Cysteinyl Leukotrienes Induce P-Selectin Expression in Human Endothelial Cells via a Non-CysLT₁ Receptor-Mediated Mechanism

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ABSTRACT

Cysteinyl leukotrienes are bioactive lipid mediators known to possess potent proinflammatory actions. Included in these are effects on vascular endothelium to promote surface expression of the adhesion molecule P-selectin. In the present study we were interested in investigating the receptor mechanism(s) involved in cysteinyl leukotriene-induced endothelial P-selectin expression. As such we examined the effect of several potent and selective cysteinyl leukotriene receptor antagonists on this response. Incubation of cultured human umbilical vein endothelial cells (HUVEC) with the cysteinyl leukotrienes leukotriene C₄ (LTC₄) or leukotriene D₄ (LTD₄) induced surface expression of P-selectin which was concentration dependent and rapid in onset. Expression of endothelial P-selectin induced by either LTC₄ or LTD₄ was not blocked however by pretreatment of HUVEC with the selective cysteinyl leukotriene-1 (CysLT₁) receptor antagonists SKF 104353, pranlukast or zafirlukast before agonist exposure. In contrast, SKF 104353 effectively antagonized the LTC₄ induced contractions in isolated human bronchial smooth muscle preparations, shifting the agonist dose-response curve to the right by some 3 log-fold in this tissue. The present results suggest that cysteinyl leukotrienes induce surface expression of endothelial P-selectin via a mechanism independent of the CysLT₁ receptor.

A central step in the migration of circulating leukocytes from blood to extravascular tissue is their adhesion to vascular endothelium. The process of leukocyte binding and transmigration is complex and involves the sequential interaction of several specific adhesion molecules located on both leukocyte and endothelial cell surfaces (Bevilacqua, 1993; Carlos and Harlan, 1994). Members of the selectin family of adhesion molecules are implicated in the initial stages of leukocyte capture (Bevilacqua and Nelson, 1993). One member of this family, P-selectin, is a glycoprotein found in the alpha granules of platelets (Hsu-Lin et al., 1984; Stenberg et al., 1985) and also in cytoplasmic granules of endothelial cells known as Weibel-Palade bodies (Bonfanti et al., 1989; McEver et al., 1989). Exposure of endothelial cells to appropriate stimuli leads to the mobilization of P-selectin from its intracellular stores and expression of this ligand on the endothelial cell surface (Hattori et al., 1989a; McEver et al., 1989). To date, expression of P-selectin has been shown to be rapidly induced (within minutes) after exposure of endothelial cells to a variety of inflammatory stimuli including histamine (Hattori et al., 1989a; McEver et al., 1989; Lorant et al., 1991), thrombin (Hattori et al., 1989a; Lorant et al., 1991) and components of complement (Hattori et al., 1989b; Foreman et al., 1994).

The cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄ are biologically active lipid mediators derived from the metabolism of arachidonic acid (Samuelsson, 1983). A substantial body of evidence now exists showing that these mediators are potent proinflammatory agents (Samuelsson et al., 1987; Henderson, 1994; Hay et al., 1995) and they have been implicated in the pathogenesis of inflammatory disease in a variety of tissues and organs (reviewed in Henderson, 1994; Hay et al., 1995; Drazen, 1995). Among the proinflammatory actions of these mediators are effects on the vasculature to cause increased vascular reactivity (Drazen et al., 1980) and permeability (Dahlen et al., 1981; Joris et al., 1987; Evans et al., 1989), as well as effects on inflammatory cell motility (Spada et al., 1994) and recruitment of leukocytes into tissue (Foster and Chan, 1991; Laitinen et al., 1993). Although the precise mechanism(s) by which cysteinyl leukotrienes cause leuko-
cyte recruitment is not yet clear, an area of emerging interest is in the ability of these mediators to affect the function and/or surface expression of cell adhesion molecules. Several observations indicate that cysteinyl leukotrienes may be involved in leukocyte recruitment via actions at the level of the endothelial cell involving P-selectin. Incubation of cultured HUVEC with LTC \(_4\) or LTD \(_4\) has been shown to induce rapid surface expression of P-selectin (Dutta et al., 1995; Papayianni et al., 1996) and increased adhesion of neutrophils to HUVEC monolayers (McIntyre et al., 1986; Papayianni et al., 1996) via a mechanism involving P-selectin (Papayianni et al., 1996). In the rolling, administration of LTC \(_4\) in vivo produces an increase in the rolling flux of leukocytes in rat mesenteric microvessels through a mechanism also dependent on P-selectin (Kanwar et al., 1995).

Cysteinyl leukotrienes mediate their biological effects via interaction with specific receptors located in target cells. Evidence is accumulating that heterogeneity within the leukotriene receptor population exists. In tissues such as guinea pig airways and ileum, findings from functional (Fleisch et al., 1982; Snyder and Krell, 1984; Gardiner et al., 1990) and receptor binding (Pong and DeHaven, 1983; Hogaboom et al., 1983) studies indicate the presence of different types of cysteinyl leukotriene receptors, one which is stimulated by LTC \(_4\) and a second which is activated by LTD \(_4\)/LTE \(_4\) (Snyder and Krell, 1984; Gardiner et al., 1990). The existence of more than one cysteinyl leukotriene receptor has also been reported in both the ferret spleen (Gardiner et al., 1994) and sheep airways (Cuthbert et al., 1991; Gardiner et al., 1994). In contrast, in airway smooth muscle preparations from human lung it is thought that only a single type of cysteinyl leukotriene receptor exists (Buckner et al., 1986). This CysLT \(_1\) receptor is characterized by contractions which are inhibited by potent CysLT \(_1\) receptor antagonists (Buckner et al., 1986, 1990; Hay et al., 1987; Fujiwara et al., 1993) and it is thought that LTC \(_4\) and LTD \(_4\) are both active at this site (Gardiner et al., 1994). There is some evidence to support the existence of leukotriene receptor subtypes in human tissue. Based on the results from inhibition studies with leukotriene receptor antagonists, distinct receptors for cysteinyl leukotrienes have been identified in human bronchial smooth muscle and pulmonary vein (Labat et al., 1992).

Little is known regarding the possible receptor mechanism(s) involved in mediating the actions of cysteinyl leukotrienes to induce endothelial P-selectin expression. Given the potential importance of adhesion molecule expression in inflammation and inflammatory disease we were interested in investigating the effects of cysteinyl leukotrienes on P-selectin expression in human endothelial cells, particularly in terms of characterizing the receptor(s) involved. In the present study we have examined the ability of cysteinyl leukotrienes to induce P-selectin expression in cultured HUVEC and have assessed the effects of a number of CysLT \(_1\) receptor antagonists on these responses. For comparison, the effects of histamine and of selective histamine receptor antagonists on histamine-induced endothelial P-selectin expression have also been reported. Our findings suggest that in human endothelial cells cysteinyl leukotriene-induced P-selectin expression is mediated via a mechanism independent of the classically defined CysLT \(_1\) receptor.

### Methods

**Endothelial cell culture.** Endothelial cells were isolated from human umbilical veins by digestion with 0.2% collagenase according to the method of Jaffe et al. (1973). The cell suspension was seeded in 25-cm\(^2\) tissue culture flasks (Nunc Inc., Naperville, IL) coated with 0.5% gelatin and 25 \(\mu\)g/ml fibronectin. Cells were cultured in M199 supplemented with 20% normal human serum, 1-glutamine (2 mM), penicillin (100 U/ml), streptomycin (196 \(\mu\)g/ml) (complete M199) and 25 \(\mu\)g/ml endothelial cell growth supplement. Cultures were allowed to grow to confluence at 37°C under 5% carbon dioxide and culture media were changed every 3 days. For adhesion experiments HUVEC at first passage were plated on eight-chamber Lab Tek slides (Nunc Inc.) coated with 0.5% gelatin and 25 \(\mu\)g/ml fibronectin. Cells were seeded at 90 to 95% confluence and used upon reaching visual confluence, typically within 24 h. Endothelial cells were characterized by a cobbled stone appearance, and all experiments were performed with cells that had been passaged only once.

**Detection of P-selectin expression on the endothelial cell surface.** Given the surface expression of P-selectin on cultured endothelial cells was performed by modification of a method described previously (Birch et al., 1994). Polyclonal goat antiserum IgG-coated magnetic, 4.5 \(\mu\)m, polystyrene beads (Dynal Inc, Great Neck, NY) were secondarily coated with either an anti-P-selectin mAb (GA6, Becton Dickinson, San Jose, CA) or with an irrelevant murine IgG \(_\alpha\) mAb (Couler Corp., Hialeah, FL) as a control. Beads were coated with the appropriate antibody by incubation in DPBS containing 0.25% HSA overnight at 4°C with gentle rotation at a final concentration of 2.8 \(\mu\)g antibody/mg beads. Beads were then washed a total of four times for 30 min each with DPBS + 0.25% HSA at 4°C and then resuspended in DPBS containing 0.2% HSA (DPBS + 0.2% HSA) such that each well to be assayed received 300 \(\mu\)l of antibody-coated bead suspension (final concentration, 1.5 mg beads/ml). The bead suspension was mixed well and allowed to warm to room temperature (22°C).

Confluent monolayers of HUVEC in eight-chamber slides were washed twice with M199 and complete M199 added to each well. HUVEC were then incubated with either LTC \(_4\), LTD \(_4\) or LTE \(_4\) (each at 1 \(\times\) \(10^{-6}\) M) or histamine (1 \(\times\) \(10^{-6}\) M) for 10 min at 37°C in a final incubation volume of 300 \(\mu\)l. In preliminary experiments cells were treated with LTC \(_4\) or LTD \(_4\) (1 \(\times\) \(10^{-7}\) M) for 5 to 30 min, and P-selectin expression was found to be maximal by 10 min. This time point was then used in all subsequent experiments. In each experiment exposure of cells to histamine (1 \(\times\) \(10^{-6}\) M) served as a positive control for P-selectin expression while a set of unstimulated cells served as the negative control.

To examine the effect of selective CysLT \(_1\) receptor antagonists on the expression of endothelial P-selectin induced by cysteinyl leukotrienes, HUVEC were incubated with LTC \(_4\) or LTD \(_4\) in the presence of the CysLT \(_1\) receptor antagonists SKF 104353 (1 \(\times\) \(10^{-5}\) M) (Hay et al., 1987), pranlukast (ONO-1078) (1 \(\times\) \(10^{-5}\) M) (Fujiiwa et al., 1993) or zafirlukast (ICI 204,219) (1 \(\times\) \(10^{-5}\) M) (Buckner et al., 1990). In these experiments the antagonists were added to cell cultures for 15 min at 37°C before administration of the agonist. In several experiments the effects of selective histamine receptor antagonists on histamine-induced P-selectin expression in HUVEC were also examined. In these experiments the selective H\(_1\) receptor antagonist pyrilamine (1 \(\times\) \(10^{-5}\) M) or the H\(_2/H\(_4\) receptor antagonist burimamide (1 \(\times\) \(10^{-5}\) M) was added to HUVEC 15 min before administration of histamine. Control cultures for the effects of agonist or antagonist vehicle on P-selectin expression received an equivalent volume of vehicle in place of the agonist or antagonist.

At the conclusion of the agonist incubation period the culture medium was removed, cells were washed once with DPBS + 0.2% HSA and 300 \(\mu\)l of bead suspension added to each well. Cells were then exposed to the antibody-coated beads for 20 min at room temperature on a rocking plate set at 30 rpm. The plastic chambers were removed, and the slide was washed manually in DPBS + 0.2% HSA.
until adherence of beads on the negative control was visibly negligible while remaining high on the positive control. Slides were then placed in 2% glutaraldehyde-PBS (4°C, pH 7.4) for 12 h after which cells were mounted in aqueous mounting medium (Crystal Mount; Biomedia, Foster City, CA) and visualized on an Olympus CK inverted microscope.

Quantitation of antibody-labeled beads adhered to HUVEC was achieved by image analysis coupled with manual counting. Slides were viewed with a color video camera (Sony; DVCX151A) attached to a Zeiss Axioslab photomicroscope using a 40 x objective lens (Zeiss; Acroplan). The video signal was processed through a camera adapter (Sony CMA-D2) to a Macintosh (PowerPC) microcomputer containing the image analysis software. The software (NIH Image, Bethesda, MD) controlled image capture, display and storage. For each well of the eight-chamber slide, eight fields of cells were viewed per well and images of these fields captured and printed by use of the computer-controlled imaging system. Using the 40 x objective each image captured corresponded to an area of 29,875 μm² on the slide. The number of beads in each image was then counted and bead densities expressed as beads/field.

Isolation of human lung tissues. Macroscopically normal human lung tissue was obtained from two patients undergoing surgical resection and four organ donors (supplied by the International Institute for the Advancement of Medicine, Exton, PA or the Anatomical Gift Foundation, Woodbine, GA). The surgical specimens were from patients with lung carcinoma, whereas the organ donor specimens (mean age, 23.5 ± 5.5 years; three males, one female) were mainly from victims of head trauma (gun shot wounds, motor vehicle accidents, closed head injury). Surgical specimens were placed in RPMI 1640 solution at 4°C within 90 min of resection for the 15 min transfer to the laboratory. Organ donor specimens were placed in cooled (4°C) tissue preservation solution (Viaspan; Du Pont Merck Pharmaceutical Co., Wilmington, DE) and transferred to the laboratory overnight. On reaching the laboratory all tissues were immediately placed in 4 liters of modified Krebs’ bicarbonate solution of the following composition (mM): NaCl, 118; KCl, 5.4; NaH2PO4, 1.0; MgSO4, 1.2; CaCl2, 1.9; NaHCO3, 25; and D-glucose, 11.1 and gassed following composition (mM): NaCl, 118; KCl, 5.4; NaH2PO4, 1.0; MgSO4, 1.2; CaCl2, 1.9; NaHCO3, 25; and d-glucose, 11.1 and gassed while remaining high on the positive control. Slides were then placed in 2% glutaraldehyde-PBS (4°C, pH 7.4) for 12 h after which cells were mounted in aqueous mounting medium (Crystal Mount; Biomedia, Foster City, CA) and visualized on an Olympus CK inverted microscope.

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Reagents. RPMI 1640, M199, L-glutamine, penicillin and streptomycin were obtained from Gibco BRL (Gaithersburg, MD); endothelial growth supplement was from Collaborative Biomedical Products (Bedford, MA); collagenase was obtained from Worthington Biochemical Corp. (Freehold, NJ); DPBS was from Biofluids (Rockville, MD); normal human serum, HSA, fibronectin, histamine diphosphate salt and pyrilamine maleate were purchased from Sigma Chemical Co. (St. Louis, MO); LTC4, LTD4, LTE4 and LTB4, SKF 104353, pranlukast, zafirlukast and burimamide were a generous gift from SmithKline Beecham Pharmaceuticals (King of Prussia, PA). Stock solutions (10⁻² M) of leukotrienes were previously prepared by dissolving LTC4 in methanol and LTD4 or LTE4 in ethanol. These were aliquoted and stored at −70°C until used. SKF 104353, pranlukast and zafirlukast were prepared by dissolving these compounds in distilled water, dimethyl sulfoxide and 0.1 N sodium hydroxide, respectively. All other drugs were dissolved in DPBS. Subsequent dilutions of drugs and stock solutions were made in DPBS with all drug dilutions prepared fresh on the day of experimentation.

Results

Effect of cysteinyl leukotrienes on P-selectin expression in HUVEC and contraction of airway smooth muscle preparations. Exposure of human endothelial cells to LTC4 or LTD4 for 10 min caused a concentration-related increase in the adherence of anti-P-selectin labeled beads to HUVEC monolayers (fig. 1a). This concentration-related increase in binding of beads to stimulated HUVEC was evident both by determination of the density of beads bound per field of cells and was also readily observable by phase-contrast microscopy. After exposure of HUVEC to 10⁻² M LTC4 mean bead density was 332.4 ± 31.7 beads/field (average of eight fields from each of n = 6 separate experiments). In control
cultures which received the vehicle for LTC4 in place of agonist, mean bead density was only 4.1 ± 1.2 beads/field (average of eight fields from each of n = 6 separate experiments). Extremely low levels of bead adherence to HUVEC were also observed in cultures where cells were stimulated with LTD4 at 10^-6 M and then exposed to beads which were either unlabeled or labeled with the irrelevant murine IgG1 mAb. Under these conditions mean bead density was found to be 2.4 ± 0.9 and 2.7 ± 0.6 beads/field, respectively (average of eight fields from each of n = 3 separate experiments).

Stimulation of endothelial cells with LTD4 also caused a concentration-related increase in the adherence of anti-P-selectin labeled beads to endothelial cells (fig. 1a). Because complete dose-response curves were not obtained with the concentrations of LTD4 and LTC4 studied it was not possible to critically determine the relative potency of LTD4 to LTC4. Nevertheless, under the present conditions at both 10^-6 M and 10^-7 M LTD4 was significantly less effective than LTC4 at inducing the increased adherence of anti-P-selectin labeled beads to HUVEC (fig. 1a, P < .05). In addition, exposure of HUVEC to LTE4 over the same concentration range was without apparent effect (fig. 1a). For example, at 10^-6 M LTE4 mean bead density was only 3.6 ± 1.4 beads/field (average of eight fields from each of n = 5 separate experiments) which was not different from control levels. Increased adherence of anti-P-selectin labeled beads to endothelial cells was also not observed after cells were stimulated with LTB4 (10^-7–10^-6 M) (data not shown).

Both LTC4 and LTD4 were more potent in causing human bronchial smooth muscle contraction than P-selectin expression (fig. 1b). Moreover unlike HUVEC P-selectin expression, the LTC4 and LTD4 concentration-effect curves for muscle contraction were superimposable (fig. 1b).

**Effect of CysLT1 receptor antagonists on leukotriene-induced responses in HUVEC and isolated airway smooth muscle preparations.** The effects of three structurally unrelated selective CysLT1 receptor antagonists, SKF 104353, pranlukast and zafirlukast, on LTC4-induced P-selectin expression in HUVEC are shown in figure 2. The concentration of each antagonist used was 10^-5 M, which is some 1000 times greater than their Kd values for the CysLT1 receptor (Hay et al., 1987; Buckner et al., 1990; Fujiwara et al., 1993). Thus, this concentration of antagonist would be predicted to cause a greater than 3 log-fold shift to the right of the agonist dose-response curve if CysLT1 receptors were involved. However pretreatment of HUVEC with either pranlukast, zafirlukast or SKF 104353 failed to significantly modify the adherence of anti-P-selectin labeled beads induced by LTC4 (fig. 2, a, b and c). Neither the maximal response nor the log (M) EC50 values (taking the response to 1 µM as a maximal response) for LTC4 were altered in the presence of these antagonists. As illustrated in figure 2, in all cases the LTC4 concentration-effect curves in the absence and presence of antagonist were superimposable. By contrast, pretreatment of isolated human airway preparations with a 10-fold lower concentration of SKF 104353 significantly antagonized the contractile effect of LTC4, which caused the expected 3 log-fold rightward shift in the agonist dose-response curve (fig. 2d).

Pretreatment of HUVEC with 10^-5 M SKF 104353 (fig. 2d), pranlukast or zafirlukast (not shown) also failed to significantly modify the surface expression of endothelial P-selectin induced by LTD4. As for LTC4, neither the maximal response nor the log (M) EC50 values (taking the response to 1 µM as a maximal response) for LTD4 were altered in the presence of these antagonists. Maximal responses for LTD4 were: control, 154.7 ± 30.0 beads/field; SKF 104353, 154.2 ± 22.4 beads/field; pranlukast, 158.7 ± 46.8 beads/field; and zafirlukast, 154.3 ± 51.1 beads/field; whereas the log (M) EC50 values for LTD4 were: control, 6.62 ± 0.02; SKF 104353, 6.58 ± 0.08; pranlukast, 6.30 ± 0.29; and zafirlukast, 6.34 ± 0.23, in paired samples from five separate experiments.

In addition to examining the ability of cysteinyl leukotrienes to induce surface expression of endothelial P-selectin, in several experiments we also examined the effect of histamine on P-selectin expression as well as the effect of selective histamine receptor antagonists on this response. Results obtained with histamine were useful for two purposes. They
allowed a direct comparison of the relative efficacies of cysteinyl leukotrienes and histamine, and also an evaluation of the actions of classically defined competitive receptor antagonists within the current model. Exposure of HUVEC to histamine for 10 min caused a concentration-related increase in the adherence of anti-P-selectin labeled beads (fig. 3). The maximum response to histamine was 377.2 ± 64.2 beads/field (average of eight fields from each of three separate experiments), which was similar to the maximum response observed for LTC4 (see above), although histamine was some 10-fold less potent than LTC4 in this regard. In the current model, pretreatment of HUVEC with the selective histamine H1 receptor antagonist pyrilamine (10⁻⁵ M) for 15 min before histamine exposure blocked the increased adherence of anti-P-selectin labeled beads induced by this agonist (fig 3; P < .05). In contrast, no significant alteration of the histamine response was observed when HUVEC were pretreated with the combined histamine H2/H3 receptor antagonist burimamide (10⁻⁵ M) (fig. 3).

Discussion

In the present study we have shown that the cysteinyl leukotrienes LTC4 and LTD4 induce surface expression of P-selectin in HUVEC. Comparison of the responses obtained with LTC4 and histamine show that LTC4 was about 10-fold more potent than and equally efficacious as histamine, a mediator well known to induce surface expression of endothelial P-selectin (Hattori et al., 1989a; McEver et al., 1989; Lorant et al., 1991). The findings that LTC4 and LTD4 cause surface expression of P-selectin in cultured human endothelial cells (Datta et al., 1995; Papayianni et al., 1996) and accumulation of inflammatory cells in vivo (Foster and Chan, 1991; Laitinen et al., 1993), suggest that these medi-
The identification of cysteinyl leukotrienes as mediators that may play a role in leukocyte recruitment during inflammation.

In the present study surface expression of endothelial P-selectin was detected through the use of small polystyrene beads coated with an anti-P-selectin mAb. This method not only allowed for ligand detection but also lent itself to quantification of the response by determining the density of beads adherent to cultured endothelial cells (Birch et al., 1994; Datta et al., 1995). Stimulation of human endothelial cells with either LTC4 or LTD4 produced surface expression of P-selectin which was rapid and concentration dependent. Under the present conditions LTC4 was found to be more effective than LTD4 at inducing P-selectin expression, whereas LTE4 and LTB4 were ineffective. This pattern of response for LTC4 and LTD4 was similar to that observed by Papayianni and co-workers (1996), who used a radiolabeled secondary antibody to detect endothelial P-selectin; it is also similar to the order of potency reported for the ability of LTC4 and LTD4 to cause another endothelial-related event, namely the secretion of von Willebrand factor from HUVEC (Datta et al., 1995). Both LTC4 and LTD4 were shown to induce eosinophil recruitment into guinea pig airways in vivo, whereas LTE4 and LTB4 were ineffective (Foster and Chan, 1991), although the site of action of the cysteinyl leukotrienes in this animal model is unknown.

The activity profile of the cysteinyl leukotrienes with respect to endothelial P-selectin expression contrasts with the effectiveness of these agonists in causing contraction in isolated human airway smooth muscle preparations. In these latter tissues, LTC4 and LTD4 produce similar dose-response curves as shown in this study and by others (Buckner et al., 1986, 1990; Labat et al., 1992). In isolated human bronchi the actions of cysteinyl leukotrienes to cause airway smooth muscle contraction have been attributed to activation of the CysLT1 receptor. In this tissue preparation the contractile effects of cysteinyl leukotrienes are readily blocked by selective CysLT1 receptor antagonists (Buckner et al., 1986; Hay et al., 1987; Labat et al., 1992; Fujiiwara et al., 1993). The characteristics of the receptor responsible for cysteinyl leukotriene-induced P-selectin expression in human endothelial cells are unclear, however. In our investigation we used three structurally unrelated, potent and selective CysLT1 receptor antagonists. The observation that, at concentrations 1000-fold greater than their estimated $K_d$ values for the CysLT1 receptor, SKF 104353, pranlukast and zafirlukast failed to inhibit the LTC4- or LTD4-induced P-selectin expression suggests that the agonists were acting by a mechanism independent of the CysLT1 receptor. Thus as graphically depicted in figure 2, it appears that the leukotriene receptor subtype responsible for LTC4- and LTD4-induced P-selectin expression in human endothelial cells is different from that which mediates leukotriene-induced human airway smooth muscle contraction. Although the characteristics of the receptor responsible for mediating cysteinyl leukotriene-induced P-selectin expression are unclear, within the current system results obtained for the histamine receptor antagonists are consistent with the hypothesis that histamine acts via the classical H1 receptor subtype to evoke endothelial P-selectin expression (Kubes and Kanwar, 1994).

Experiments with isolated human and animal tissues have already provided evidence for the existence of more than one leukotriene receptor (Fleisch et al., 1982; Snyder and Krell, 1984; Labat et al., 1992; Gardiner et al., 1994). Several studies with leukotriene receptor antagonists have demonstrated that there are least two cysteinyl leukotriene receptor types, one sensitive to a range of leukotriene antagonists and another which is insensitive to such compounds (Snyder and Krell, 1984; Gardiner et al., 1990, 1994; Cuthbert et al., 1991). In a comparative study on the contractile effects of cysteinyl leukotrienes in human airway and pulmonary vein smooth muscle it was reported that there were two types of leukotriene receptors in human lung, one found on airways which was inhibited by CysLT2 antagonists and a second found on pulmonary veins which was resistant to the CysLT1 antagonists (Labat et al., 1992). The antagonist data presented in the current study are in accord with such findings.

It should be noted that leukotriene receptor antagonists have been shown to be effective against cysteinyl leukotriene-induced endothelial responses. In two studies with cultured human endothelial cells, SKF 104353 was shown to inhibit LTD4-induced secretion of von Willebrand factor (Datta et al., 1995) and the LTC4- or LTD4-induced increase in endothelial cell cytosolic calcium (Heimburger and Palmblad, 1996). However in both these cases high concentrations of the antagonist ($\geq 1 \mu M$) were required for effect. In addition, CysLT1 antagonists are also effective at inhibiting the increase in microvascular permeability induced by cysteinyl leukotrienes in animal models (Nakagawa et al., 1992; Bochnowicz and Underwood, 1995). In certain tissues such as the guinea pig trachea (Snyder and Krell, 1984) and ileum (Gardiner et al., 1990) it appears that more than one type of functional leukotriene receptor is present. Whether such a situation exists in endothelial cells is unknown.
man airway smooth muscle which show that cysteinyl leukotriene-induced contractions could be inhibited by selective leukotriene receptor antagonists has lead to the development of a range of compounds whose antagonist activity profiles are based on their effectiveness in this tissue preparation. It is now apparent that such antagonists are not effective against all cysteinyl leukotriene-induced responses, nor are the actions of cysteinyl leukotrienes limited to effects in airway smooth muscle. It was the major finding of this study that the cysteinyl leukotrienes LTC4 and LTD4 induce surface expression of P-selectin in human endothelial cells via a mechanism that appears independent of the classical CysLT1 receptor. More specific information concerning the nature of the CysLT1 receptor mediating endothelial P-selectin expression must await better pharmacological and molecular biological tools. Nevertheless, it can be hypothesized that the development of selective antagonists for this putative receptor subtype may have a therapeutic advantage over selective CysLT1 receptor antagonists in inhibiting some of the proinflammatory effects of cysteinyl leukotrienes.

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References


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