene; ICAM-1, intercellular adhesion molecule-1; MAC-1, membrane attack complex-1; PG, prostaglandin; PMN, polymorphonuclear; PUFA, polyunsaturated fatty acid; SC19200, 8-chloro-dibenzo[ b,f][1,4]oxazepine-10(11H)-carboxylic acid; UT5302, 6-[5-(3-hydroxy-1E,5Z-undecadienyl)-2-pyridinyl]-1,5-hexanediol; LY255283, 1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1H-tetrazol-5-yl)heptyloxy]phenyl]ethane; AH 8809, 6-isopropoxy-9-oxoxanthene-2-carboxylic acid; AH 23848, (4Z)-7-[(rel-1S,2S,5R)-5-((1,1’-biphenyl-4-yl)methoxy)-2-(4-morpholinyl)-3-oxyocyclopentyl]-4-heptenoic acid hemicalcium salt hydrate.
Although phospholipases A₂ can discriminate between different fatty acid chains, it hydrolyzes AA and EPA equally well from phospholipids (Nieves and Moreno, 2006). The preferred substrate for cyclooxygenase (COX) catalysis by COX-1 and COX-2 to produce 2-series prostanoids is AA. In a similar form, AA is metabolized by lipooxygenases to release 4-series leukotrienes. EPA was also reported as being a substrate for AA cascade enzyme pathways, in which it induced the production of other eicosanoids such as 3-series prostanooids and 5-series leukotrienes (Smith, 2005). Although similar in structure and stability, the AA-derived eicosanoids are considered to be more proinflammatory than EPA metabolites. Hawkes et al. (1992) reported that PGE₂ had fewer edematogenic effects than PGE₃ and that LTB₅ showed 10% of the chemotactic potency of LTB₄ (Heidel et al., 1989). Thus, supplementing diets with ω-3 instead of ω-6 fatty acids is considered to be a therapeutic approach for a number of inflammatory processes. However, studies are lacking that directly compare the effects of both families of eicosanoids on cellular functions involved in inflammation development. If EPA-derived eicosanoids do possess anti-inflammatory properties, they could serve as a therapeutic agent in a number of inflammatory diseases.

In this study, we compared the effects of PGE₂ and PGE₃ on endothelium permeability, and the effects of LTB₄ and LTB₅ on endothelial permeability and mononuclear adhesion and migration.

**Materials and Methods**

**Materials.** Evans blue, 2-acetylhydrazide 10(11H)-carboxylic acid (albumin), SC19200, U75302, and 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetic acid (indomethacin) were provided by Sigma-Aldrich (St Louis, MO). PGE₂, PGE₃, LTB₄, and LTB₅ were provided by Cayman Chemical (Ann Arbor, MI). LY255283 was from Torcis (Bristol, UK). AH 6809 and AH 23848 were kindly supplied by GlaxoSmithKline (Uxbridge, Middlesex, UK) and ONO-AE3-240 (chemical structure not exhibited by ONO). These compounds were dissolved in dimethyl sulfoxide and diluted in medium to keep the final concentration of dimethyl sulfoxide below 0.1%. Control cells were incubated with the vehicle. All other chemicals were of the highest quality that was commercially available.

**Evaluation of Endothelial Monolayer Permeability to Albumin.** Human umbilical vein endothelial cells (ECV304) were obtained from the European Collection of Cell Cultures (Salisbury, UK) and were grown in medium 199 with Earl’s salts, 25 mM HEPES supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum. Cells were split at confluence 1 to 3 or at higher passages 1 to 2 every 5 to 7 days and plated on gelatin-coated flasks up to passage 20.

Evans blue was added in a 1:1 M ratio to a 4% bovine serum albumin solution in M199 to obtain a final concentration of 0.67 mg/ml. The solution was incubated at 37°C for 15 min and then dialyzed against water for 24 h. The dialysate was used immediately.

The experimental system consisted of two chambers separated by an ECV monolayer grown on 12-mm polycarbonate filters (Transwells) with a pore size of 0.4 μm. The upper chamber contained 700 μl of the Evans blue-albumin (EBA) solution. The system was left to stabilize for 1 h. The lower chamber was sampled (300 μl) every 5 min for a 30-min period to evaluate basal ECA permeability to EBA. Eicosanoids were then added in the upper chamber containing the cells, and aliquots were withdrawn for an additional 30 min. The EBA content was then determined by measuring the absorbance of Evans blue dye at 620 nm. The permeability coefficient (PC) was determined as described by Casnocha et al. (1989) by use of the formula derived from Fick’s law of diffusion:

$$\ln[1/1 - (V_f + V_i)C_{i}(t)/TP(t)] = PC[A(V_f + V_i)/V_f V_i]$$

where A represents the area of membrane, $V_f$ is the volume of luminal chamber, $r$ is sample time, $V_i$ is albumin concentration in subluminal chamber, $C_{i}(t)$ is albumin concentration at each sample time, and $TP(t)$ is total mass of albumin at each sample time. Results are expressed as the percentage difference between the PC before and after adding eicosanoids.

**Polymorphonuclear Granulocyte Isolation.** Blood samples were mixed with 1% dextran sulfate in 0.9% NaCl at a ratio of 1:1 (v/v) and left for 60 min at 37°C for erythrocyte sedimentation to occur. The leukocyte-rich plasma was layered over an equal volume of Histopaque-1077 (Sigma-Aldrich), and gradient centrifugation was carried out for 30 min at 700g. The pellet comprised polymorphonuclear (PMN) cells and some contaminating erythrocytes. Erythrocytes were eliminated by lysis with 0.98% ammonium chloride, and the PMN cells were washed twice with Eagle’s minimal essential medium and resuspended at a final concentration of $1 \times 10^6$ PMN cells/ml.

**Polymorphonuclear Granulocyte Adhesion to Endothelial Cells.** ECV304 cells were plated and grown to confluence (around $5 \times 10^5$ cells/well). Thereafter, endothelial cells were washed and incubated with polymorphonuclear granulocytes ($2 \times 10^6$ cells/well) in the presence of LTs and allowed to attach at 37°C for 3 h. Non-adherent cells were then removed, and the cells were washed three times with cell culture medium. Adherent cells were fixed in 3.7% phosphate-buffered formalin. The PMN cells that adhered to ECV304 cells were counted under a phase-contrast microscope (Nikon, Melville, NY) by use of a digital photograph system (Sony, Tokyo, Japan), which measured an area of 0.16 mm²/field.

**Analysis of adhesion molecules.** PMN or ECV304 monolayers were incubated with leukotrienes at 37°C for 1 or 4 h. Then, PMN and ECV304 cells were scraped off, were pelleted and resuspended with diluted specific primary antibody solution, respectively. PMN ($2 \times 10^6$ cells/ml) were incubated with antibodies for LFA-1 and MAC-1 (Boehringer Ingelheim, Ingelheim, Germany; dilution 1:50). ECV304 cells ($2 \times 10^5$ cells/ml) were examined for ICAM-1 (1:10) and E-selectin (1:25) (R&D Systems, Minneapolis, MN). After 30 to 45 min at 4°C, cells were washed twice with 1% bovine serum albumin in phosphate-buffered saline, and fluorescein-labeled goat anti mouse antibody (Boehringer Ingelheim) was added for 30 min at 4°C. The cells were then washed with phosphate-buffered saline, fixed in 1% paraformaldehyde in phosphate-buffered saline, and analyzed with a fluorescein-activated cell sorter analyzer (FACScan; BD Biosciences, San Jose, CA) as described in Hauser et al. (1993). Antibody binding was determined as mean fluorescence intensity after gating for cells by their characteristic forward and side scatter properties. The results are shown in relative fluorescence units (RFUs). RFU = $F_{\text{UExperimental}} - F_{\text{UIsotype}} \times 100/F_{\text{UControl}}$ - $F_{\text{UIsotype}}$, where $F_{\text{UExperimental}}$ and $F_{\text{UControl}}$ are the fluorescence intensities of stimulated and control cells, respectively, and $F_{\text{UIsotype}}$ is the fluorescence intensity of class-matched irrelevant antibody.

**Polymorphonuclear Granulocyte Chemotaxis.** PMN chemotaxis was quantified by use of the modified Boyden chamber technique (Boyden, 1962). In brief, PMN cells were suspended in Eagle’s minimal essential medium containing 20 mM HEPES, pH 7.3, and 5 × 10³/0.5 ml pipetted into the top chamber of the Transwell (Costar, Cambridge, MA), which was separated from the lower chamber by a 3-μm pore filter with a thickness of 150 μm (Millipore, Billerica, MA). Chemotactic agents were incubated in this lower chamber for 3 h at 37°C in atmospheric conditions of 5% CO₂ and 95% air at high humidity. The filters were fixed with 70% ethanol and stained with methylene blue. To evaluate PMN cell locomotion, cells were counted at every 10-μm level starting from the proximal surface (monolayer) to the distal surface. The number of cells...
counted was multiplied by the distance of that level from the monolayer, and the products obtained from all levels were added. The number obtained was the total distance migrated by the counted cells. To obtain the average distance migrated per cell, or locomotion index, the total distance was divided by the total number of cells counted at all levels (Maderazo and Woronick, 1978) and was expressed as the migration index compared with the control (control = 1). For each filter, three fields were counted and indexes were calculated as mentioned earlier.

**Statistical and Data Analysis.** Results are expressed as means±S.E.M. Differences between control and treated cultures were assessed by analysis of variance test, Student’s t test, or a one-way analysis of variance followed by the least-significant difference test when appropriate.

### Results

**PGE\textsubscript{2} and PGE\textsubscript{3} Increase Endothelial Monolayer Permeability to Albumin.** To determine the role of PGE\textsubscript{2} and PGE\textsubscript{3} in edema formation, ECV304 monolayers were exposed to both prostaglandins and EBA extravasation across endothelial cell cultures was measured. Results are expressed as a function of the percentage increase in the **trans**-endothelial albumin clearance rate after the addition of prostaglandin compared with basal levels. Vehicle (medium) caused a slight change in permeability (from 6.9 ± 1.2 × 10^{-6} cm/s to 8.2 ± 1.1 × 10^{-6} cm/s), whereas both prostaglandins increased the permeability of **trans**-endothelial Evans blue-albumin in a concentration-dependent manner (Fig. 1A). Both prostaglandins reached a maximum plateau effect at 100 nM. The effect of PGE\textsubscript{3} was significantly more pronounced than that of PGE\textsubscript{2}.

PGE\textsubscript{2} and PGE\textsubscript{3} bind to four plasma membrane receptors (EP\textsubscript{1}–EP\textsubscript{4}). The role of EP receptors in changing of albumin permeability induced by PGE\textsubscript{2}/PGE\textsubscript{3} was investigated by use of specific EP antagonist. We used SC19200 as EP\textsubscript{1} antagonist (Funk et al., 1993), AH 6809 as EP\textsubscript{2} antagonist (Woodward et al., 1995), ONO-AE3-240 as EP\textsubscript{3} antagonist (Sugimoto and Narumiya, 2007), and AH 23484 as EP\textsubscript{3} antagonist (Davis and Sharif, 2000). Considering the affinity of these antagonists, all compounds were used at a concentration of 1 μM, which markedly blocked the binding of PGE\textsubscript{2} to EP receptors. Our results reveal a significant decrease in Evans blue-albumin permeability induced by PGE\textsubscript{2}/PGE\textsubscript{3} in the presence of EP\textsubscript{1} and EP\textsubscript{2} antagonists (Fig. 1B). These findings demonstrate the role of these PGE\textsubscript{2} receptors in the enhancement of endothelial permeability induced by PGE\textsubscript{2}/PGE\textsubscript{3}.

In contrast to prostaglandin effects, LTB\textsubscript{4} and LTB\textsubscript{5} showed only a slight effect on EBA extravasation (Fig. 2A). However, we also observed that both leukotrienes were able to induce EBA clearance through endothelial cultures in the presence of PMN (Fig. 2B), indicating that this permeability response was PMN-dependent. It is interesting that we observed that LTB\textsubscript{5} was 100 times more active on PMN adhesion and, consequently, on PMN-dependent changes in permeability than LTB\textsubscript{5} was. In addition, we observed that these PMN-dependent changes in endothelial permeability induced by LTB\textsubscript{4}/LTB\textsubscript{5} were significantly inhibited by a cyclooxygenase inhibitor, such as indomethacin, and EP\textsubscript{1} and EP\textsubscript{2} antagonists (Fig. 2C) suggested a role of prostaglandins in these events.

**LTB\textsubscript{5} Induced a Lower Ratio of PMN Adhesion to ECV304 Cells than LTB\textsubscript{4}.** In comparison with control conditions, LTB\textsubscript{4} (1–100 nM) caused significant increases in the number of PMN cells adhering to ECV304 cultures, whereas LTB\textsubscript{5} (1–100 nM) was unable to induce significant PMN adhesion to endothelial cells (Fig. 3A). LTB\textsubscript{4} acts via two specific plasma membrane receptors named BLT1 (high affinity) and BLT2 (low affinity). To investigate the role of both receptors in LTB\textsubscript{4}-induced PMN adhesion, we used U75302 as a BLT1 antagonist and LY255283 as a BLT2 antagonist (Yokomizo et al., 2000). U75302 treatment reduced cell adhesion, whereas LY255283 did not have any effect indicating that only BLT1 is involved in the adhesion of PMN cells to endothelium induced by LTB\textsubscript{4}. LTB\textsubscript{5} was less effective than LTB\textsubscript{4} but was sensitive to the presence of U75302 presence (Fig. 3B). Our findings also demonstrated that the effects of LTB\textsubscript{4}/LTB\textsubscript{5} on PMN adhesion were not appreciably modified by indomethacin (Fig. 3B).

**PMN Adhesion to Endothelial Cells Induced by LTB\textsubscript{4} May Be Mediated through the Enhanced Expression of Adhesion Molecules.** To address the hypothesis that the effect of LTB\textsubscript{4} on PMN/endothelium adhesion was the result of adhesion molecule expression on both cells, LFA-1 and MAC-1, and E-selectin and ICAM-1 expression were deter-
mained in the surface of PMN or ECV304 cells, respectively. We observed that all adhesion molecules were markedly upregulated by LTB4, whereas LTB5 caused only a minor effect. Thus, LFA-1, MAC-1, and E-selectin expression increased by 500%, 445%, and 200% respectively, 1 h after LTB4 (100 nM) challenge, whereas LTB5 only increased the expression of these adhesion molecules by 180%, 45%, and 40%, respectively (Figs. 4, A–C). ICAM-1 expression was highest 4 h after LTB4 (10 nM) incubation, whereas the concentration of LTB5 did not significantly increase ICAM-1 expression (Fig. 4D). These findings correspond with the higher effect on PMN/endothelial cell adhesion of LTB4 derived from AA than with that of LTB5 derived from EPA.

**LTB4 Is a Highly Potent Chemoattractant, whereas LTB5 Has a Weaker Effect.** PMN cells migrated across filters in response to LTB4 (Fig. 5A). LTB4 induced maximum migration at a concentration of 100 nM (migration index, 1.7), whereas at 1000 nM LTB5 caused only a minor PMN migration (migration index, 1.2). PGE2 and PGE3 up to 100 nM did not have chemoattractant activity, and these prostanoids did not potentiate LTB4 effects. Our findings also demonstrate that these chemoattractant effects were reversed by a BLT1 antagonist, U75302, in a concentration-dependent manner (Fig. 5B). Moreover, we observed that COX inhibition by indomethacin and BLT2 antagonist did not reduce the migration index induced by LTB4 (Fig. 3C).

**Discussion**

PGE2 and LTB4 are important mediators in the development of both acute and chronic inflammation. Thus, the
inhibition of eicosanoid synthesis prevented tissue edema, leukocyte infiltration, and hyperalgesia, which clearly demonstrates that these eicosanoids are necessary for the progression of inflammation.

Manipulation of dietary PUFA has been used both experimentally and clinically as a means to modulate inflammatory diseases. In an effort to understand the mechanism underlying the effectiveness of dietary fatty acid modulation in altering inflammation, the current study compared the effects of AA-derived eicosanoids (PGE2 and LTB4) and EPA-derived eicosanoids (PGE3 and LTB5) on important events in the development of inflammatory responses such as endothelial permeability, leukocyte adhesion to endothelial cells, and leukocyte chemotaxis.

PGE2 plays a critical role in the development of edema in acute inflammatory processes (Portanova et al., 1996). Several reports have shown that n-3 fatty acid supplementation can lead to the increased synthesis of 3-series PG in vivo (Fischer et al., 1988; Vanamala et al., 2008); however, the effects of AA-derived eicosanoids (PGE2 and LTB4) and EPA-derived eicosanoids (PGE3 and LTB5) on important events in the development of inflammatory responses such as endothelial permeability, leukocyte adhesion to endothelial cells, and leukocyte chemotaxis.

**Fig. 4.** Effect of LTB4 and LTB5 on adhesion molecule expression. The effects of LTB4 and LTB5 on LFA-1 (A) and MAC-1 (B) expression on PMN and on E-selectin (C), and the effects of ICAM-1 (D) expression on ECV304 cells were measured. The effects of LFA-1, MAC-1, and E-selectin expression were measured 1 h after LTB4 (■)/LTB5 (○) incubation, whereas ICAM-1 expression was assayed 4 h after leukotriene incubation. Results are shown as means ± S.E.M. of three independent experiments performed in triplicate. *P < 0.05 for comparison with control group.

**Fig. 5.** PMN migration across Boyden chamber membrane in response to LTB4 or LTB5. A, PMN cells were incubated with LTB4 (○) and LTB5 (●) and allowed to migrate for 3 h across the membrane as described in Materials and Methods. An average locomotion index was quantified as expressed in comparison with the control (control = 1). The effect of LTB4 (○) and LTB5 (●) on PMN migration was also determined in the presence of U75302 (0–10 μM) (B) and in the presence of LY2555283 (5 μM) and indomethacin (Indo, 10 μM) (C). We also determined the chemotactic effect of PGE2 and PGE3 (100 nM). Results are shown as means ± S.E.M. of three independent experiments performed in triplicate. *P < 0.05 for comparison with control group (A) and the LTB4/LTB5 group (B).
cellular effects of increasing the synthesis of 3-series PG have not been investigated. Moreover, very few studies have focused on the effects of PGE\textsubscript{3} on plasma extravasation, and consequently on edema formation. Our results demonstrate that in a concentration-dependent manner, PGE\textsubscript{2} and PGE\textsubscript{3} increase the permeability of an ECV304 monolayer to albumin, without the participation of any other mediator or cell type. Moreover, the concentration of prostaglandins that induced endothelial permeability was similar to the prostaglandin concentration in inflammatory focus (Moreno 1993; Lloret and Moreno 1995).

EP\textsubscript{1}, EP\textsubscript{2}, EP\textsubscript{3}, and EP\textsubscript{4} have been identified as PGE\textsubscript{2} receptors (Coleman et al., 1994). Our results show that EP\textsubscript{1} and EP\textsubscript{2} antagonists impaired the observed increase in permeability induced by PGE\textsubscript{2} and PGE\textsubscript{3}, which confirmed the role of both receptors in the effect of PGE\textsubscript{2}/PGE\textsubscript{3} on endothelial permeability. PGs induced changes in the cell cytoskeleton, which lead to cell retraction, and extracellular gaps formation (Mark et al., 2001). Consequently, this effect might be enhanced by the permeability of the monolayer to albumin through this mechanism.

In one study of mice, PGE\textsubscript{3} had lower edemagenic effects than PGE\textsubscript{2} (Hawkes et al., 1992); however, results presented here suggest a similar mechanism of action for PGE\textsubscript{3} with a higher effect than PGE\textsubscript{2}. With the exception of the EP\textsubscript{2} receptor, there is no quantitative information or direct evidence that can be used to clarify the differences between the EP receptors in their responses to PGE\textsubscript{2} and PGE\textsubscript{3}. One explanation for our findings may be that PGE\textsubscript{3} has a slightly higher affinity for the EP\textsubscript{2} receptor than PGE\textsubscript{2}, which was recently proposed by Bagga et al. (2003). This could explain the slightly greater effects of PGE\textsubscript{3} than PGE\textsubscript{2}.

PGE\textsubscript{3} also exhibits regulatory function to control immune response, such as proinflammatory cytokine secretion, and consequently chronic inflammatory processes, and to control tissue repair and fibrosis processes, important events in the resolution phase of inflammation (Harizi et al., 2002; Vancheri et al., 2004). We must consider that PGE\textsubscript{3} and other EPA or DHA metabolites such as resolvins (Serhan et al., 2008) might have a role in the beneficial effects of dietary supplementation with n-3 PUFA through these mechanisms.

Nevertheless, our results show that endothelial monolayers exposed to LTB\textsubscript{4} and LTB\textsubscript{5} did not increase permeability to albumin, whereas the assay performed in the presence of PMN cells induced an increase in endothelial permeability. Findings that suggest that LTB\textsubscript{4}-induced increase in endothelial permeability is related to PMN adhesion, PMN activation, and probably mediator release by PMN, but not by LTB\textsubscript{5}, have a direct effect, in agreement with Di Gennaro et al. (2009), who reported an indirect activity of LTB\textsubscript{4} on vessel wall barrier function, with heparin-binding protein playing the role of effector molecule. In this way, the effects of a COX inhibitor and EP\textsubscript{1} and EP\textsubscript{4} antagonists suggest the participation of PGE\textsubscript{2} through EP\textsubscript{1}/EP\textsubscript{4} interaction in these events, and consequently the interplay of PGE\textsubscript{2}/PGE\textsubscript{3} in the effects of LTB\textsubscript{4}/LTB\textsubscript{5} on endothelial permeability in a coculture model of endothelial and PMN cells. From our results, LTB\textsubscript{4} had a lower effect on PMN adhesion than LTB\textsubscript{5} did. Consequently, LTB\textsubscript{5} modifies endothelial permeability to a minor extent.

Enhanced transmigration of circulating blood cells across the vascular endothelium is considered an important contribution to the pathogenesis of acute and chronic inflammatory diseases. This leukocyte recruitment to inflamed areas requires a precise sequence of events that initially involves the interaction of leukocytes with activated endothelial cells via the regulated expression of surface adhesion molecules. The contact with inflamed endothelium causes rolling and arrest of circulating leukocytes on the endothelium via adhesion molecules. Several adhesion molecules have been shown to be involved in these events, including β-integrins, selectins, and intercellular adhesion molecules (Andrew et al., 1998; Reinhardt and Kubes, 1998). Sperling et al. (1993) reported that ω-3 PUFA supplementation reduces neutrophil adhesion and migration. This indicates that these events take place after the reduction of LTB\textsubscript{4} formation and the increase of LTB\textsubscript{5} levels.

To our knowledge, the comparative effects of LTB\textsubscript{4} and LTB\textsubscript{5} on adhesion molecule expression and leukocyte adhesion to endothelial cells have not yet been described. Thus, an important finding of the present study is that LTB\textsubscript{5} presents a markedly minor induction of PMN adhesion to endothelial cells with respect to LTB\textsubscript{4} effects. This might be explained by the minor expression of the adhesion molecule on PMN/ endothelial cell surface in the presence of LTB\textsubscript{5}. Our results confirm that LTB\textsubscript{4} induced a slight LFA-1/MAC-1 or ICAM-1/E-selectin expression on PMN or endothelial cells, respectively, whereas LTB\textsubscript{5} induced a significant increase in the expression of four adhesion molecules. Consequently, the adhesion of PMN on endothelial cells was found to be largely increased by LTB\textsubscript{4}, but not by LTB\textsubscript{5}. This lack of effect of the EPA-derived leukotriene could be related to the suppression of leukocyte adhesion by EPA supplementation (Mayer et al., 2002).

This study provides the first description of an in vitro assay in which LTB\textsubscript{5} has a markedly lower chemotactrant effect than LTB\textsubscript{4}. In addition, we observed that PGE\textsubscript{2} and PGE\textsubscript{3} were not able to induce chemotaxis across filters. LTB\textsubscript{4} exerts its biological actions via two distinct receptors: LTB\textsubscript{4} receptor type 1 (BLT1), which is highly expressed in leukocytes, and LTB\textsubscript{4} receptor type 2 (BLT2), which is expressed more pervasively than BLT1 in human tissues. Both receptors can transmit LTB\textsubscript{4} signals, but BLT1 has ~20-fold higher affinity for LTB\textsubscript{4} than BLT2 has (Yokomizo et al., 2000). The lack of effect of LTB\textsubscript{5} on molecule adhesion expression on mononuclear and endothelial cell surfaces, mononuclear adhesion to endothelium and mononuclear migration, might be correlated with the minor affinity of LTB\textsubscript{5} by LTB\textsubscript{4} receptors and especially by BLT1. Therefore, the difference in the binding affinities of LTB\textsubscript{4} and LTB\textsubscript{5} to the LTB\textsubscript{4} receptor has been proposed to explain the differences in their biological activities (Seya et al., 1988). One study reported that the pretreatment of PMN with U75302, a specific BLT1 antagonist (Yokomizo et al., 2000), inhibited the LTB\textsubscript{4}/LTB\textsubscript{5}-triggered adhesion and migration of mononuclear cells. This demonstrates that PMN adhesion and the chemotactrant effect of both leukotrienes were mediated by BLT1 on mononuclear cells, as was also reported for monocytes (Friedrich et al., 2003), whereas BLT2 and prostaglandins seem not to be involved in these events. In line with this, BLT1 has been shown to play an important role in the recruitment of leukocytes into inflamed tissues (Kim et al., 2006).

In summary, the present results illustrate the effects of LTB\textsubscript{4} and LTB\textsubscript{5} on adhesion molecule expression and on
PMN adhesion and migration, and demonstrate that, unlike LTB₄, LTB₅ does not modulate expression of adhesion molecules. LTB₄ consequently does not induce PMN adhesion to endothelial cells. The reduced activity of LTB₅ in adhesion molecule expression and PMN cell adhesion to endothelial cells, together with the marginal chemotactic activity of the LTB₅, which possesses approximately 10% of the chemotactic activity of LTB₄, could be explained by the different in vivo effects of both leukotrienes on tissue leukocyte infiltration. It is interesting that these differences were similar to those achieved in vivo. Heidel et al. (1989) observed that 1 nmol of LTB₄ was required to achieve a cellular influx equivalent to that elicited by 30 pmol of LTB₅.

In conclusion, understanding the differences in the effects of LTB₄/LTB₅ on the main steps of PMN transmigration may help to explain the benefits of ω-3 PUFA in inflammatory processes. We have also demonstrated for the first time that the PGE₂ and PGF₃α induce similar effects on the endothelial permeability to macromolecules such as albumin.

References

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