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

JUAN J. MORENO

Department of Physiology, Faculty of Pharmacy, University of Barcelona, Avda. Joan XXIII s/n, E-08028 Barcelona, Spain.
Running title: n-3 and n-6 Eicosanoids and PMN Transmigration

Corresponding author: Juan J. Moreno. Department of Physiology, Faculty of Pharmacy, University of Barcelona, Avda. Joan XXIII s/n, E-08028 Barcelona, Spain. Tel. 3493 4024505, FAX 3493 4035901, e-mail: jjmoreno@ub.edu

Number of text pages: 26
Number of tables: 0
Number of figures: 5
Number of references: 37
Number of words in the abstract: 251
Number of words in the Introduction: 439
Number of words in the discussion: 1414

ABBREVIATIONS: AA, arachidonic acid; DHA, docosahexaenoic acid; EBA, Evans blue-albumin; EPA, eicosapentaenoic acid; FCS, fetal calf serum; 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

Recommended section: Inflammation, Immunopharmacology and Asthma
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 pro-inflammatory than AA metabolites. However, the molecular basis of this action is poorly understood. In this study, we compared the effects of PGE\textsubscript{2} and PGE\textsubscript{3} on endothelium permeability, and the effects of LTB\textsubscript{4} and LTB\textsubscript{5} 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. Interestingly, the effect of PGE\textsubscript{3} was significantly more pronounced than of PGE\textsubscript{2} and both were antagonized by EP\textsubscript{1} and EP\textsubscript{2} antagonists. LTB\textsubscript{4} and LTB\textsubscript{5} had a slight effect on EBA extravasation. However, we observed the enhancement of endothelial permeability in presence of polymorphonuclear (PMN), probably consequence of an interplay between leukotriene and prostanoid effects. LTB\textsubscript{4} caused significant increases in the number of PMN adhering to endothelial cells whereas LTB\textsubscript{5} did not induce a significant effect. This effect of LTB\textsubscript{4} appears BLT1 receptor dependent and was mediated through the enhancement of LFA-1, MAC-1, E-selectin and ICAM-1 expression. Finally, we observed that unlike LTB\textsubscript{5}, which had a weak effect, LTB\textsubscript{4} was a highly potent chemoattractant. An understanding of the differences in the effects of LTB\textsubscript{4}/LTB\textsubscript{5} on PMN adhesion and migration may help to explain the beneficial impact of \(\omega\)-3 fatty acids in inflammatory processes.
Introduction

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 anti-carcinogenic action (Jho et al., 2004), and major positive effects on lipid profile, plaque formation, and the reduction of infarction from coronary atherosclerosis (Kris-Etherton 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 PGE_2 (Moreno et al., 2001), whereas the presence of EPA/DHA in membrane phospholipids is increased.

Although phospholipases A_2 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 metabolised by lipoxygenases 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 prostanoids and 5-series leukotrienes (Smith, 2005). Although similar in structure and stability, the AA-derived eicosanoids are considered to be more pro-inflammatory than EPA metabolites. Hawkes et al. (1992) reported that PGE₃ had less oedematogenic effects than PGE₂ and that LTB₅ showed 10% of the chemotactic potency of LTB₄ (Heidel et al., 1989). Thus, supplementating diets with ω-3 instead of ω-6 fatty acids-rich is considered to be a therapeutic approach for a number of inflammatory processes. However, there is a lack of studies that directly compares 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 endothelium permeability and on mononuclear adhesion and migration.
Methods

Materials. Evans blue, albumin, 2-Acetylhydrazide 10(11H)-carboxylic acid; 8-Chloro-dibenzo[b,f][1,4]oxazepine-10(11H)-carboxylic acid (SC 19200), 6-[6-(3-Hydroxy-1E,5Z-undecadienyl)-2-pyridinyl]-1,5-hexanediol (U 75302), and 1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetic acid (indomethacin) were provided by Sigma Chemical Co. (St Louis, MO). PGE2, PGE3, LTB4 and LTB5 were provided by Cayman Chemical Co (Ann Arbor, MI). 1-[5-Ethyl-2-hydroxy-4-[(6-methyl-6-(1H-tetrazol-5-yl)heptyl]oxy]phenyl]ethanone (LY255283) from Tocris (Bristol, UK). 6-Isoproxy-9-oxoxanthene-2-carboxylic acid (AH 6809) and (4Z)-7-((rel-1S,2S,5R)-5-(1,1’-Biphenyl-4-yl)methoxy)-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid hemicalcium salt hydrate (AH 23848) were kindly supplied by Glaxo Wellcome 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% FCS. Cells were split at confluence 1 to 3 or at higher passages 1 to 2 every 5 to 7 days and plated on gelatine-coated flasks up to passage 20.

Evans blue was added in a 1:1 molar 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 dialyzate 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 ECV 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) using the formula derived from Fick’s law of diffusion:

\[
\ln \left[ \frac{1}{1-(V_s + V_l) C_s(t)/T_P (t)} \right] = PC \left[ A \left( V_s + V_l \right) / V_l / V_s \right]
\]

where (A) represents the area of membrane, (Vl), the volume of luminal chamber, (t), sample times, (Vs), albumin concentration in subluminal chamber, (Cs(t)), albumin concentration at each sample time and (TP (t)) total mass of albumin at each sample time.

Results are expressed as the percent difference between the PC before and after adding eicosanoid.

**Polymorphonuclear granulocyte isolation.** Blood samples were mixed with 1% dextran sulphate 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, and gradient centrifugation was carried out for 30 min at 700 g. The pellet comprised polymorphonuclear (PMN) cells and some contaminating erythrocytes. Erythrocytes were eliminated by lysis with 0.98% ammonium chloride, and the PMN were washed twice with Eagle’s minimal essential medium (MEM) and resuspended at a final concentration of 1 x 10⁶ PMN/ml.
Polymorphonuclear granulocyte adhesion to endothelial cells. ECV304 cells were plated and grown to confluence (around 5 x 10^5 cells/well). Thereafter, endothelial cells were washed and incubated with polymorphonuclear granulocytes (2 x 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) using 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 x 10^6 cells/ml) were incubated with antibodies for LFA-1 and MAC-1 (Boehringer Ingelheim, Ingelheim, Germany; dilution 1:50). ECV304 cells (2 x 10^6 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 PBS, fixed in 1% paraformaldehyde in PBS, and analyzed with a fluorescein-activated cell sorter analyzer (FACScan, Becton Dickinson, Mountain View, 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 (RFU). RFU = FU_{experimental} - FU_{isotype} x 100/FU_{control} - FU_{isotype} where FU_{experimental} and FU_{control} are the
fluorescence intensities of stimulated and control cells, respectively, and FUisotype is the fluorescence intensity of class-matched irrelevant antibody.

**Polymorphonuclear granulocyte chemotaxis.** PMN chemotaxis was quantified using the modified Boyden chamber technique (Boyden, 1962). Briefly, PMN were suspended in Eagle’s MEM containing 20 mM HEPES (pH 7.3) and 5 x 10⁵/0.5 ml pipeted 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, Bedford, MA). Chemoattractant 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 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 levels 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 in comparison to the control (control = 1). For each filter, three fields were counted and indexes were calculated as above was mentioned.

**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$_2$ and PGE$_3$ increase endothelial monolayer permeability to albumin.** To determine the role of PGE$_2$ and PGE$_3$ in oedema formation, ECV304 monolayers were exposed to both prostaglandins and EBA extravasation across endothelial cell cultures was measured. Results are expressed as a function of percent increase in the transendothelial albumin clearance rate after the addition of prostaglandin compared to basal levels. Vehicle (medium) caused a slight change in permeability (from 6.9 ± 1.2 10^{-6} \text{ cm/s} to 8.2 ± 1.1 10^{-6} \text{ cm/s}), whereas both prostaglandins increased the permeability of trans-endothelial Evans blue-albumin in a concentration-dependent manner (Fig. 1A). Both prostanoids reached a maximum plateau effect at 100 nM. Interestingly, the effect of PGE$_3$ was significantly more pronounced than that of PGE$_2$.

PGE$_2$ and PGE$_3$ bind to four plasma membrane receptors (EP$_1$-EP$_4$). The role of EP receptors in changing of albumin permeability induced by PGE$_2$/PGE$_3$ was investigated using specific EP antagonist. We used SC19200 as EP$_1$ antagonist (Funk et al. 1993), AH 6809 as EP$_2$ antagonist (Woodward et al., 1995), ONO-AE3-240 as EP$_3$ antagonist (Sugimoto and Narumiya, 2007) and AH 23848 as EP$_4$ antagonist (Davis et al., 2000). Considering the affinity of these antagonists, all compounds were used at a concentration of 1 \text{ µM}, which markedly blocked the binding of PGE$_2$ to EP receptors. Our results reveal a significant decrease in Evans blue-albumin permeability induced by PGE$_2$ and PGE$_3$ in the presence of EP$_1$ and EP$_2$ antagonists (Fig. 1B). These finding demonstrate the role of these PGE$_2$ receptors in the enhancement of endothelial permeability induced by PGE$_2$/PGE$_3$.

In contrast to prostaglandin effects, LTB$_4$ and LTB$_5$ showed only a slight effect on EBA extravasation (Fig. 2A). However, we also observed that both leukotrienes were able to induce EBA clearance though endothelial cultures in the presence of PMN (Fig. 2B),
indicating that this permeability response was PMN-dependent. Interestingly, we observed that LTB₄ was 100 times more active on PMN adhesion and consequently on PMN-dependent changes in permeability than LTB₅ was. In addition, we observed that these PMN-dependent changes in endothelial permeability induced by LTB₄/LTB₅ were significantly inhibited by a cyclooxygenase inhibitor such as indomethacin as well as EP₁ and EP₂ antagonist (Fig. 2C) suggested a role of prostaglandins in these events.

**LTB₅ induced a lower ratio of PMN adhesion to ECV304 cells than LTB₄.** In comparison with control conditions, LTB₄ (1-100 nM) caused significant increases in the number of PMN adhering to ECV304 cultures, whereas LTB₅ (1-100 nM) was unable to induce significant PMN adhesion to endothelial cells (Fig. 3A). LTB₄ acts via two specific plasma membrane receptors named BLT1 (high affinity) and BLT2 (low affinity). To investigate the role of both receptors in LTB₄-induced PMN adhesion, we used U-75302 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 to endothelium induced by LTB₄. LTB₅ was less effective than LTB₄ but was sensitive to the presence of U-75302 presence (Fig. 3B). Our findings also demonstrated that the effects of LTB₄/LTB₅ on PMN adhesion were not appreciably modified by indomethacin (Fig. 3B).

**PMN adhesion to endothelial cells induced by LTB₄ may be mediated through the enhanced expression of adhesion molecules.** To address the hypothesis that the effect of LTB₄ 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 determined in the surface of PMN or ECV304, respectively. We observed that all adhesion molecules were markedly up-regulated 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 (Figures 4A, 4B and 4C). ICAM-1 expression was highest 4 h after LTB₄ (10 nM) incubation, whereas the concentration of LTB₅ did not significantly increase ICAM-1 expression (Figure 4D). These findings correspond with the higher effect on PMN/endothelial 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 migrated across filters in response to LTB₄ (Fig. 5A). LTB₄ induced maximum migration at a concentration of 100 nM (migration index around 1.7), whereas at 1000 nM LTB₅ caused only a minor PMN migration (migration index around 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, U 75302, in a concentration-dependent manner (Fig. 5B). Moreover, we observed that COX inhibition by indomethacin and BLT2 antagonist did not reduce migration index induced by LTB₄ (Fig. 3C).
Discussion

PGE$_2$ and LTB$_4$ are important mediators in the development of both acute and chronic inflammation. Thus, the inhibition of eicosanoid synthesis prevented tissue oedema, 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 (PGE$_2$ and LTB$_4$) and EPA-derived eicosanoids (PGE$_3$ and LTB$_5$) on important events in the development of inflammatory responses such as endothelium permeability, leukocyte adhesion to endothelial cells, and leukocyte chemotaxis.

PGE$_2$ plays a critical role in the development of oedema in acute inflammatory processes (Portanova et al. 1998). Several reports have shown that ω-3 fatty acid supplementation can lead to the increased synthesis of 3-series PG in vivo (Fisher et al., 1988; Vanamala et al., 2008); however, the 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$_3$ on plasma extravasation, and consequently on oedema formation. Our results demonstrate that in a concentration dependent manner, PGE$_2$ and PGE$_3$ 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$_1$, EP$_2$, EP$_3$ and EP$_4$ have been identified as PGE$_2$ receptors (Coleman et al., 1994). Our results show that EP$_1$ and EP$_2$ antagonists impaired the observed increase in
permeability induced by PGE$_2$ and PGE$_3$, which confirmed the role of both receptors in
the effect of PGE$_2$/PGE$_3$ 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 though this mechanism.

In one study using mice, PGE$_3$ had lower oedematogenic effects than PGE$_2$ (Hawkes
et al., 1992); however, results presented here suggest a similar mechanism of action for
PGE$_3$ with a higher effect than PGE$_2$. With the exception of the EP$_2$ 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$_2$ and PGE$_3$. One explanation for our
findings may be that PGE$_3$ has a slightly higher affinity for the EP$_2$ receptor than PGE$_2$,
which was recently proposed by Bagga et al. (2003). This could explain the slightly
greater effects of PGE$_3$ than PGE$_2$.

PGE$_2$ also exhibits regulatory function to control immune response such as pro-
inflammatory cytokines secretion and consequently on 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$_3$ as well as 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$_4$ and
LTB$_5$ did not increase permeability to albumin, whereas the assay performed in
presence of PMN induced an increase in endothelium permeability. Findings that
suggest that LTB$_4$ induced increase in endothelial permeability is related to PMN
adhesion, PMN activation and probably mediators release by PMN but not by LTB$_4$
directly effect. In agree with Di Gennaro et al. (2009) who reported an indirect activity of LTB₄ on vessel wall barrier function, with hepain-binding protein playing the role of effector molecule. In this way, the effects of a COX inhibitor as well as EP₁ and EP₂ antagonist suggest the participation of PGE₂ through EP₁/EP₂ interaction in these events, and consequently the interplay of PGE₂/PGE₃ in the effects of LTB₄/LTB₅ on endothelial permeability in a co-culture model of endothelial and PMN cells. From our results, LTB₅ had a lower effect on PMN adhesion than LTB₄ did. Consequently LTB₅ 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 (ICAM) (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 following the reduction of LTB₄ formation and the increase of LTB₅ levels.

To our knowledge, the comparative effects of LTB₄ and LTB₅ 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 LTB₅ present a markedly minor induction of PMN adhesion to endothelial cells with respect to LTB₄ effects. This might be explained by the minor expression of the adhesion molecule on PMN/endothelial cell
surface in the presence of LTBs. Our results confirm that LTB5 induced a slight LFA-1/MAC-1 or ICAM-1/E-selectin expression on PMN or endothelial cells, respectively, whereas LTB4 induced a significant increase in the expression of four adhesion molecules adhesion. Consequently, the adhesion of PMN on endothelial cells was found to be largely increased by LTB4, but not by LTB5. 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 LTB5 has a markedly lower chemoattractant effect than LTB4. In addition, we observed that PGE2 and PGE3 were not able to induce chemotaxis across filters. LTB4 exerts its biological actions via two distinct receptors: LTB4 receptor type 1 (BLT1), which is highly expressed in leukocytes, and LTB4 receptor type 2 (BLT2), which is expressed more pervasively than BLT1 in human tissues. Both receptors can transmit LTB4 signals, but BLT1 has ~20-fold higher affinity for LTB4 than BLT2 has (Yokomizo et al., 2000). The lack of effect of LTB5 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 LTB5 by LTB4 receptors and especially by BLT1. Therefore, the difference in the binding affinities of LTB4 and LTB5 to the LTB4 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 U-75302, a specific BLT1 antagonist (Yokomizo et al. 2000), inhibited the LTB4/LTB5-triggered adhesion and migration of mononuclear cells. This demonstrates that PMN adhesion and the chemoattractant 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 appear to be not 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 LTB₄ and LTB₅ on adhesion molecule expression and on PMN adhesion and migration, and demonstrate that unlike LTB₄, LTB₅ does not modulate adhesion molecules expression. Consequently LTB₅ does not induce PMN adhesion to endothelial cells. The reduced activity of LTB₅ in adhesion molecule expression and PMN 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. Interestingly, these differences were similar to those achieved in in vivo. Heidel et al (1989) observed that 1 nmole of LTB₅ was required to achieve a cellular influx equivalent to that elicited by 30 picomoles of LTB₄.

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. Interestingly, we have also demonstrated for the first time that PGE₂ and PGE₃ induce similar effects on the endothelial permeability to macromolecules such as albumin.
References


Footnotes

This work was supported by the Ministerio de Ciencia y Tecnología [Grants BFU2004-04960/BFI, BFU2007-61727/BFI]; and the Generalitat de Catalunya [Grant SGR20050269]

Send reprint requests to: Dr. Juan J. Moreno. University of Barcelona, Faculty of Pharmacy, Department of Physiology, Avda. Joan XXIII s/n, E-08028, Barcelona, Spain. E-mail: jjmoreno@ub.edu
Legends for Figures

Fig. 1. Effect of PGE$_2$ and PGE$_3$ on endothelial permeability to albumin. (A) ECV304 monolayers were incubated in the presence of PGE$_2$ (○) and PGE$_3$ (●) (0.1-1000 nM) for 30 min and permeability to albumin was measured. (B) The effect of PGE$_2$ (100 nM, white bars) and PGE$_3$ (100 nM, black bars) on endothelial permeability was also measured for 30 min in the presence of SC19200 (EP$_1$ antagonist), AH6809 (EP$_2$ antagonist), ONO-AE3-240 (EP$_3$ antagonist), and AH23848 (EP$_4$ antagonist) at 1 µM. Values are shown as means ± S.E.M. of three independent experiments performed in duplicate. * P < 0.05 for comparison with control group (in figure A) and the PGE$_2$/PGE$_3$ group (in figure B). § P < 0.05 for comparison with PGE$_2$ group.

Fig. 2. Effect of LTB$_4$ and LTB$_5$ on endothelial permeability to albumin. (A) ECV304 monolayers were incubated in presence of LTB$_4$ (○) or LTB$_5$ (●) (0.1-1000 nM) for 30 min and permeability to albumin was measured. (B) The effect of LTB$_4$ (○) or LTB$_5$ (●) (0.1-10000 nM) was also studied for 30 min in ECV302 monolayers in presence of PMN (2 x 10$^6$ cells/well). (C) Effect of indomethacin (Indo, 10 µM), SC19200 (SC, 1 µM), AH6809 (AH68, 1 µM) and SC19200 (1 µM) plus AH6809 (1 µM) on changes of endothelial permeability induced by LTB$_4$ (100 nM, white bars) or LTB$_5$ (100 nM, black bars) in a co-culture of ECV304 cell monolayers with PMN (2 x 10$^6$ cells/well). Compounds were preincubated 10 min and leukotriene incubations were performed for 30 min. Values are shown as means ± S.E.M. of three independent experiments performed in duplicate. * P < 0.05 for comparison with control group (figures 2A, 2B) or with LTB$_4$/LTB$_5$ group (Figure 2C). § P < 0.05 for comparison with co-cultures treated with LTB$_4$/LTB$_5$ in presence of SC19200.
Fig. 3. Effects of LTB$_4$ or LTB$_5$ on PMN adhesion to endothelial cells. (A) PMN (2 x 10$^6$ cells/well) were incubated into ECV304 monolayer in the presence of leukotrienes (1-100 nM) for 3 h and PMN adhesion was then determined. (B) The effect of LTB$_4$ (white bars) and LTB$_5$ (black bars) on PMN adhesion to endothelial cells was also performed in the presence of U75302 (1-10 µM), LY255283 (5 µM) or indomethacin (Indo, 10 µM). Values are shown as means ± S.E.M. of three independent experiments performed in duplicate. * P < 0.05 for comparison with the control group and § P < 0.05 for comparison with the LTB$_4$/LTB$_5$ group.

Fig. 4. Effect of LTB$_4$ and LTB$_5$ on adhesion molecule expression. The effects of LTB$_4$ and LTB$_5$ on LFA-1 (A) and MAC-1 (B) expression on PMN and on ICAM-1 (C) and the effects of E-selectin (D) expression on ECV304 cells were measured. LFA-1, MAC-1, and E-selectin expression were measured 1 h after LTB$_4$ (white bars)/LTB$_5$ (black bars) incubation, while 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 were incubated with LTB₄ (○) and LTB₅ (●) and allowed to migrate for 3 h across the membrane as described in Material and methods section. An average locomotion index was quantified as expressed in comparison to the control (control = 1). (B) The effect of LTB₄ (○) and LTB₅ (●) on PMN migration was also determined in the presence of U75302 (0-10 µM) and (C) in presence of LY255283 (5 µM) and indomethacin (Indo, 10 µM). 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 (in figure A) and the LTB₄/LTB₅ group (in figure B).
Figure 1

A

B

- Concentration (nM)
- % Permeability
- SC AH68 ONO AH23
- PGE$_2$
- PGE$_3$

Bars with asterisks indicate significant differences.
Figure 2

A

ECV304 cells

% Permeability

0 40 80 120 160

Concentration (nM)

0.1 1 10 100 1000

B

ECV304 cells + PMN

% Permeability

0 50 100 150 200 250

Concentration (nM)

0.1 1 10 100 1000

C

ECV304 cells + PMN

% Permeability

0 50 100 150 200

Indo SC AH68 SC+AH68

* * § *
Figure 5

A

B

C

Migration index vs. Concentration (nM)

Migration index vs. U 75302 (μM)

Migration index vs. Treatment

Control  U75  LY255  Indo  PGE₂  PGE₃  LTB₄  LTB₄ + PGE₂