Molecular cloning and characterization of another leukotriene B₄ receptor

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Running title: Molecular cloning and characterization of another LTB₄ receptor
SUMMARY

Leukotriene $B_4$ is a potent lipid mediator known to be implicated mainly in inflammatory actions. Previous pharmacological studies indicated the existence of only one class of G protein-coupled receptor for leukotriene $B_4$, for which a candidate gene, namely BLT, had been identified. Here we report the isolation of another gene encoding a functional G protein-coupled receptor for leukotriene $B_4$, named JULF2. JULF2 is a novel G protein-coupled receptor of 358 amino acids which shares 36.6% amino acid identity with human BLT. According to genomic information, JULF2 gene is located on the chromosome 14, about 4 kb upstream of the BLT gene. During screening of endogenous ligands for JULF2, we found that leukotriene $B_4$ induced inhibition of forskolin-stimulated cAMP accumulation in Chinese hamster ovary cells, stably expressing JULF2. Additionally, Chinese hamster ovary cells expressing exogenous JULF2 showed chemotactic responses with leukotriene $B_4$ in a pertussis-toxin-sensitive manner. A large amount of JULF2 mRNA was detected in the human spleen and the peripheral blood leukocytes. Furthermore, JULF2 mRNA was expressed in mononuclear lymphocytes, in which BLT mRNA was barely detected. The discovery of this second leukotriene $B_4$ receptor will eventually lead to a better understanding of the classification of leukotriene $B_4$ receptors and reconsideration of the pathophysiological role of leukotriene $B_4$. 
INTRODUCTION

Leukotriene B₄ (LTB₄) and cysteinyl-leukotrienes (LTC₄, LTD₄, and LTE₄) derived from arachidonic acid metabolism are synthesized in a sequence, first by 5-lipoxygenase followed by LTA₄ hydrolase or LTC₄ synthase, respectively (1). LTB₄ is one of the most potent chemoattractant mediators, acting mainly on neutrophils but also on related myeloid cells, mast cells, and endothelial cells (2, 3). LTB₄, therefore has been implicated in a number of allergic and inflammatory diseases such as asthma (4), allergic encephalomyelitis (5), psoriasis (6), rheumatoid arthritis (7, 8), and inflammatory bowel disease (9). Whilst the development of specific and highly potent LTB₄ receptor antagonists using human leukocyte membrane preparations has been accelerated, these antagonists have not been shown to be effective in the treatment of certain human inflammatory disorders. Although neutrophils are thought to have a role at some stage during the course of these diseases, it is possible that the recruitment of inflammatory leukocytes involves more than one mediator, such as C5a, interleukin-8 and tumor necrosis factor α (TNFα) (10).

Chemoattractants direct the movement of leukocytes by signaling through specific surface receptors. The chemoattractant receptors constitute a superfamily of G-protein coupled receptors (GPCRs) including C5a (11), PAF (12), fMLP (13), CysLT1 (14, 15), and chemokine receptors (16-18). In 1997, a cDNA clone was isolated for BLT, a human LTB₄ receptor, from retinoic acid-differentiated HL-60 cells (19), and in 1998, the mouse orthologue was isolated (20). Both receptors were predicted to contain a seven transmembrane-spanning domain, and the recombinant human and mouse BLT showed specific binding of LTB₄. We searched for novel chemoattractant receptors using a computational survey of public dbEST and genomic databases, and identified numerous sequences that encode structural characteristics common to GPCRs. One clone, JULF2, is predicted to be a novel GPCR encoding a 358-amino acid glycoprotein that is 36.6% identical
to the human BLT receptor. We report, evidence that JULF2 is an additional receptor for LTB₄. Analysis of its unique pharmacological features will aid in the classification of LTB₄ receptors, while preliminary data on its tissue distribution suggest that the pathophysiological role of JULF2 should be reassessed.
EXPERIMENTAL PROCEDURES

Cloning of BLT-like, JULF2

Using the TBLASTN algorithm, dbEST and genomic databases (National Center for Biotechnology Information, NCBI/NIH) were queried with the amino acid sequence of BLT. One genomic draft-sequence (GenBank Accession No. AL096870) derived from chromosome 14 was predicted to encode BLT and a novel GPCR, located approximately 4 kb upstream of the BLT gene. The novel GPCR had some similarity to BLT. Primers were generated from the novel GPCR sequence and the full-length cDNA was amplified from Human Leukocyte Marathon-ready cDNA library and Human Spleen Marathon-ready cDNA library (Clontech) library (21) by 5’- and 3’-rapid amplification of the cDNA ends (RACE) (21). The amplified RACE products were sequenced directly and a 1077 bp open reading frame (ORF) for AB044402 (JULF2) was found. Using primers 5’-AAGGATGTCGGTCTGCTACCGTCCCCCA-3’ and 5’-GCTGTCAAAGGTCCCATTCCGGAC-3’ determined from the RACE products, the ORF was amplified from human spleen cDNA under previously described high GC conditions (22).

Preparation of Chinese hamster ovary (CHO) cell line expressing JULF2

The JULF2 cDNA was cloned into an expression vector plasmid, pEF-BOS (dhfr) (23) using the XbaI sites (pEF-JULF2). pEF-JULF2 or vector alone (mock) was transfected into CHO (dhfr-) cells using FuGENE6 (Boehringer Mannheim). CHO cells expressing JULF2 were selected using a selection medium deficient in nucleotides (100 nM methotrexate).

Assay for inhibition of forskolin-induced intracellular accumulation of cAMP

CHO cells expressing JULF2 were plated in 24-well plates at $1 \times 10^5$ cells/well and
incubated for 24 h. The cells were washed with α-MEM medium containing 1 mM 3-isobutyl-1-methylxanthine (Sigma) and 0.1% (w/v) bovine serum albumin. Cells were treated with appropriate ligands and 1 µM forskolin (dissolved in medium). After incubation at 37°C in 5% CO2 for 30 min, intracellular cAMP was extracted with 20% (v/v) perchloric acid for 30 min. The extracted cAMP was measured using an enzyme-linked immunoassay kit (Amersham Pharmacia Biotech). For pertussis toxin sensitivity, cells were pretreated for 20 h with 50 ng/ml pertussis toxin (Sigma).

**Calcium mobilization experiments**

Calcium mobilization studies were conducted using Fluo 3-loaded CHO cells and a microtiter plate-based assay, using FLIPR (Molecular Devices). CHO cells were grown to confluence and allowed to adhere to the FLIPR microtiter plates, growth medium was removed and replaced with 1 µM Fluo-3 AM fluorescent indicator dye (Molecular Probes) in Hanks’ balanced salt solution containing 10 mM HEPES, 200 µM CaCl2, 0.1% (w/v) BSA and 2.5 mM probenecid. After incubation for 1 h (37°C, 5% CO2), the cells were washed four times with the same buffer. Initially, fluorescence was read every 1 sec for 1 min and then every 6 sec for the following minute. Agonist was added after 10 sec and the concentration-response curves were obtained by calculating the maximal fluorescence counts above background after addition of each concentration of agonist.

**Northern blot analysis**

Northern membranes containing 2 µg poly A+ RNA from different human tissues were purchased from Clontech. Northern analysis of JULF2 transcripts was performed under high stringency conditions as described previously (24). A JULF2 probe was prepared by PCR, using primers to position 22-615 in the nucleotide sequence (AB044402). After stripping the Northern blot, it was reprobed with a -actin control (supplied by Clontech). The
membrane was exposed to X-ray film with an intensifying screen for autoradiography.

Quantitative analysis of JULF2 and BLT mRNA by reverse transcription-polymerase chain reaction (RT-PCR)

Human mononuclear cells, neutrophils and eosinophils were isolated from the heparinized blood of healthy volunteers using Ficoll-Paque (Amersham Pharmacia Biotech) technique and CD16 microbeads (MACS reagents, Miltenyi Biotech). Total RNA preparations and cDNA syntheses were performed as described previously (25). We quantified JULF2 and BLT mRNA with the Prism 7700 Sequence Detector (ABI) using primer sets (5’-CTCTGACCCTTTCTGTGC-3’ and 5’-CAGCGTCACGCTGTAGCA-3’ for JULF2; 5’-GCCAAGGCACCTGGAGA-3’ and 5’-ACTCCGCAGACATAGTGACACA-3’ for BLT) and fluorescent-labeled probes (5’-(FAM)-TCCTTTCGGGCTGATGCTCGG-(TAMRA)-3’ for JULF2; 5’-(FAM)-TTGGACTGGCTGGGTGCCGC-(TAMRA)-3’ for BLT). RT-PCR was carried out in a 25 µl reaction mixture prepared with a TaqMan PCR Core Reagent Kit (ABI) containing an appropriately diluted cDNA solution, 0.2 µM of each primer, and 0.1 µM of the probe. To obtain a calibration curve, we amplified a known amount of human genomic DNA using the same method as above. The number of mRNA copies per 1 µl of total RNA was calculated using the equation as described previously (26). Human β-actin mRNA was also measured as an internal control.

Chemotaxis assay

Polycarbonate filters with 8 µm pores (Neuroprobe) were coated with 10 µg/ml fibronectin (IWAKI) in PBS, overnight at 4°C. A dry coated filter was sandwiched in a 96-blind well chamber (Neuroprobe), with ligands in the lower wells and the CHO cells (200 µl, 2 x 10⁵ per well) in the top wells. The ligand solution and cell suspension were prepared in the
same buffer (α-MEM medium containing 0.1% (w/v) BSA). After incubation at 37°C in 5% CO₂ for 4 h, the filter was disassembled, the cells on the filter were fixed with methanol and stained with a Diff-Quik staining kit (International Reagents Corp.). Cells were then scraped from the upper side of the filter. The number of cells that had migrated to the lower side was determined by measuring optical densities at 595 nm, using a 96-well microplate reader. For pertussis toxin sensitivity, cells were pretreated for 20 h with 50 ng/ml pertussis toxin before seeding.

Chemicals

LTB₄, LTC₄, LTD₄, LTE₄, 20-hydroxy-LTB₄, 20-carboxy-LTB₄, 6-trans-LTB₄, 5(S)-HETE, (±)12-HETE, 5-oxo-HETE, (±)5,6-DiHETE, (±)8,9-DiHETE, (±)11,12-DiHETE, (±)14,15-DiHETE, 8(S),15(S)-DiHETE were purchased from CAYMAN Chemical.
RESULTS

Identification and molecular characterization of a novel BLT-like receptor, JULF2

The human JULF2 gene was identified using a combination of genomic database screening and 5'- and 3'-RACE. Screening of genomic databases, using the human BLT sequence as a query, revealed a genomic draft-sequence (GenBank Accession No. AL096870) encoding a novel GPCR. After identification of a corresponding mRNA in the human placenta by RT-PCR, the ORF was amplified by 5'- and 3'-RACE from human spleen and PBLs derived cDNA. The ORF encodes a unique putative GPCR protein of 358 amino acids. This receptor was designated JULF2. Sequence comparison revealed that JULF2 shares a high level of amino acid sequence similarity with human BLT (36.6%) (Fig. 1), compared to other GPCRs. Genomic sequence analysis revealed that the JULF2 gene is present as a single copy in the human genome and has no intron in its ORF. Interestingly, both JULF2 and BLT genes map to chromosome 14 with JULF2 gene located upstream, adjacent of the BLT gene and is transcribed in the same direction.

Pharmacological characterization of JULF2

To identify possible ligands for JULF2, changes in intracellular cAMP accumulation and calcium mobilization were monitored following addition of various bioactive lipids in CHO cells transfected with JULF2 cDNA. Among the lipoygenase metabolites tested, LTB4 and its derivatives, such as, LTB3, LTB5, and 12-epi-LTB4, but not CysLTs (LTC4, LTD4 and LTE4) nor 5(S)-hydroxyeicosatetraenoic acid (5(S)-HETE), induced significant inhibition of forskolin-stimulated intracellular cAMP accumulation (Fig. 2a, Table 1). CHO-JULF2 cells responded to LTB4 > 12-epi-LTB4 > LTB5 > LTB3, with EC50 values of 8.1, 16.8, 24.3 and 48.3 nM respectively. Cells transfected with the vector alone did not respond to stimulation by LTB4 (Fig. 2b). Interestingly, LTB4 metabolites, 20-hydroxy-LTB4, 20-carboxy-LTB4 and 6-
trans-LTB₄, did not elicit specific cAMP changes in CHO-JULF2 cells. Additionally, the DiHETE series, consisting of four diastereomeric dihydroxy acids with varying locations of two hydroxyl groups, were also negative (data not shown).

BLT is known to elevate intracellular calcium by agonist stimulation (19, 20, 27, 28), however, calcium mobilization responses was not observed when CHO-JULF2 cells were treated with LTB₄ in the presence and the absence of Gα₁₅ or Gα₁₆ (data not shown). Adenyl cyclase inhibition was completely blocked by prior treatment with 50 ng/ml of pertussis toxin, suggesting the involvement of Gαi protein, as shown in Fig. 2b.

**Tissue distribution of JULF2**

Expression of JULF2 mRNA in human tissues was evaluated using Northern blot analysis. A significant quantity of JULF2 message was detected at 2.4 kb in the human spleen, the peripheral blood leukocytes and the ovary (Fig. 3a). Also, a message of 2.4 kb was detected in the pancreas and the heart at reduced levels. In the peripheral leukocytes, 3 kb, 5 kb and 8 kb mRNAs were detected at low levels. 5 kb mRNA expression was higher in the spleen than in the peripheral leukocytes, whereas an 8 kb mRNA was detected uniquely in the peripheral leukocytes. Previous reports have indicated that BLT is highly expressed and is restricted to human peripheral blood leukocytes (19, 20, 28, 29).

The expression patterns of JULF2 and BLT mRNA, in the purified sub-populations of leukocytes from peripheral blood, were examined using real time quantitative RT-PCR (TaqMan®) analysis. As shown in Fig. 3b, JULF2 mRNA was detected in both mononuclear and polymorphonuclear populations of leukocytes, whereas BLT mRNA expression was restricted to the polymorphonuclear leukocytes. There was a clear difference in the expression levels between JULF2 and BLT in human mononuclear cell population.

*Effect of LTB₄ on chemotaxis of JULF2 expressing CHO cells*
To examine the chemotactic activities of CHO-JULF2 cells, we performed a chemotaxis assay using a Boyden chamber. As Fig. 4a indicates, LTB4-induced chemotactic activity exhibited a bell-shaped dose dependence, with the maximal activity at 30 nM LTB4. In CHO-BLT cells, the maximal dose was previously reported at 1-10 nM (19, 28). LTB4-induced chemotaxis was completely inhibited by pretreatment of the cells with 50 ng/ml pertussis toxin for 20 h. As shown in Fig. 4b, other lipoxygenase metabolites did not induce CHO-JULF2 cell migration, except for 12-epi-LTB4 which indicated reduced induction. None of the tested eicosanoids induced migration in mock-transfected CHO cells (data not shown).
DISCUSSION

We have identified a novel receptor, termed JULF2, a functional GPCR for LTB₄. The deduced amino acid sequence of JULF2 shows the highest homology to BLT with 36.6% amino acid identity. Other GPCRs, including those for prostanoids, platelet-activating factor, purines and chemokines, showed weaker homology (< 25.9%) to JULF2 as well as to BLT. The JULF2 gene is located 4 kb upstream of the BLT gene on human chromosome 14q11.2-q12, suggesting that both genes may have been generated by a tandem duplication during an early evolutionary event and that both receptors may constitute an unique GPCR family. Structurally, JULF2 shares many features with GPCR proteins (30), including seven transmembrane (TM)-spanning domains, potential N-linked glycosylation sites in the amino terminal extracellular domain, and cysteines in the first and second extracellular loops, which may form a disulfide bridge. Also there is an asparagine in TM I, an aspartate in TM II, tryptophans in TM IV and VI, prolines in TM IV, V, VI and VII, and the highly conserved NPXXY motif in TM VII. However, JULF2 does possess unique characteristics distinct from other GPCRs such as a glutamine substituting the aspartate or the glutamate, and a cysteine replacing the tyrosine usually found in the (D/E)RY motif in the amino-terminus of the second cytoplasmic loop. This unusual replacement of the (D/E)RY motif is novel, suggesting that JULF2 may have unique signal transduction and/or structural conformation.

Inhibition of intracellular cAMP accumulation and induction of cell migration were induced by LTB₄ in JULF2 expressing CHO cells. Both activities were completely blocked by pertussis toxin. Unlike BLT, no calcium mobilization was found in CHO-JULF2 cells following LTB₄ treatment whether in presence or in absence of Gα₃₅ or Gα₁₆. Previous studies have indicated that BLT has coupled to Gα₃₅, Gα₄ and Gα₁₆ proteins to augment specific signals (19, 27), whereas JULF2 is thought to couple specifically to the Gα₁₁ protein. Also, our studies for the ligand screening of JULF2 revealed that the ligand selectivities of JULF2 and
BLT differed on 20-hydroxy-LTB₄ and 12-HETE. JULF2 showed no response against 20-hydroxy-LTB₄ or (±)12-HETE unlike BLT, for which 20-hydroxy-LTB₄ is a potent agonist (19, 28) and 12(R)-HETE, a weak agonist (31). Furthermore, CHO-JULF2 cells responded to LTB₄, 12-epi-LTB₄, LTB₅, and LTB₃, but not to 20-hydroxy-LTB₄, (±)12-HETE (Table 1), or DiHETEs, suggesting that the ligand selectivity of JULF2 may be unusually specific, as it was easily influenced by the double bonds, the location and the configuration of the two hydroxyl groups, and the modification of carbon in position 20 of ligands.

Goldman et al. delineated two subsets of the LTB₄ receptor and showed that the high-affinity class of LTB₄ receptor is present at 4,400 sites, and the low-affinity class of LTB₄ receptor is present at 270,000 sites on human polymorphonuclear leukocytes (32). They also demonstrated that the high-affinity subset was linked to chemotaxis and the low-affinity subset to different functional responses of neutrophils to LTB₄ (33). These observations were confirmed by Showell et al. using the specific BLT antagonist CP-105696 (34, 35). Both forms of the LTB₄ receptor are thought to be the same GPCR, but it has not been proven whether the recombinant BLT can take on the two different functional conformations. Our data illustrates that the maximum migration of CHO-JULF2 cells was observed at 30 nM of LTB₄, about one-tenth of that for BLT (19, 28). The EC₅₀ value of LTB₄-evoked inhibition of forskolin-induced adenylyl cyclase activity in JULF2-CHO cells was 8.1 nM, whereas in guinea-pig BLT-CHO cells it was 0.56 nM (28). These findings suggest the possibility that JULF2 might be the previously identified low-affinity form of LTB₄.

The BLT gene has already been cloned in the human (19), the mouse (20), the rat (29), and the guinea pig (27, 28). These BLT mRNA was found to be restricted to the peripheral leukocytes. The localization of JULF2 mRNA was different from that of human BLT. Abundant mRNA of JULF2 was detected in the human spleen and the peripheral leukocytes. In leukocytes, JULF2 mRNA was easily detected in a mononuclear leukocyte population in comparison to BLT mRNA, which was barely detectable. These data suggest that JULF2 may
be the major LTB₄ receptor in mononuclear leukocytes, mainly the lymphocytes, as described by Payan et al. (36), and BLT the major LTB₄ receptor in neutrophils. The differences of distribution between JULF2 and BLT suggest the need for further exploration of the roles of LTB₄ in the pathogenesis of related diseases.

In summary, we have cloned a second functional LTB₄ receptor, JULF2, from the genomic databases. JULF2 exhibited differential signalling, ligand selectivity, and tissue distribution compared to BLT. However, there was no pharmacological or biological evidence that the diverse effects of LTB₄ were mediated via an interaction with two receptors that belong to the superfamily of GPCRs. The discovery of the JULF2 gene will probably offer a new cellular system for screening of JULF2 selective agonists/antagonists, which lead to the elucidation of the role of the pathophysiological LTB₄. Studies to this end are currently ongoing in our laboratory.
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REFERENCES


25. Matsumoto, M., Kamohara, M., Sugimoto, T., Hidaka, K., Takisaki, J., Saito, T., Okada,


The abbreviations used are: GPCR, G protein-coupled receptor; EST, expression sequence tag; ORF, open reading frame; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-polymerase chain reaction; LTB₄, leukotriene B₄; CysLTs, cysteinyl leukotrienes; HETEs, hydroxy-eicosatetraenoic acids; DiHETEs, dihydroxy-eicosatetraenoic acids; PTX, pertussis toxin; EC₅₀, median effective concentration; TM, trans-membrane.

The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB044402.
FIGURE LEGENDS

Figure 1  Putative amino acid sequence of the JULF2 clone compared to the sequence of the human BLT receptor. Residues that are identical in JULF2 and BLT receptors are enclosed in solid boxes. Putative transmembrane domains are indicated by underlines. The GenBank accession number of the JULF2 is AB044402.

Figure 2  LTB₄ inhibition of forskolin-induced cAMP accumulation. (a). Effects of LTB₄ and its derivatives on cAMP accumulation in CHO-JULF2 cells. (b). Effects of pertussis toxin (PTX, 50 ng/ml, 20 h) on cAMP accumulation in CHO-JULF2 cells. Forskolin induced cAMP accumulation is defined as 100%. Intact cell cAMP level is defined as 0%. Data represent the mean ± standard deviation of duplicate determinations.

Figure 3  Expression of JULF2 in human tissue. (a). Northern blot analysis was performed. Membrane was first probed by JULF2, then stripped and reprobed by β-actin. The molecular size markers are shown in kilobases. (b). The cDNA corresponding to 1 ng total RNA from human leukocytes was assessed for JULF2 and BLT mRNA by TaqMan PCR. The control mRNA was β-actin. Data are presented as the mean ± standard deviation of three individual mRNA levels for each population.

Figure 4  Chemotactic response of CHO-JULF2 cells induced by LTB₄. (a). Migration assays were performed in a 96-well Boyden chamber, on cells stably expressing JULF2, control mock transfected cells and pertussis toxin (PTX, 50 ng/ml, 20 h) pretreated CHO-JULF2 cells. (b). Selectivity of lipoxygenase metabolites in chemotactic response of CHO-JULF2 cells.
Table 1  Potencies of leukotrienes for human JULF2

CHO cells expressing JULF2 were treated with ligands and 1 µM forskolin (37°C in 5% CO₂ for 30 min), then the extracted cAMP was measured by an enzyme-linked immunoassay. The EC₅₀ values were determined from the concentration-response curves.

<table>
<thead>
<tr>
<th>Leukotriene</th>
<th>EC₅₀ (nM)</th>
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<tbody>
<tr>
<td>LTB₄</td>
<td>8.1 ± 1.6 (n = 9)</td>
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<tr>
<td>12-epi-LTB₄</td>
<td>16.2, 17.3</td>
</tr>
<tr>
<td>LTB₅</td>
<td>17.8, 30.7</td>
</tr>
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<tr>
<td>(±) 12-HETE</td>
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Fig. 2a

![Graph showing the effect of various leukotrienes on forskolin elevated cAMP](image)

- LTB₄
- LTB₅
- 20-hydroxy-LTB₄
- 20-carboxy-LTB₄
- 6-trans-LTB₄
- 12-epi-LTB₄

leukotrienes (-log M)

12 11 10 9 8 7 6

0 20 40 60 80 100 120 Forskolin elevated cAMP (%)

Fig. 2b

![Graph showing the effect of JULF2 and LTB₄ on forskolin elevated cAMP](image)

- JULF2
- JULF2 + PTX
- MOCK

LTB₄ (-log M)

10 9 8 7 6

0 20 40 60 80 100 120 Forskolin elevated cAMP (%)
Fig. 3b

Quantitative analysis of mRNA expression for JULF2, BLT, and β-actin in mononuclear cells, neutrophils, and eosinophils from two donors (A and B).
Fig. 4a

![Graph showing absorbance at 595 nm vs. LTB4 (-log M)]

- JULF2
- JULF2 + PTX
- MOCK

Fig. 4b

![Graph showing absorbance at 595 nm vs. ligands (-log M)]

- LTB4
- 12-epi-LTB4
- LTD4
- 5-oxo-ETE
- 5(S)-HETE