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(54) **USE OF LIPOXIN COMPOUNDS FOR INHIBITING OF TNF-ALPHA INITIATED NEUTROPHIL RESPONSE**

VERWENDUNG VON LIPOXINEN ZUR INHIBIERUNG VON TNF-ALPHA INITIIERTER
GEGENREAKTION

UTILISATION DE COMPOSES DE LIPOXINE AFIN D'INHIBER LA REPONSE DE NEUTROPHILE
INDUITE PAR TNF-ALPHA

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**WO-A-94/29262 WO-A-95/01179
WO-A-98/11049 US-A- 5 441 951**

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- **T. TAKANO ET AL: "Aspirin-triggered 15-Epi-Lipoxin (LXA4) and LXA4 Stable Analogues Are Potent Inhibitors of Acute Inflammation: Evidence for Anti-inflammatory Receptors" JOURNAL OF EXPERIMENTAL MEDICINE , vol. 185, no. 9, 5 May 1997 (1997-05-05), pages 1693-1704, XP002141526 cited in the application**
- **T. TAKANO ET AL: "Neutrophil-mediated Changes in Vascular Permeability Are Inhibited by Topical Application of Aspirin-triggered 15-epi-lipoxin A4 and Novel Lipoxin B4 Stable Analogues" JOURNAL OF CLINICAL INVESTIGATIONS, vol. 101, no. 4, February 1998 (1998-02), pages 819-826, XP002141527**
- **A. T. GEWIRZ ET AL: "Pathogen-induced Chemokine Secretion from Model Intestinal Epithelium is Inhibited by Lipoxin A4 Analogs" JOURNAL OF CLINICAL INVESTIGATIONS, vol. 101, no. 9, May 1998 (1998-05), pages 1860-1869, XP002141528**

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EP 1 165 066 B1

Description

BACKGROUND OF THE INVENTION

[0001] Lipid and protein mediators of inflammation such as cytokines and chemokines have a profound impact on the formation and actions of each other (Serhan, C.N., J.Z. Haeggström, and C.C. Leslie. 1996. Lipid mediator networks in cell signaling: update and impact of cytokines. *FASEB J.* 10:1147-1158). In-particular, the cytokines $\text{TNF}\alpha$ and $\text{IL-1}\beta$ play major roles in inflammation, septic shock and tissue injury. PMN perform a range of well-appreciated specialized functions, including chemotaxis, generation of reactive oxygen species and biosynthesis of potent lipid mediators (Weiss, S.J. 1989. Tissue destruction by neutrophils. *N. Engl. J. Med.* 320:365-376). In this regard, $\text{TNF}\alpha$ stimulates PMN to transcribe and release cytokines such as $\text{IL-1}\beta$, enhances leukotriene biosynthesis, and up-regulates adhesion molecules (Marucha, P.T., R.A. Zeff, and D.L. Kreutzer. 1991. Cytokine-induced $\text{IL-1}\beta$ gene expression in the human polymorphonuclear leukocyte: transcriptional and post-transcriptional regulation by tumor necrosis factor and IL-1 . *J. Immunol.* 147:2603-2608). Since PMN represent approximately 70% of the peripheral blood leukocytes and are in many instances the initial cell type recruited to interstitial sites, they are now considered a significant source of "proinflammatory" cytokines including $\text{TNF}\alpha$ and $\text{IL-1}\beta$. These as well as other PMN-derived cytokines and chemokines can, in turn, affect the course of inflammatory and immune responses (Lloyd, A.R., and J.J. Oppenheim. 1992. Poly's lament: the neglected role of the polymorphonuclear neutrophil in the afferent limb of the immune response. *Immunology Today* 13:169-172). In certain clinical settings, including respiratory distress syndrome, myocardial reperfusion injury, gout and rheumatoid arthritis, PMN contribute to ongoing damage of host tissues (Weiss, S.J. 1989. Tissue destruction by neutrophils. *N. Engl. J. Med.* 320:365-376; Hachicha, M., P.H. Naccache, and S.R. McColl. 1995. Inflammatory microcrystals differentially regulate the secretion of macrophage inflammatory protein-1 and interleukin-8 by human neutrophils: A possible mechanism of neutrophil recruitment to sites of inflammation in synovitis. *J. Exp. Med.* 182: 2019-2025; Hansen, P.R. 1995. Role of neutrophils in myocardial ischemia and reperfusion. *Circulation* 91:1872-1885). Thus, it is of interest to understand the complex relationships between lipid mediators and $\text{TNF}\alpha$ -evoked PMN responses in order to gain insight for new approaches in controlling these events.

SUMMARY OF THE INVENTION

[0002] The present invention pertains to the use of a lipoxin analog defined below for preparing a pharmaceutical agent for treating seborrheic dermatitis, pustular dermatosis or dandruff associated with $\text{TNF}\alpha$ initiated polymorphonuclear (PMN) inflammation.

[0003] In a preferred embodiment, the present invention relates to said use, wherein the pharmaceutical agent is in the form of a shampoo or a body cleansing product.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] The invention will be more fully understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Figure 1 shows that LXA_4 and ATL stable analogs inhibit $\text{TNF}\alpha$ -stimulated superoxide generation by human neutrophils. Human PMN were incubated with either vehicle alone or indicated concentrations of LXA_4 , 15 R/S-methyl- LXA_4 or 16-phenoxy- LXA_4 for 5 min and then with $\text{TNF}\alpha$ (50 ng/ml) for an additional 10 min. Values are the mean \pm SEM for LXA_4 (n=3), 15 R/S-methyl- LXA_4 (n=4) or 16-phenoxy- LXA_4 (n=3). LXA_4 and analogs, at all concentrations tested, led to a statistically significant inhibition of $\text{TNF}\alpha$ -induced $\text{IL-1}\beta$ appearance ($p<0.01$).

Figure 2 shows that LXA_4 and stable analogs inhibit $\text{TNF}\alpha$ -induced $\text{IL-1}\beta$ production in human neutrophils. A) PMN were incubated ($\text{TNF}\alpha$ (10 ng/ml) plus vehicle or $\text{TNF}\alpha$ plus LXA_4 (100 nM)) as denoted for 20 h at 37 °C and 5% CO_2 . Supernatants were collected, and $\text{IL-1}\beta$ was quantitated by ELISA. Results are expressed as mean \pm SD of duplicates, and are from one experiment representative of n=3. B) PMN were incubated for indicated periods of time in the presence of increasing concentrations of 15 R/S-methyl- LXA_4 . Values represent the mean \pm SEM, n=3. At all time intervals tested, $\text{TNF}\alpha$ induced a significant appearance of $\text{IL-1}\beta$ over vehicle-treated cells ($*p<0.01$).

Figure 3 demonstrates 15 R/S-methyl-LXA₄ downregulates $\text{TNF}\alpha$ -triggered $\text{IL-1}\beta$ gene expression. PMN were incubated with either 0.1 % ethanol (vehicle) or 15 R/S-methyl- LXA_4 at 10, 100, and 1000 nM, in the presence or absence of $\text{TNF}\alpha$ (10ng/ml), for 6 h at 37 °C. Northern blots were performed in order to detect $\text{IL-1}\beta$ messenger RNA. The results presented are from one experiment which is representative of two others performed with different donors.

Figure 4 depicts the involvement of the LXA_4 receptor. PMN were incubated with either IgG purified from pre-

immune serum (50 µg/ml) or anti-LXA₄ receptor (50 µg/ml) for 1 h at 4 °C, then exposed to agonists for 12 h at 37 °C and 5% CO₂. Values are expressed as mean ± SD from an experiment performed in triplicate, which is representative of three distinct experiments each performed with different donors (**p*<0.01).

Figure 5 shows the inhibition of TNFα-induced PMN infiltration in murine air pouches. One ml of sterile PBS containing either 0.1% ethanol, TNFα, 15 R/S-methyl-LXA₄, or TNFα plus 15 R/S-methyl-LXA₄ was injected into the pouches, and the exudates were collected at indicated time periods. The total number of leukocytes was counted as in *Materials & Methods*. The results are expressed as mean ± SEM from three different mice for each point. At all time intervals, TNFα induced a significant leukocyte infiltration into the air pouch cavity (*P*<0.05). *Statistically different from TNFα-treated cells; and from vehicle- or 15 R/S-methyl-LXA₄-treated cells (*p*<0.01).

Figure 6 shows that 15 R/S-methyl-LXA₄ redirects the TNFα-induced cytokine/chemokine profile *in vivo*. Experiments were conducted as described in Figure 5 legend. Quantitation for IL-1β, IL-4, IL-10, IL-13 and MIP-2 was performed using ELISA with air pouch cell-free exudates. The results are expressed as mean ± SEM from three different mice for each point. Changes in IL-1β, MIP-2 and IL-4 were significant at all tested time intervals (*p*<0.01).

DETAILED DESCRIPTION OF THE INVENTION

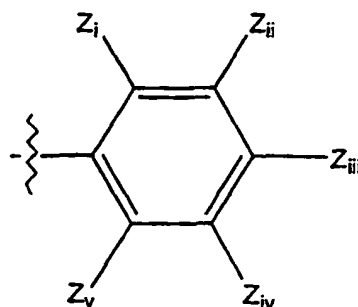
[0005] The features and other details of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention as defined by the claims.

[0006] The impact of lipoxin A₄ (LXA₄) and aspirin-triggered-lipoxins (ATL) was investigated in tumor necrosis factor (TNFα)-initiated neutrophil (PMN) responses *in vitro* and *in vivo* using metabolically stable LX analogs. At concentrations as low as 1-10 nM, the LXA₄ and ATL analogs each inhibited TNFα-stimulated superoxide anion generation and IL-1β release by human PMN. These LXA₄-ATL actions were time- and concentration-dependent and proved selective for TNFα, since these responses were not altered with either GM-CSF or zymosan-stimulated cells. TNFα-induced IL-1β gene expression was also regulated by both anti-LXA₄-receptor antibodies and LXA₄-ATL analogs. In murine air pouches, 15 R/S-methyl-LXA₄ dramatically inhibited TNFα-stimulated leukocyte trafficking, as well as the appearance of both macrophage inflammatory peptide-2 and IL-1β, while concomitantly stimulating IL-4 in pouch exudates. Together, these results indicate that both LXA₄ and ATL regulate TNFα directed neutrophil actions *in vitro* and *in vivo* and stimulate IL-4 in exudates, which plays a pivotal role in immune responses.

[0007] Abbreviations used in this application: ATL, aspirin-triggered-lipoxin; ATL analog, 15R/S-methyl-LXA₄-methyl ester; LX, lipoxin; LXA₄, 5S, 6R, 15S-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid; LXA₄ analog, 16 phenoxy-lipoxin A₄ methyl ester, 15-*epi*-LXA₄, 5S, 6R, 15R-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid; LT, leukotriene; MIP, macrophage inflammatory peptide; RA, rheumatoid arthritis. Suitable methods of preparation of lipoxin compounds can also be found, for example, in U.S. Patent Nos. 5,411,951, 5,648,512, 5,650,435 and 5,750,354.

[0008] The contribution of leukotriene (LT¹)B₄ in inflammation is well established in view of its potent ability to attract PMN. Another series of bioactive lipid mediators termed lipoxins (LX) and aspirin-triggered lipoxins (ATL) each, within the nanomolar range, inhibits fMLP and LTB₄ stimulated PMN adhesion and transmigration and hence are proposed counter-regulatory signals operative in the resolution of inflammatory sites (Serhan, C.N., J.Z. Haeggström, and C.C. Leslie. 1996. Lipid mediator networks in cell signaling: update and impact of cytokines. *FASEB J.* 10:1147-1158; Takano, T., S. Fiore, J.F. Maddox, H.R. Brady, N.A. Petasis, and C.N. Serhan. 1997. Aspirin-triggered 15-*epi*-lipoxin A₄ and LXA₄ stable analogs are potent inhibitors of acute inflammation: Evidence for anti-inflammatory receptors. *J. Exp. Med.* 185:1693-1704; Clària, J., and C.N. Serhan. 1995. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc. Natl. Acad. Sci. USA* 92:9475-9479; Lee, T.H., C.E. Horton, U. Kyan-Aung, D. Haskard, A.E. Crea, and B.W. Spur. 1989. Lipoxin A₄ and lipoxin B₄ inhibit chemotactic responses of human neutrophils stimulated by leukotriene B₄ and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Clin. Sci.* 77: 195-203; Serhan, C.N. 1994. Lipoxin biosynthesis and its impact in inflammatory and vascular events. *Biochim. Biophys. Acta* 1212:1-25); In human tissues, three main pathways are known for LX generation. An intraluminal source of LX is exemplified by PMN-platelet interactions that utilize sequential transcellular biosynthetic routes with the PMN 5-lipoxygenase (LO) product LTA₄ and platelet 12-LO. The mucosal and/or interstitial source of these eicosanoids involves cell-cell interactions with leukocyte 5-LO and 15-LO present in, for example, eosinophils, gastrointestinal or tracheal epithelium that is controlled by IL-4 and IL-13 (reviewed in (Serhan, C.N., J.Z. Haeggström, and C.C. Leslie. 1996. Lipid mediator networks in cell signaling: update and impact of cytokines. *FASEB J.* 10:1147-1158)). The third and most recently elucidated also represents a novel mechanism of action for aspirin that triggers the endogenous biosynthesis of 15 R epimers of native LX, termed aspirin-triggered lipoxins (ATL), generated via transcellular biosynthesis (Clària, J., and C.N. Serhan. 1995. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc. Natl. Acad. Sci. USA* 92:9475-9479).

[0009] LX are generated during cell-cell interactions via transcellular biosynthesis, and are produced *in vivo* during



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} , and Z_v , are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen atom, halogen, methyl, $-\text{OR}_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl;

(vii) a detectable label molecule; or

(viii) a straight or branched chain alkenyl of 2 to 8 carbon atoms, inclusive;

wherein Q_1 is $\text{C}=\text{O}$, SO_2 or CN , provided when Q_1 is CN , then X is absent;

wherein Q_3 and Q_4 are each independently O or NH ;

wherein one of R_2 and R_3 is a hydrogen atom and the other is

(a) H ;

(b) an alkyl of 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched;

(c) a cycloalkyl of 3 to 6 carbon atoms, inclusive;

(d) an alkenyl of 2 to 8 carbon atoms, inclusive, which may be straight chain or branched; or

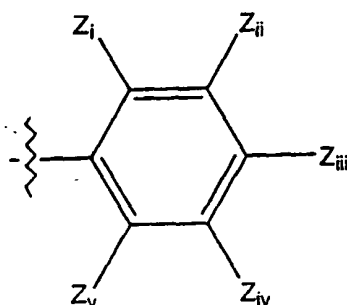
(e) $\text{R}_a\text{Q}_2\text{R}_b$ wherein Q_2 is $-\text{O}-$ or $-\text{S}-$; wherein R_a is alkylene of 0 to 6 carbons atoms, inclusive, which may be straight chain or branched and wherein R_b is alkyl of 0 to 8 carbon atoms, inclusive, which may be straight chain or branched, provided when R_b is 0, then R_b is a hydrogen atom;

wherein R_4 is

(a) H ;

(b) an alkyl of 1 to 6 carbon atoms, inclusive, which may be a straight chain or branched;

wherein R_5 is



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} , and Z_v are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen atom, halogen, methyl, $-\text{OR}_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl or a substituted or unsubstituted, branched or unbranched alkyl group;

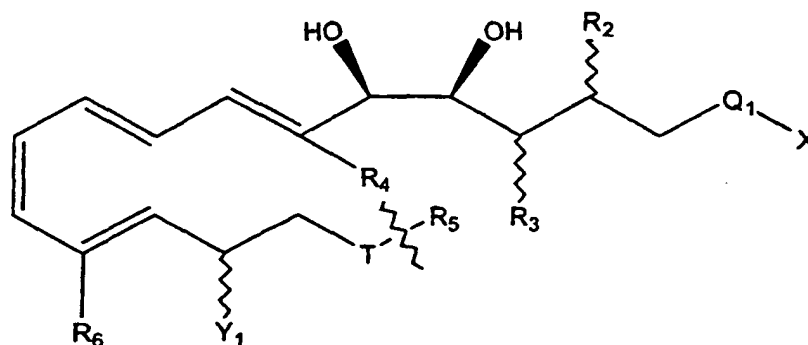
wherein Y_1 is $-\text{OH}$, methyl, $-\text{SH}$, an alkyl of 2 to 4 carbon atoms, inclusive, straight chain or branched, an alkoxy of 1 to 4 carbon atoms, inclusive, or CH_aZ_b where $a+b=3$, $a=0$ to 3, $b=0$ to 3 and Z is cyano, nitro or a halogen;

wherein R_6 is

- (a) H;
 (b) an alkyl from 1 to 4 carbon atoms, inclusive, straight chain or branched;

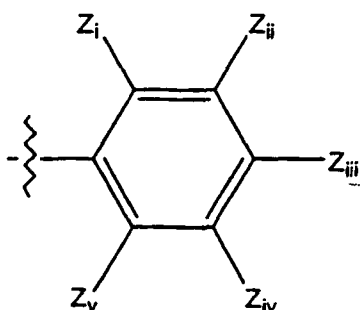
wherein T is O or S, and pharmaceutically acceptable salts thereof.

[0013] In another embodiment, compounds useful in the invention have the formula (II)



wherein X is R₁, OR₁, or SR₁;
 wherein R₁ is

- (i) a hydrogen atom;
 (ii) an alkyl of 1 to 8 carbon atoms, inclusive, which may be straight chain or branched;
 (iii) a cycloalkyl of 3 to 10 carbon atoms;
 (iv) an aralkyl of 7 to 12 carbon atoms;
 (v) phenyl;
 (vi) substituted phenyl



wherein Zi, Zij, Ziii, Ziv, and Zv are each independently selected from -NO₂, -CN, -C(=O)-R₁, -SO₃H, a hydrogen atom, halogen, methyl, -OR_x, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl;

- (vii) a detectable label molecule; or
 (viii) a straight or branched chain alkenyl of 2 to 8 carbon atoms, inclusive;

wherein Q₁ is (C=O), SO₂ or (CN), provided when Q₁ is CN, then X is absent;
 wherein one of R₂ and R₃ is a hydrogen atom and the other is

- (a) H;
 (b) an alkyl of 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched;
 (c) a cycloalkyl of 3 to 6 carbon atoms, inclusive;
 (d) an alkenyl of 2 to 8 carbon atoms, inclusive, which may be straight chain or branched; or

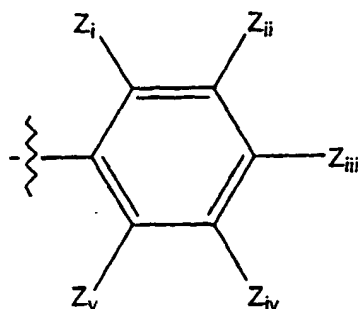
(e) $R_a Q_2 R_b$ wherein Q_2 is -O- or -S-; wherein R_a is alkylene of 0 to 6 carbons atoms, inclusive, which may be straight chain or branched and wherein R_b is alkyl of 0 to 8 carbon atoms, inclusive, which may be straight chain or branched, provided when R_b is 0, then R_b is a hydrogen atom;

wherein R_4 is

(a) H;

(b) an alkyl of 1 to 6 carbon atoms, inclusive, which may be a straight chain or branched;

wherein R_5 is



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} and Z_v are each independently selected from -NO₂, -CN, -C(=O)-R₁, -SO₃H, a hydrogen atom, halogen, methyl, -OR_x, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl or a substituted or unsubstituted, branched or unbranched alkyl group;

wherein Y_1 is -OH, methyl, -SH, an alkyl of 2 to 4 carbon atoms, inclusive, straight chain or branched, an alkoxy of 1 to 4 carbon atoms, inclusive, or CH₂Z_b where a+b=3, a=0 to 3, b=0 to 3 and Z is cyano, nitro or a halogen;

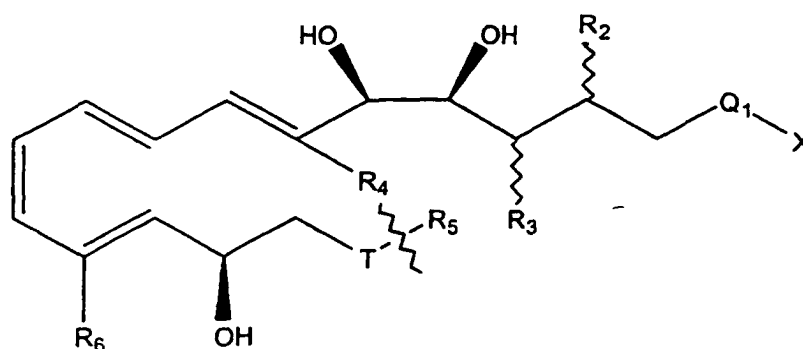
wherein R_6 is

(a) H;

(b) an alkyl from 1 to 4 carbon atoms, inclusive, straight chain or branched;

wherein T is O or S, and pharmaceutically acceptable salts thereof.

[0014] The invention is also directed to useful lipoxin compounds having the formula (III)



wherein X is R₁, OR₁, or SR₁;

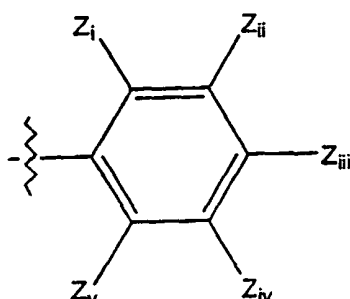
wherein R₁ is

(i) a hydrogen atom;

(ii) an alkyl of 1 to 8 carbons atoms, inclusive, which may be straight chain or branched;

(iii) a cycloalkyl of 3 to 10 carbon atoms;

- (iv) an aralkyl of 7 to 12 carbon atoms;
- (v) phenyl;
- (vi) substituted phenyl



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} , and Z_v , are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen atom, halogen, methyl, $-\text{OR}_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl;

- (vii) a detectable label molecule; or
- (viii) a straight or branched chain alkenyl of 2 to 8 carbon atoms, inclusive;

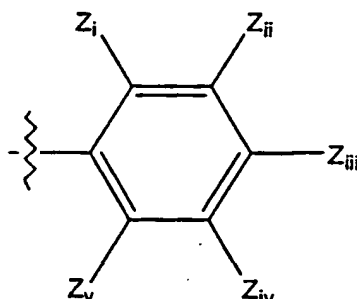
wherein Q_1 is $\text{C}=\text{O}$, SO_2 or CN , provided when Q_1 is CN , then X is absent;
wherein one of R_2 and R_3 is a hydrogen atom and the other is

- (a) H ;
- (b) an alkyl of 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched;
- (c) a cycloalkyl of 3 to 6 carbon atoms, inclusive;
- (d) an alkenyl of 2 to 8 carbon atoms, inclusive, which may be straight chain or branched; or
- (e) $\text{R}_a\text{Q}_2\text{R}_b$ wherein Q_2 is $-\text{O}-$ or $-\text{S}-$; wherein R_a is alkylene of 0 to 6 carbons atoms, inclusive, which may be straight chain or branched and wherein R_b is alkyl of 0 to 8 carbon atoms, inclusive, which may be straight chain or branched, provided when R_b is 0, then R_b is a hydrogen atom;

wherein R_4 is

- (a) H ;
- (b) an alkyl of 1 to 6 carbon atoms, inclusive, which may be a straight chain or branched;

wherein R_5 is



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} and Z_v are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen atom, halogen, methyl, $-\text{OR}_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl or a substituted or unsubstituted, branched or unbranched alkyl group;

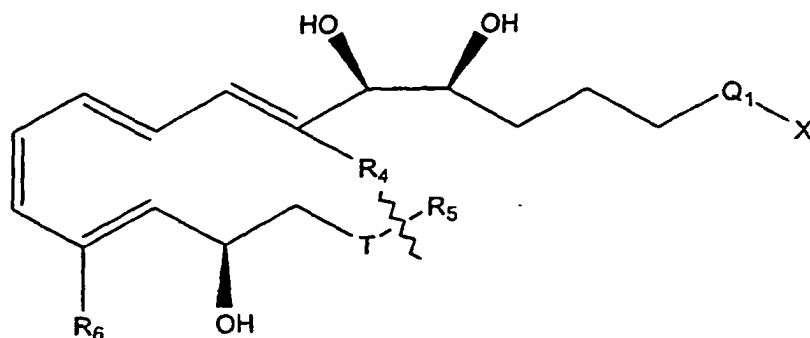
wherein R_6 is

(a) H;

(b) an alkyl from 1 to 4 carbon atoms, inclusive, straight chain or branched;

wherein T is O or S, and pharmaceutically acceptable salts thereof.

[0015] The invention is further directed to useful lipoxin compounds having the formula (IV)



wherein X is R_1 , OR_1 , or SR_1 ;

wherein R_1 is

(i) a hydrogen atom;

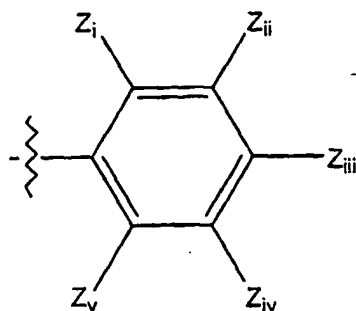
(ii) an alkyl of 1 to 8 carbon atoms, inclusive, which may be straight chain or branched;

(iii) a cycloalkyl of 3 to 10 carbon atoms;

(iv) an aralkyl of 7 to 12 carbon atoms;

(v) phenyl;

(vi) substituted phenyl



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} and Z_v are each independently selected from $-NO_2$, $-CN$, $-C(=O)-R_1$, $-SO_3H$, a hydrogen atom, halogen, methyl, $-OR_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl;

(vii) a detectable label molecule; or

(viii) a straight or branched chain alkenyl of 2 to 8 carbon atoms, inclusive;

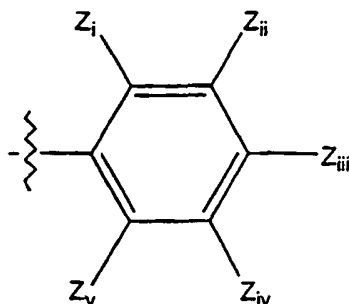
wherein Q_1 is $(C=O)$, SO_2 or (CN) , provided when Q_1 is CN , then X is absent;

wherein R_4 is

(a) H;

(b) an alkyl of 1 to 6 carbon atoms, inclusive, which may be a straight chain or branched;

wherein R_5 is



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} and Z_v are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen atom, halogen, methyl, $-\text{OR}_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl or a substituted or unsubstituted, branched or unbranched alkyl group;

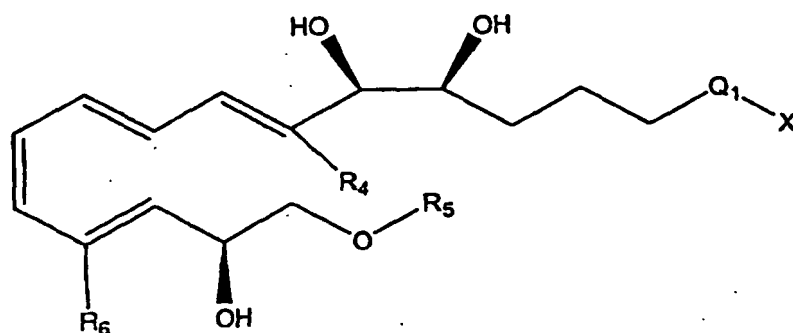
wherein R_6 is

(a) H;

(b) an alkyl from 1 to 4 carbon atoms, inclusive, straight chain or branched;

wherein T is O or S, and pharmaceutically acceptable salts thereof.

[0016] The invention is further directed to useful lipoxin compounds having the formula (V)



wherein X is R_1 , OR_1 , or SR_1 ;

wherein R_1 is

(i) a hydrogen atom;

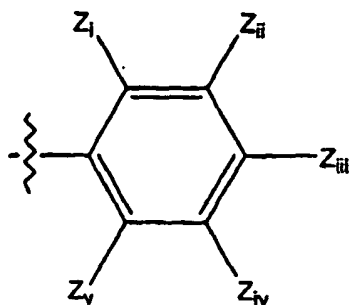
(ii) an alkyl of 1 to 8 carbon atoms, inclusive, which may be straight chain or branched;

(iii) a cycloalkyl of 3 to 10 carbon atoms;

(iv) an aralkyl of 7 to 12 carbon atoms;

(v) phenyl;

(vi) substituted phenyl



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} and Z_v are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen atom, halogen, methyl, $-\text{OR}_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl;

(vii) a detectable label molecule; or

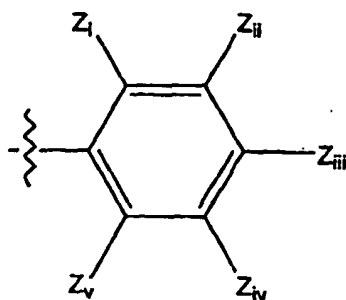
(viii) a straight or branched chain alkenyl of 2 to 8 carbon atoms, inclusive;

wherein Q_1 is $\text{C}=\text{O}$, SO_2 or CN , provided when Q_1 is CN , then X is absent;
wherein R_4 is

(a) H ;

(b) an alkyl of 1 to 6 carbon atoms, inclusive, which may be a straight chain or branched;

wherein R_5 is



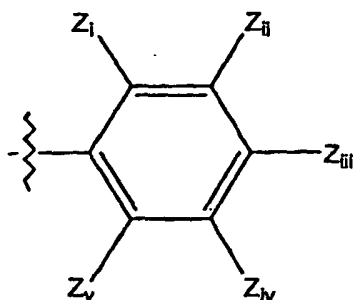
wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} and Z_v are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen atom, halogen, methyl, $-\text{OR}_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl or a substituted or unsubstituted, branched or unbranched alkyl group;
wherein R_6 is

(a) H ;

(b) an alkyl from 1 to 4 carbon atoms, inclusive, straight chain or branched; and

pharmaceutically acceptable salts thereof.

[0017] In preferred embodiments, X is OR_1 wherein R_1 is a hydrogen atom, an alkyl group of 1 to 4 carbon atoms or a pharmaceutically acceptable salt, Q_1 is $\text{C}=\text{O}$, R_2 and R_3 , if present, are hydrogen atoms, R_4 is a hydrogen atom or methyl, Q_3 and (Q_4 , if present, are both O , R_6 , if present, is a hydrogen atom, Y_1 , if present, is OH , T is O and R_5 is a substituted phenyl, e.g.,



wherein Z_I , Z_{II} , Z_{III} , Z_{IV} and Z_V are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen atom, halogen, methyl, $-\text{OR}_x$, wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl.

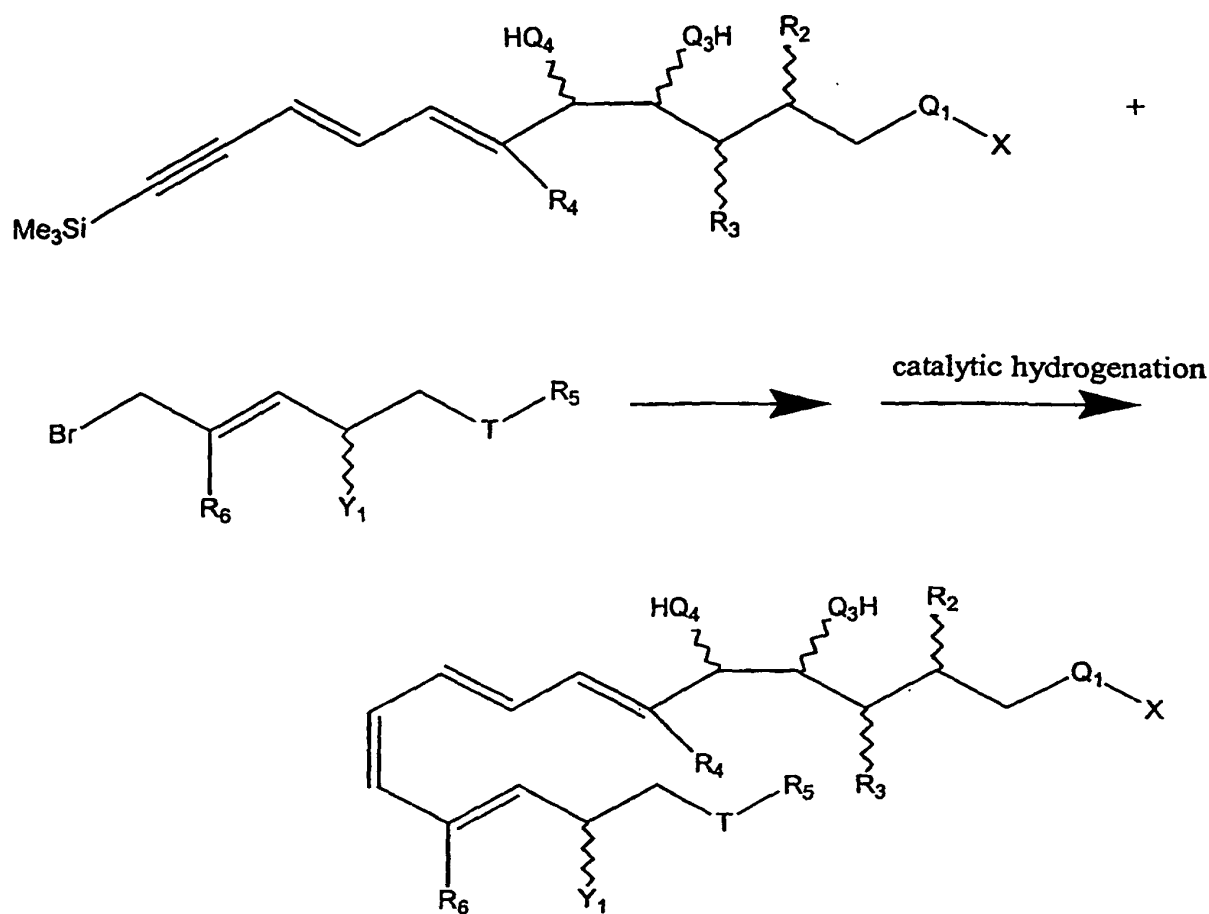
[0018] In preferred embodiments, Y_1 is a hydroxyl and the carbon bearing the hydroxyl can have an R or S configuration. In most preferred embodiments, the chiral carbon bearing the hydroxyl group, e.g., Y_1 , is designated as a 15-epi-lipoxin as is known in the art.

[0019] In certain embodiments the chirality of the carbons bearing the R_2 , R_3 , Q_3 and Q_4 groups can each independently be either R or S. In preferred embodiments, Q_3 and Q_4 have the chiralities shown in structures II, III, IV or V.

[0020] In preferred embodiments, R_4 is a hydrogen. In other preferred embodiments, R_6 is a hydrogen.

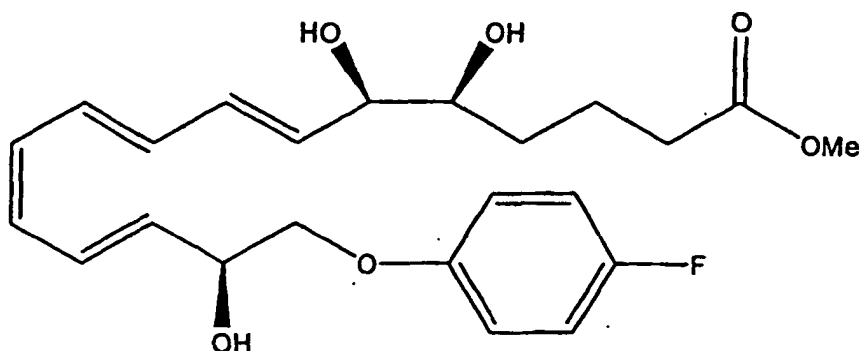
[0021] Additionally, R_5 can be a substituted or unsubstituted, branched or unbranched alkyl group having between 1 and about 6 carbon atoms, preferably between 1 and 4 carbon atoms, most preferably between 1 and 3, and preferably one or two carbon atoms. The carbon atoms can have substituents which include halogen atoms, hydroxyl groups, or ether groups.

[0022] The compounds useful in the present invention can be prepared by the following synthetic scheme:



wherein X, Q₁, Q₃, Q₄, R₂, R₃, R₄, R₅, R₆, Y₁ and T are as defined above. Suitable methods known in the art to can be used to produce each fragment. For example, the acetylenic fragment can be prepared by the methods discussed in Nicolaou, K.C. et al. (1991) *Angew. Chem. Int. Ed. Engl.* 30:1100; Nicolaou, K.C. et al. (1989) *J. Org. Chem.* 54: 5527; Webber, S.E. et al. (1988) *Adv. Exp. Med. Biol.* 229:61; and U.S.-Patent 5,441;951. The second fragment can be prepared by the methods of Raduchel, B. and Vorbruggen, H. (1985) *Adv. Prostaglandin Thromboxane Leukotriene Res.* 14:263.

[0023] The lipoxin analog can be used according to the present invention in pharmaceutical compositions including compounds having the above-described formulae and a pharmaceutically acceptable carrier. In one embodiment, a preferred compound is



[0024] In a preferred embodiment, the pharmaceutical carrier is not a ketone, e.g., acetone.

[0025] In one embodiment, the pharmaceutical agent is in the form of a shampoo or a body cleansing product, e.g., a soap, for cleansing of the scalp and/or body. The use of these compounds in a shampoo or soap product can be used to treat seborrheic dermatitis, pustular dermatosis and dandruff. Thus the compounds are useful for modulating TNF α PMN or cytokine inflammation associated with such conditions.

[0026] A "lipoxin analog" shall mean a compound which has an "active region" that functions like the active region of a "natural lipoxin", but which has a "metabolic transformation region" that differs from natural lipoxin. Lipoxin analogs include compounds which are structurally similar to a natural lipoxin, compounds which share the same receptor recognition site, compounds which share the same or similar lipoxin metabolic transformation region as lipoxin, and compounds which are art recognized as being analogs of lipoxin. Lipoxin analogs include lipoxin analog metabolites. The compounds disclosed herein may contain one or more centers of asymmetry. Where asymmetric carbon atoms are present, more than one stereoisomer is possible, and all possible isomeric forms are intended to be included within the structural representations shown. Optically active (R) and (S) isomers may be resolved using conventional techniques known to the ordinarily skilled artisan. The present invention is intended to include the possible diastereomers as well as the racemic and optically resolved isomers.

[0027] The terms "corresponding lipoxin" and "natural lipoxin" refer to a naturally-occurring lipoxin or lipoxin metabolite. Where an analog has activity for a lipoxin-specific receptor, the corresponding or natural lipoxin is the normal ligand for that receptor. For example, where an analog is a LXA₄ specific receptor on differentiated HL-60 cells, the corresponding lipoxin is LXA₄. Where an analog has activity as an antagonist to another compound (such as a leukotriene), which is antagonized by a naturally-occurring lipoxin, that natural lipoxin is the corresponding lipoxin.

[0028] "Active region" shall mean the region of a natural lipoxin or lipoxin analog, which is associated with in vivo cellular interactions. The active region may bind the "recognition site" of a cellular lipoxin receptor or a macromolecule or complex of macromolecules, including an enzyme and its cofactor. Preferred lipoxin A₄ analogs have an active region comprising C₅-C₁₅ of natural lipoxin A₄. Preferred lipoxin B₄ analogs have an active region comprising C₅-C₁₄ of natural lipoxin B₄.

[0029] The term "recognition site" or receptor is art-recognized and is intended to refer generally to a functional macromolecule or complex of macromolecules with which certain groups of cellular messengers, such as hormones, leukotrienes, and lipoxins, must first interact before the biochemical and physiological responses to those messengers are initiated. As used in this application, a receptor may be isolated, on an intact or permeabilized cell, or in tissue, including an organ. A receptor may be from or in a living subject, or it may be cloned. A receptor may normally exist or it may be induced by a disease state, by an injury, or by artificial means. A compound of this invention may bind reversibly, irreversibly, competitively, noncompetitively, or uncompetitively with respect to the natural substrate of a recognition site.

[0030] The term "metabolic transformation region" is intended to refer generally to that portion of a lipoxin, a lipoxin

metabolite, or lipoxin analog including a lipoxin analog metabolite, upon which an enzyme or an enzyme and its cofactor attempts to perform one or more metabolic transformations which that enzyme or enzyme and cofactor normally transform on lipoxins. The metabolic transformation region may or may not be susceptible to the transformation. A nonlimiting example of a metabolic transformation region of a lipoxin is a portion of LXA₄ that includes the C-13,14 double bond or the C-15 hydroxyl group, or both.

[0031] The term "detectable label molecule" is meant to include fluorescent, phosphorescent, and radiolabeled molecules used to trace, track, or identify the compound or receptor recognition site to which the detectable label molecule is bound. The label molecule may be detected by any of the several methods known in the art.

[0032] The term "labeled lipoxin analog" is further understood to encompass compounds which are labeled with radioactive isotopes, such as but not limited to tritium (³H), deuterium (²H), carbon (¹⁴C), or otherwise labeled (e.g. fluorescently). The compounds of this invention may be labeled or derivatized, for example, for kinetic binding experiments, for further elucidating metabolic pathways and enzymatic mechanisms, or for characterization by methods known in the art of analytical chemistry.

[0033] The term "inhibits metabolism" means the blocking or reduction of activity of an enzyme which metabolizes a native lipoxin. The blockage or reduction may occur by covalent bonding, by irreversible binding, by reversible binding which has a practical effect of irreversible binding, or by any other means which prevents the enzyme from operating in its usual manner on another lipoxin analog, including a lipoxin analog metabolite, a lipoxin, or a lipoxin metabolite.

[0034] The term "resists metabolism" is meant to include failing to undergo one or more of the metabolic degradative transformations by at least one of the enzymes which metabolize lipoxins. Two nonlimiting examples of LXA₄ analog that resists metabolism are 1) a structure which can not be oxidized to the 15-oxo form, and 2) a structure which may be oxidized to the 15-oxo form, but is not susceptible to enzymatic reduction to the 13,14-dihydro form.

[0035] The term "more slowly undergoes metabolism" means having slower reaction kinetics, or requiring more time for the completion of the series of metabolic transformations by one or more of the enzymes which metabolize lipoxin. A nonlimiting example of a LXA₄ analog which more slowly undergoes metabolism is a structure which has a higher transition state energy for C-15 dehydrogenation than does LXA₄ because the analog is sterically hindered at the C-16.

[0036] The term "tissue" is intended to include intact cells, blood, blood preparations such as plasma and serum, bones, joints, muscles, smooth muscles, and organs.

[0037] The term "halogen" is meant to include fluorine, chlorine, bromine and iodine, or fluoro, chloro, bromo, and iodo. In certain aspects, the compounds of the invention do not include halogenated compounds, e.g., fluorinated compounds.

[0038] The term "subject" is intended to include living organisms susceptible to conditions or diseases caused or contributed to by inflammation, inflammatory responses, vasoconstriction, and myeloid suppression. Examples of subjects include humans, dogs, cats, cows, goats, and mice. The term subject is further intended to include transgenic species.

[0039] When the compounds of the present invention are administered as pharmaceuticals, to humans and mammals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

[0040] The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a compound(s) of the present invention within or to the subject such that it can perform its intended function. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0041] In certain embodiments, the compounds used in the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Represent-

ative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

[0042] The term "pharmaceutically acceptable esters" refers to the relatively non-toxic, esterified products of the compounds of the present invention. These esters can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Carboxylic acids can be converted into esters via treatment with an alcohol in the presence of a catalyst. The term is further intended to include lower hydrocarbon groups capable of being solvated under physiological conditions, e.g., alkyl esters, methyl, ethyl and propyl esters. In a preferred embodiment, the ester is not a methyl ester (See, for example, Berge et al., *supra*).

[0043] Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0044] Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[0045] Formulations of the present invention include those suitable for intravenous, oral, nasal, topical, transdermal, buccal, sublingual, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

[0046] Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[0047] Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

[0048] In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[0049] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

[0050] The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres.

They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

[0051] Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

[0052] Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

[0053] Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[0054] Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

[0055] Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

[0056] Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

[0057] The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

[0058] Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

[0059] Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active compound in a polymer matrix or gel.

[0060] Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

[0061] Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[0062] Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0063] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be

desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0064] In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

[0065] Injectable depot forms are made by forming microcapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

[0066] The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Intravenous injection administration is preferred.

[0067] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, - usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

[0068] The phrases "systemic administration," "administered systematically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

[0069] These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

[0070] Regardless of the route of administration selected, the compounds used in the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of ordinary skill in the art.

[0071] Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[0072] The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0073] A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0074] In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 100 mg per kilogram of body weight per day, more preferably from about 0.01 to about 50 mg per kg per day, and still more preferably from about 0.1 to about 40 mg per kg per day. For example, between about 0.01 microgram and 20 micrograms, between about 20 micrograms and 100 micrograms and between about 10 micrograms and 200 micrograms of the compounds of the invention are administered per 20 grams of subject weight.

[0075] If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

[0076] In the following description of experiments, embodiments, in case they are not falling under the scope of the claims, are retained since they are useful for the understanding of the invention.

Materials and Methods

[0077] Human and mouse recombinant TNF α and human recombinant GM-CSF were obtained from Boehringer Mannheim (Indianapolis, IN). Dulbecco's PBS (Mg⁺⁺ and Ca⁺⁺-free), RPMI-1640 and FCS were purchased from Bio Whittaker Inc. (Walkersville, MD). Ficoll-Hypaque was from Organon Teknika Corp. (Durham, NC) and Hank's balanced salt solution was purchased from Gibco BRL (Grand Island, NY). Serum bovine albumin, dextran, antibiotics, L-glutamine, cytochrome C, superoxide dismutase and zymosan were obtained from Sigma (St. Louis, MO). The assessment of human IL-1 β in supernatants was performed by using an immunometric assay with acetylcholine esterase (Cayman Chemical, Ann Arbor, MI). Murine IL-1 β was assessed using an ELISA from Endogen (Woburn, MA). ELISAs for IL-4 and IL-10 were from Amersham (Arlington Heights, IL); MIP-2 and IL-13 ELISAs were from R&D Systems (Minneapolis, MN). LXA₄ and ATL metabolically stable analogs were prepared and characterized, including nuclear magnetic resonance spectroscopy, as in (14). Concentrations of each LX analog were determined using an extinction coefficient of 50,000 M⁻¹.cm⁻¹ just prior to each experiment. Where indicated, statistical analyses were performed using Student's non-paired *t*-test (two-tailed), and significance (*) was considered to be attained when *P* was < 0.05.

Preparation of human PMN suspensions and superoxide-anion generation

[0078] Venous blood from healthy donors was collected using sterile conditions using acid citrate dextrose (ACD) as an anticoagulant, and PMN were isolated as in (15). PMN were suspended in cold (4 ° C) Hank's medium (supplemented with 1.6 mM Ca⁺⁺, 0.1 % FCS, 2 mM L-glutamine, 1 % penicillin, and 2% streptomycin, pH 7.4). Cell preparations were > 98 % PMN as determined by Giemsa-Wright staining; cell viability was > 98% as determined by trypan blue exclusion and light microscopy. To examine superoxide production, PMN (1.0 x 10⁶/ml) were placed at 37 ° C (3 min) and then exposed to either vehicle (0.1 % ethanol) or synthetic LXA₄, 15R/S-methyl LXA₄ or 16-phenoxy-LXA₄ for 5 min at 37 ° C. Before adding TNF α (50 ng/ml), PMN were incubated with cytochrome C (0.7 mg/ml) for 10 min at 37 ° C. Superoxide dismutase-dependent reduction of cytochrome C was terminated by rapidly placing tubes in an ice-water bath. The extent of cytochrome C reduction in each supernatant was determined at 550 nm in reference to control values obtained when superoxide dismutase was added before a stimulus or vehicle control. Cytochrome C reduction was quantitated using the extinction coefficient of 21.1/mmol/L.

RNA isolation and Northern blot analysis

[0079] Total RNA extraction and Northern blot analyses were performed as in (7). pSM320 vector containing cDNA for IL-1 β was purchased from ATCC.

Murine air pouches

[0080] Six to eight week old male BALB/c mice were obtained from Taconic Farms (Germantown, NY). Air pouches were raised on the dorsum by s.c. injection of 3 ml of sterile air on day 0 and day 3. All experiments were conducted on day 6 (16). Individual air pouches (one per mouse) were injected with either vehicle alone (0.1 % ethanol), TNF α , 15 R/S-methyl-LXA₄ or TNF α plus 15 R/S-methyl-LXA₄, and each was suspended in 1 ml endotoxin-free PBS immediately before injection into pouch cavities. At given intervals, the mice were sacrificed and individual air pouches were lavaged three times with sterile PBS (1 ml). The exudates were centrifuged at 2000 RPM (5 min) and the supernatants were removed. Cell pellets were suspended in PBS (200 μ l) for enumeration and assessed for viability. Fifty μ l of each cell suspension was mixed with 150 μ l 30% BSA and then centrifuged onto microscope slides at 500 RPM for 5 min using a cytospin centrifuge, air dried, and stained with Giemsa-Wright.

Inhibition of TNF α -stimulated superoxide generation

[0081] TNF α , although a modest agonist of O₂⁻ generation by human PMN, is a physiologically relevant stimulus for the generation of reactive oxygen species (ROS) by human PMN that can play critical roles in local tissue injury during both inflammation and reperfusion (Tsuji, M., S. Kawano, S. Tsuji, H. Sawaoka, M. Hori, and R.N. DuBois. 1998. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 93:705-716; Shibuya, H., N. Ohkohchi, S. Tsukamoto, and S. Satomi. 1997. Tumor necrosis factor-induced, superoxide-mediated neutrophil accumulation in cold ischemic/reperfused rat liver. *Hepatology* 26:113-120; Jaeschke, H., A. Farhood, and C.W. Smith. 1990. Neutrophils contribute to ischemia/reperfusion injury in rat liver *in vivo*. *FASEB J.* 4:3355-3359). In Figure 1, the impact of LXA₄ and ATL related bioactive stable analogs were evaluated on TNF α stimulated superoxide anion production. Native LXA₄ and the analogs (15 R/S-methyl-LXA₄ and 16 phenoxy-LXA₄) inhibited TNF α -stimulated superoxide anion generation in a concentration-dependent fashion. Their rank order of potency at 10 nM was 15 R/S-methyl-LXA₄ (81.3 \pm

14.1% inhibition) » 16 phenoxy-LXA₄ (93.7 ± 3.2%) > LXA₄ (34.3 ± 2.3%). 15R/S-methyl-LXA₄ covers both LXA₄ and ATL in structure, and 16-phenoxy-LXA₄ is a LXA₄ analog (see Fig. 1). Each analog competes at the LXA₄ receptor. (Takano, T., S. Fiore, J.F. Maddox, H.R. Brady, N.A. Petasis, and C.N. Serhan. 1997. Aspirin-triggered 15-epi-lipoxin A₄ and LXA₄ stable analogs are potent inhibitors of acute inflammation: Evidence for anti-inflammatory receptors. *J. Exp. Med.* 185:1693-1704.) Neither LXA₄, 15 R/S-methyl-LXA₄ nor 16 phenoxy-LXA₄, at concentrations up to 1000 nM added to cells alone, stimulated generation of ROS. 15 R/S-methyl-LXA₄ and 16 phenoxy-LXA₄ were approximately three times more potent than native LXA₄ and proved to be powerful inhibitors of TNF α -stimulated superoxide generation by PMN. However, neither LXA₄ nor its analogs inhibit PMA (100 nM; n=3; not shown) or fMLP-stimulated O₂⁻ production. Inhibition of ROS by LXA₄ and its analogs is of interest in a context of ischemia/reperfusion, where ROS are held to be primary mediators of tissue injury (15).

Suppression of TNF α -stimulated IL-1 β release

[0082] PMN express and release interleukin-1 β , which is a potent proinflammatory cytokine (Dinarello, C.A. 1996. Biologic basis for interleukin-1 in disease. *Blood* 87:2095-2147). Therefore, the actions of native LXA₄ and its analogs on TNF α -induced IL-1 β release were investigated. Incubation of PMN with physiologically relevant concentrations of TNF α , GM-CSF or phagocytic particles (zymosan) resulted in a concentration dependent increase in the levels of IL-1 β present in supernatants. Approximate EC₅₀ for each agonist were: TNF α , 10 ng/ml; GM-CSF, 10 U/ml; and zymosan, 100 μ g/ml. Native LXA₄ specifically inhibited TNF α -induced IL-1 β release (Fig. 2A), while similar amounts of IL-1 β were released in the presence or absence of LXA₄ when PMN were exposed to either GM-CSF or zymosan.

[0083] PMN were exposed to increasing concentrations of 15 R/S-methyl-LXA₄, 16-phenoxy-LXA₄ or native LXA₄ in the presence of TNF α (10 ng/ml) or vehicle alone. At a concentration of 100 nM, 15 R/S-methyl-LXA₄ inhibited ~ 60 % of IL-1 β release, and 16-phenoxy-LXA₄ at equimolar levels gave approximately 40% inhibition (values comparable to those obtained with native LXA₄). Time course and concentration dependence were carried out with 15 R/S-methyl-LXA₄ (Fig 2B). At 10 nM, 15 R/S-methyl-LXA₄ gave clear statistically significant inhibition, which was evident within 6 h and more prominent after 24 h (Fig. 2B). Inhibition of IL-1 β by these LX analogs was, at least in part, the result of a down-regulation in gene expression, since the IL-1 β messenger RNA levels in cells treated with TNF α (10 ng/ml) plus 15 R/S-methyl-LXA₄ (100 nM) were decreased by approximately 60 % when compared to cells treated with TNF α alone (Fig. 3). Therefore, since IL-1 β and TNF α are two cytokines that are considered important in inflammation, the inhibition of IL-1 β observed (Figs. 1 & 2) suggested that 15 R/S-methyl-LXA₄ might exert a potent *in vivo* anti-cytokine action (*vide infra*).

Involvement of LXA₄ receptor

[0084] To investigate whether the LXA₄ receptor (LXA₄-R) was involved in the regulation of TNF α -stimulated IL-1 β release, the rabbit polyclonal antibodies against a portion of the third extracellular domain (ASWGGTPEERLK) of LXA₄-R prepared earlier were used (Fiore, S., and C.N. Serhan. 1995. Lipoxin A₄ receptor activation is distinct from that of the formyl peptide receptor in myeloid cells: inhibition of CD11/18 expression by lipoxin A₄-lipoxin A₄ receptor interaction. *Biochemistry* 34:16678-16686). PMN were incubated with - 50 μ g/ml of either preimmune protein-A purified IgG or IgG directed against LXA₄-R for 1 h at 4 °C prior to exposure to TNF α (10 ng/ml) and 15 R/S-methyl-LXA₄ (100 nM). Anti-LXA₄-R antibodies prevented IL-1 β release by TNF α , suggesting that the third extracellular loop plays a crucial role in LXA₄ receptor activation (Fig. 4). 15 R/S-methyl-LXA₄ inhibited about 50 % of IL-1 β release. When added together, anti-LXA₄-R antibodies and 15R/S-methyl-LXA₄ in the presence of TNF α did not further inhibit IL-1 β appearance, and neither anti-LXA₄-R antibodies nor 15 R/S-LXA₄ alone stimulated significant amounts of IL-1 β to appear within supernatants. The results of these experiments are two fold: first, they indicated that the inhibitory action of 15 R/S-methyl-LXA₄ is transduced via the LXA₄ receptor and second, that the anti-LXA₄-R antibodies alone activate the LXA₄ receptor and lead to inhibition of IL-1 β release.

Inhibition of TNF α -directed leukocyte trafficking *in vivo*

[0085] Since TNF α evokes leukocyte infiltration in a chemokine-dependent fashion in the murine six day air pouch, the impact of 15 R/S-methyl-LXA₄ in this model was evaluated to determine whether LXA₄ or ATL also intersects the cytokine-chemokine axis *in vivo* (Tessier, P.A., P.H. Naccache, I. Clark-Lewis, R.P. Gladue, K.S. Neote, and S.R. McColl. 1997. Chemokine networks *in vivo*: involvement of C-X-C and C-C chemokines in neutrophil extravasation *in vivo* in response to TNF- α . *J. Immunol.* 159:3595-3602; Sin, Y.M., A.D. Sedgwick, E.P. Chea, and D.A. Willoughby. 1986. Mast cells in newly formed lining tissue during acute inflammation: a six day air pouch model in the mouse. *Ann. Rheum. Dis.* 45:873-877). 15 R/S-methyl-LXA₄ is the most subtle modification to native LXA₄ and ATL structure with addition of a methyl at carbon 15. Murine TNF α (10 ng/ml) caused a transient infiltration of leukocytes to the air pouch in a

time-dependent fashion with maximal accumulation at 4 h. 15 R/S-methyl-LXA₄ at 25 nmoles inhibited the TNF α -stimulated recruitment of leukocytes to the air pouch by 62% (Fig. 5). Inhibition was evident at 1 h, and maximal between 2 h and 4 h. At these intervals, a more than 60 % reduction in leukocyte infiltration was noted that remained significantly reduced at 8 h (Fig. 5, insert). Injection of pouches with either vehicle or the analog alone did not cause a significant leukocyte infiltration. Also, inflammatory exudates were collected 4 h after injection with vehicle alone, TNF α , 15 R/S-methyl-LXA₄ alone, or TNF α plus 15 R/S-methyl-LXA₄, and cell types were enumerated. In the six-day pouches given TNF α , PMN constituted the major cell type present within the exudates at 4 h and ranged from 80 to 85% of total cell number. Administration of both 15 R/S-methyl-LXA₄ and TNF α into the six day air pouch cavity inhibited migration of PMN and eosinophils/basophils as well as mononuclear cells (Table I). Of interest, administration of 15 R/S-methyl-LXA₄ alone evoked a small but statistically significant increase in mononuclear cell influx (Table I), a result which is consistent with earlier *in vitro* observations in which specific stimulation of monocyte and inhibition of PMN chemotaxis have been observed (Maddox, J.F., M. Hachicha, T. Takano, N.A. Petasis, V.V. Fokin, and C.N. Serhan. 1997. Lipoxin A₄ stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein linked lipoxin A₄ receptor. *J. Biol. Chem.* 272:6972-6978).

Table I

TNF α -induced leukocyte infiltration in murine air pouches: Inhibitory action of 15 R/S-methyl-LXA ₄ †.			
Injection	Number of leukocytes present in the pouch (x 10 ⁶)		
	Neutrophils	Eosinophils/ Basophils	Monocytes/ Macrophages
TNF α	2.40 \pm 0.10*	0.30 \pm 0.01*	0.20 \pm 0.01*
15 R/S-methyl-LXA ₄ + TNF α	0.98 \pm 0.10** (59.1 %)	0.13 \pm 0.01** (56.0 %)	0.10 \pm 0.01** (50%)
15 R/S-methyl-LXA ₄	0.25 \pm 0.01	0.03 \pm 0.01	0.14 \pm 0.01*
Vehicle	0.30 \pm 0.01	0.07 \pm 0.01	0.06 \pm 0.01

†Air pouches were raised as described in *Materials and Methods*. Each mouse was injected with 1 ml PBS containing either vehicle (0.1 % ethanol), TNF α (10 ng), 15 R/S-methyl-LXA₄ (25 nmoles) or TNF α plus 15 R/S-methyl-LXA₄. Leukocyte infiltration was determined 4 h post-injection. Results present the mean \pm SEM of three different mice. Percent inhibition is indicated in parentheses. Statistically different from

*vehicle-injected mice ($p < 0.01$), and from

**TNF α -injected mice ($p < 0.01$).

Cytokine chemokine profiles

[0086] Since MIP-2 is the major chemokine involved in recruiting PMN to the air pouch following injection of TNF α , the action of 15 R/S-methyl-LXA₄ in this TNF α -induced -chemokine/cytokine axis was determined. MIP-2 and IL-1 β are important proinflammatory cytokines, and IL-4, IL-10 and IL-13 possess immunomodulatory properties (Isomaki, P., and J. Punnonen. 1997. Pro- and anti-inflammatory cytokines in rheumatoid arthritis. *Ann. Med.* 29:499-507; Volpert, O.V., T. Fong, A.E. Koch, J.D. Peterson, C. Waltenbaugh, R.I. Tepper, and N.P. Bouck. 1998. Inhibition of angiogenesis by interleukin 4. *J. Exp. Med.* 188:1039-1046). Exudates from selected time intervals were collected and cell-free supernatants assessed for the presence of these murine cytokines. TNF α induced maximal detectable amounts of MIP-2 and IL-1 β within 90 minutes (not shown). 15 R/S-methyl-LXA₄ (25 nmoles) inhibited TNF α -stimulated MIP-2 and IL-1 β release by 48 % and 30 % respectively (Fig. 6). 15 R/S-methyl-LXA₄ alone in the air pouch did not stimulate MIP-2 or IL-1 β release. In sharp contrast, 15 R/S-methyl-LXA₄ stimulated the appearance of IL-4 within the exudates. This stimulation of IL-4 was observed both in the absence as well as presence of TNF α . Neither IL-10 nor IL-13 was detected within the pouch exudates. These results demonstrate that administration of 15 R/S-methyl-LXA₄ modified the cytokine-chemokine axis in TNF α -initiated acute inflammation, and of interest this re-orientation of the cytokine-chemokine axis paralleled the reduction in leukocyte infiltration.

[0087] Several different strategies have been explored in an attempt to attenuate undesirable action of TNF α in inflammatory diseases and ischemia/reperfusion injury including treatment of patients suffering from rheumatoid arthritis with specific Fc portion of monoclonal antibodies directed against TNF α -receptor (26). Different steroidal and nonsteroidal drugs to alleviate the pain and the severity of inflammatory responses are extensively used (Marriott, J. B., M. Westby, and A.G. Dalgleish. 1997. Therapeutic potential of TNF- α inhibitors old and new. *DDT* 2:273-282). However, certain clinical settings such as reperfusion injury are still not well controlled, and new therapeutic agents are needed. The present results indicate that LXA₄ and ATL, as evidenced by the actions of their metabolically stable analogs (16-phenoxyl-LXA₄ and 15 R/S-methyl-LXA₄), are potent cytokine-regulating lipid mediators that can also impact the course of inflammation initiated by TNF α and IL-1 β . These two cytokines are considered to be key components in orchestrating the rapid inflammatory-like events in ischemia/reperfusion (within minutes to hours), and are major

cytokines in rheumatoid arthritis and many other chronic diseases. Of interest, in an exudate and skin wound model, 15 R/S-methyl-LXA₄ not only inhibited the TNF α -elicited appearance of IL-1 β and MIP-2, but also concomitantly stimulated IL-4 (Figs. 5-6). This represents the first observation that lipoxins induce upregulation of a potential "anti-inflammatory" cytokine such as IL-4. Hence, it is of particular interest that IL-4 inhibits PMN influx in acute antibody-mediated inflammation and inhibits H₂O₂ production by IFN γ -treated human monocytes (Saleem, S., Z. Dai, S.N. Coelho, B.T. Konieczny, K.J.M. Assmann, F.K. Baddoura, and F.G. Lakkis. 1998. IL-4 is an endogenous inhibitor of neutrophil influx and subsequent pathology in acute antibody-mediated inflammation. *J. Immunol.* 160:979-984; Lehn, M., W.Y. Weiser, S. Engelhom, S. Gillis, and H.G. Remold. 1989. IL-4 inhibits H₂O₂ production and antileishmanial capacity of human cultured monocytes mediated by IFN- γ . *J. Immunol.* 143:3020-3024). IL-4 is also an active antitumor agent and most recently was shown to be a potent inhibitor of angiogenesis (Volpert, O.V., T. Fong, A.E. Koch, J.D. Peterson, C. Waltenbaugh, R.I. Tepper, and N.P. Bouck. 1998. Inhibition of angiogenesis by interleukin 4. *J. Exp. Med* 188: 1039-1046). It is thus likely that the increase in IL-4 levels stimulated by metabolically stable LX analogs may mediate in part some of the *in vivo* impact of LXA₄ and aspirin-triggered 15-epi-LXA₄, a finding that opens a new understanding of the relationship between "antiinflammatory" cytokines and lipid mediators.

[0088] In conclusion, LXA₄ and aspirin-triggered-LXA₄ appear to be involved in controlling both acute as well as chronic inflammatory responses. The results presented here support the notion that aspirin may exert in part its beneficial action via the biosynthesis of endogenous aspirin-triggered-LXA₄ that can in turn act directly on PMN and/or the appearance of IL-4. Thus, LX-ATL can protect host tissues via multi-level regulation of proinflammatory signals.

References

[0089]

1. Serhan, C.N., J.Z. Haeggström, and C.C. Leslie. 1996. Lipid mediator networks in cell signaling: update and impact of cytokines. *FASEB J.* 10:1147-1158.
2. Weiss, S.J. 1989. Tissue destruction by neutrophils. *N. Engl.J. Med.* 320:365-376.
3. Marucha, P.T., R.A. Zeff, and D.L. Kreutzer. 1991. Cytokine-induced IL-1 β gene expression in the human polymorphonuclear leukocyte: transcriptional and post-transcriptional regulation by tumor necrosis factor and 1L-1. *J. Immunol.* 147:2603-2608.
4. Lloyd, A.R., and J.J. Oppenheim. 1992. Poly's lament: the neglected role of the polymorphonuclear neutrophil in the afferent limb of the immune response. *Immunology Today* 13:169-172.
5. Hachicha, M., P.H. Naccache, and S.R. McColl. 1995. Inflammatory microcrystals differentially regulate the secretion of macrophage inflammatory protein-1 and interleukin-8 by human neutrophils: A possible mechanism of neutrophil recruitment to sites of inflammation in synovitis. *J. Exp. Med.* 182:2019-2025.
6. Hansen, P.R. 1995. Role of neutrophils in myocardial ischemia and reperfusion. *Circulation* 91:1872-1885.
7. Takano, T., S. Fiore, J.F. Maddox, H.R. Brady, N.A. Petasis, and C.N. Serhan. 1997. Aspirin-triggered 15-epi-lipoxin A₄ and LXA₄ stable analogs are potent inhibitors of acute inflammation: Evidence for anti-inflammatory receptors. *J. Exp. Med* 185:1693-1704.
8. Clària, J., and C.N. Serhan. 1995. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc. Natl. Acad. Sci. USA* 92:9475-9479.
9. Lee, T.H., C.E. Horton, U. Kyan-Aung, D. Haskard, A.E. Crea, and B.W. Spur. 1989. Lipoxin A₄ and lipoxin B₄ inhibit chemotactic responses of human neutrophils stimulated by leukotriene B₄ and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Clin. Sci.* 77:195-203.
10. Serhan, C.N. 1994. Lipoxin biosynthesis and its impact in inflammatory and vascular events. *Biochim. Biophys. Acta* 1212:1-25.
11. Papayianni, A., C.N. Serhan, M.L. Phillips, H.G. Rennke, and H.R. Brady. 1995. Transcellular biosynthesis of lipoxin A₄ during adhesion of platelets and neutrophils in experimental immune complex glomerulonephritis. *Kidney Int.* 47:1295-1302.
12. Chavis, C., I. Vachier, P. Chanez, J. Bousquet, and P. Godard. 1996. 5(S),15(S)-Dihydroxyeicosatetraenoic acid and lipoacin generation in human polymorphonuclear cells: dual specificity of 5-lipoxygenase towards endogenous and exogenous precursors. *J. Exp. Med* 183:1633-1643.
13. Thomas, E., J.L. Leroux, F. Blotman, and C. Chavis. 1995. Conversion of endogenous arachidonic acid to 5,15-diHETE and lipoxins by polymorphonuclear cells from patients with rheumatoid arthritis. *Inflamm. Res.* 44: 121-124.
14. Serhan, C.N., J.F. Maddox, N.A. Petasis, I. Akritopoulou-Zanze, A. Papayianni, H.R. Brady, S.P. Colgan, and J.L. Madara. 1995. Design of lipoxin A₄ stable analogs that block transmigration and adhesion of human neutrophils. *Biochemistry* 34:14609-14615.
15. Gronert, K., S.P. Colgan, and C.N. Serhan. 1998. Characterization of human neutrophil and endothelial cell

ligand-operated extracellular acidification rate by microphysiometry: impact of reoxygenation. *J. Pharmacol. Exp. Ther.* 285:252-261.

16. Tessier, P.A., P.H. Naccache, I. Clark-Lewis, R.P. Gladue, K.S. Neote, and S.R. McColl. 1997. Chemokine networks in vivo: involvement of C-X-C and C-C chemokines in neutrophil extravasation in vivo in response to TNF- α . *J. Immunol.* 159:3595-3602.

17. Tsujii, M., S. Kawano, S. Tsuji, H. Sawaoka, M. Hori, and R.N. DuBois. 1998. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 93:705-716.

18. Shibuya, H., N. Ohkohchi, S. Tsukamoto, and S. Satomi. 1997. Tumor necrosis factor-induced, superoxide-mediated neutrophil accumulation in cold ischemic/reperfused rat liver. *Hepatology* 26:113-120.

19. Jaeschke, H., A. Farhood, and C.W. Smith. 1990. Neutrophils contribute to ischemia/reperfusion injury in rat liver *in vivo*. *FASEB J.* 4:3355-3359.

20. Dinarello, C.A. 1996. Biologic basis for interleukin-1 in disease. *Blood* 87:2095-2147.

21. Fiore, S., and C.N. Serhan. 1995. Lipoxin A₄ receptor activation is distinct from that of the formyl peptide receptor in myeloid cells: inhibition of CD11/18 expression by lipoxin A₄-lipoxin A₄ receptor interaction. *Biochemistry* 34:16678-16686.

22. Sin, Y.M., A.D. Sedgwick, E.P. Chea, and D.A. Willoughby. 1986. Mast cells in newly formed lining tissue during acute inflammation: a six day air pouch model in the mouse. *Ann. Rheum. Dis.* 45:873-877.

23. Maddox, J.F., M. Hachicha, T. Takano, N.A. Petasis, V.V. Fokin, and C.N. Serhan. 1997. Lipoxin A₄ stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein linked lipoxin A₄ receptor. *J. Biol. Chem.* 272:6972-6978.

24. Isomaki, P., and J. Punnonen. 1997. Pro- and anti-inflammatory cytokines in rheumatoid arthritis. *Ann. Med.* 29:499-507.

25. Volpert, O.V., T. Fong, A.E. Koch, J.D. Peterson, C. Waltenbaugh, R.I. Tepper, and N.P. Bouck. 1998. Inhibition of angiogenesis by interleukin 4. *J. Exp. Med.* 188:1039-1046.

26. Moreland, L.W., S.W. Baumgartner, M.H. Schiff, E.A. Tindall, R.M. Fleischmann, A.L. Weaver, R.E. Ettlinger, S. Cohen, W.J. Koopman, K. Mohler, M.B. Widmer, and C.M. Bloch. 1997. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N. Engl. J. Med.* 337:141-147.

27. Marriott, J.B., M. Westby, and A.G. Dalgleish. 1997. Therapeutic potential of TNF- α inhibitors old and new. *DDT* 2:273-282.

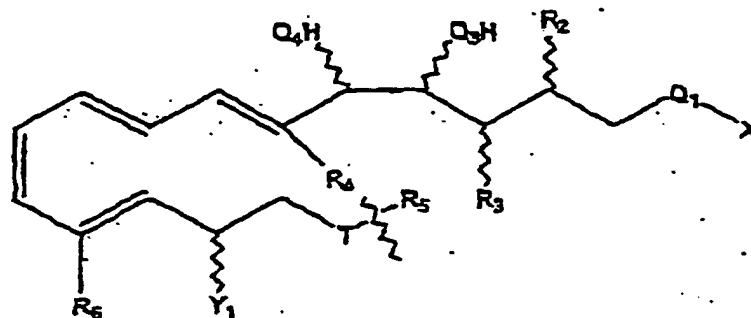
28. Saleem, S., Z. Dai, S.N. Coelho, B.T. Konieczny, K.J.M. Assmann, F.K. Baddoura, and F.G. Lakkis. 1998. IL-4 is an endogenous inhibitor of neutrophil influx and subsequent pathology in acute antibody-mediated inflammation. *J. Immunol.* 160:979-984.

29. Lehn, M., W.Y. Weiser, S. Engelhom, S. Gillis, and H.G. Remold. 1989. IL-4 inhibits H₂O₂ production and antileishmanial capacity of human cultured monocytes mediated by IFN- γ . *J. Immunol.* 143:3020-3024.

[0090] One having ordinary skill in the art will appreciate further features and advantages of the invention based on the above-described embodiments. Accordingly, the invention is not to be limited by what has been particularly shown and described, except as indicated by the appended claims.

Claims

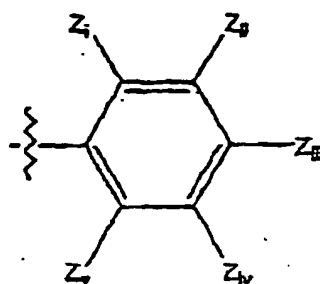
1. The use of a lipoxin analog having the formula



wherein X is R_1 , OR_1 or SR_1 ;

wherein R_1 is

- (i) a hydrogen atom;
- (ii) an alkyl of 1 to 8 carbon atoms, inclusive, which may be straight chain or branched;
- (iii) a cycloalkyl of 3 to 10 carbon atoms;
- (iv) an aralkyl of 7 to 12 carbon atoms;
- (v) phenyl;
- (vi) substituted phenyl



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} and Z_v are each independently selected from $-NO_2$, $-CN$, $-C(=O)-R_1$, $-SO_3H$, a hydrogen atom, halogen, methyl, $-OR_4$

wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl;

- (vii) a detectable label molecule, or
- (viii) a straight or branched chain alkenyl of 2 to 8 carbon atoms, inclusive,

wherein Q_1 is $(C=O)$, SO_2 or (CN) , provided when Q_1 is CN , then X is absent;

wherein Q_3 and Q_4 are each independently O, S or NH;

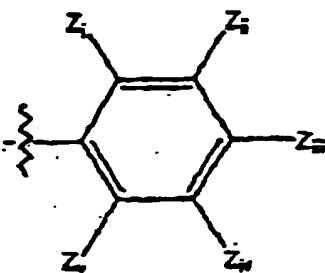
wherein one of R_2 and R_3 is a hydrogen atom and the other is

- (a) H;
- (b) an alkyl of 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched;
- (c) acycloalkyl of 3 to 6 carbon atoms, inclusive;
- (d) an alkenyl of 2 to 8 carbon atoms, inclusive, which may be straight chain or branched; or
- (e) $R_aQ_2R_b$ wherein Q_2 is $-O-$ or $-S-$; wherein R_a is alkylene of 0 to 6 carbon atoms, inclusive, which may be straight chain or branched and wherein R_b is alkyl of 0 to 8 carbon atoms, inclusive which may be straight chain or branched, provided when R_b is 0, then R_b is a hydrogen atom;

wherein R_4 is

- (a) H;
- (b) an alkyl of 1 to 6 carbon atoms, inclusive, which may be a straight chain or branched;

wherein R_5 is



wherein Z_i , Z_{ii} , Z_{iii} , Z_{iv} and Z_i are each independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, a hydrogen

hologen, methyl, $-\text{OR}_x$,
 wherein R_x is 1 to 8 carbon atoms, inclusive, which may be a straight chain or branched, and hydroxyl or a

substituted or unsubstituted, branched or unbranched alkyl group;
 wherein Y_1 is $-\text{OH}$, methyl, SH , an alkyl of 2 to 4 carbon atoms, inclusive, straight chain or branched, an

alkoxyl of 1 to 4 carbon atoms, inclusive, or CH_2Z_b where $a+b=3$, $a=0$ to 3, $b=0$ to 3 and Z is cyano, nitro or a halogen;
 wherein R_6 is

(a) H ;

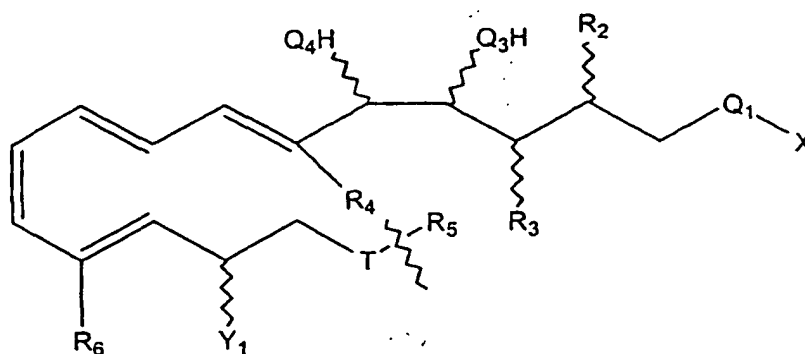
(b) an alkyl from 1 to 4 carbon atoms, inclusive, straight chain or branched;

wherein T is O or S , and pharmaceutically acceptable salts thereof; for preparing a pharmaceutical agent
 for treating seborrheic dermatitis, pustular dermatosis or dandruff associated with $\text{TNF}\alpha$ initiated polymorphonuclear (PMN) infiltration.

2. The use according to claim 1, wherein the pharmaceutical agent is in the form of a shampoo or a body cleansing product.

Patentansprüche

1. Verwendung eines Lipoxinanalogs mit der Formel



wobei X R_1 , OR_1 oder SR_1 ist;

wobei R_1

(i) ein Wasserstoffatom

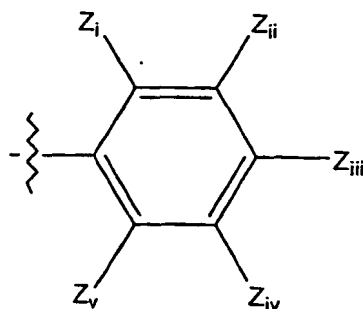
(ii) ein Alkyl mit 1 bis einschließlich 8 Kohlenstoffatomen, welches geradkettig oder verzweigt sein kann,

(iii) ein Cycloalkyl mit 3 bis 10 Kohlenstoffatomen;

(iv) ein Aralkyl mit 7 bis 12 Kohlenstoffatomen;

(v) Phenyl;

(vi) substituiertes Phenyl



wobei Z_i , Z_{ii} , Z_{iii} , Z_{iv} und Z_v jeweils unabhängig aus $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, einem Wasserstoffatom, Halogen, Methyl, $-\text{OR}_x$, wobei R_x 1 bis einschließlich 8 Kohlenstoffatome aufweist und geradkettig oder verzweigt sein kann, sowie Hydroxyl ausgewählt ist;

(vii) ein detektierbares Markierungsmolekül; oder

(viii) ein gerades oder verzweigt-kettiges Alkenyl mit 2 bis einschließlich 8 Kohlenstoffen, ist;

wobei Q_1 ($\text{C}=\text{O}$), SO_2 oder (CN) bedeutet, mit der Maßgabe, daß wenn Q_1 CN ist, X fehlt;

wobei Q_3 und Q_4 jeweils unabhängig O , S oder NH sind;

wobei eines von R_2 und R_3 ein Wasserstoffatom ist und das andere

(a) H ;

(b) ein Alkyl mit 1 bis einschließlich 8 Kohlenstoffatomen, das geradkettig oder verzweigt sein kann;

(c) ein Cycloalkyl mit 3 bis einschließlich 6 Kohlenstoffatomen;

(d) ein Alkenyl mit 2 bis einschließlich 8 Kohlenstoffatomen, welches geradkettig oder verzweigt sein kann; oder

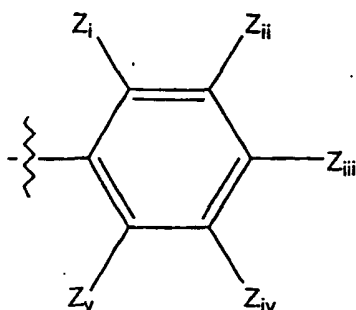
(e) $\text{R}_a\text{Q}_2\text{R}_b$, wobei Q_2 $-\text{O}-$ oder $-\text{S}-$ ist; wobei R_a ein Alkyl mit 0 bis einschließlich 6 Kohlenstoffatomen ist, welches geradkettig oder verzweigt sein kann, und wobei R_b ein Alkyl mit 0 bis einschließlich 8 Kohlenstoffatomen ist, welches geradkettig oder verzweigt sein kann, vorausgesetzt, dass wenn R_b O ist, R_b ein Wasserstoffatom ist, ist;

wobei R_4

(a) H ;

(b) ein Alkyl mit 1 bis einschließlich 6 Kohlenstoffatomen, welches geradkettig oder verzweigt sein kann, ist;

wobei R_5 ist



wobei Z_i , Z_{ii} , Z_{iii} , Z_{iv} und Z_v jeweils unabhängig aus $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(=\text{O})-\text{R}_1$, $-\text{SO}_3\text{H}$, einem Wasserstoffatom, Halogen, Methyl, $-\text{OR}_x$, wobei R_x 1 bis einschließlich 8 Kohlenstoffatome aufweist und geradkettig oder verzweigt sein kann, sowie Hydroxyl oder einer substituierten oder unsubstituierten, verzweigten oder nicht verzweigten Alkylgruppe ausgewählt ist;

wobei Y_1 -OH, Methyl, -SH, ein geradkettiges oder verzweigtes Alkyl mit 2 bis einschließlich 4 Kohlenstoffatomen, Alkoxy mit 1 bis einschließlich 4 Kohlenstoffatomen oder CH_aZ_b ist, wobei $a+b=3$, $a=0$ bis 3, $b=0$ bis 3 ist, und Z Cyano, Nitro oder ein Halogen ist;

wobei R_6

(a) H;

(b) ein Alkyl mit 1 bis einschließlich 4 Kohlenstoffatomen, das geradkettig oder verzweigt ist, ist;

wobei T O oder S ist,

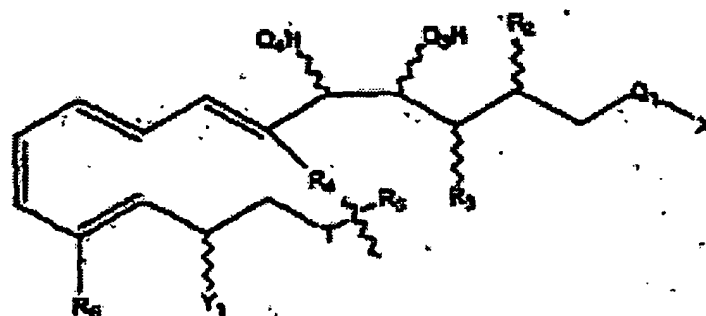
sowie pharmazeutisch akzeptabler Salze davon,

zur Herstellung eines pharmazeutischen Mittels zur Behandlung von Dermatitis seborrhoica, pustulärer Dermatitis oder Schuppen, einhergehend mit einer TNF α -initiierten Polymorphoneutrophilen (PMN)-Infiltrierung.

2. Verwendung nach Anspruch 1, wobei das pharmazeutische Mittel in Form eines Shampoos oder Körperreinigungsprodukts vorliegt.

Revendications

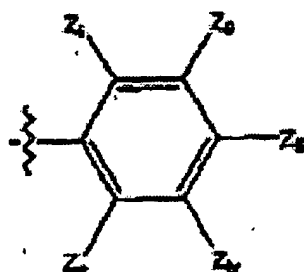
1. Utilisation d'un analogue de lipoxine ayant la formule



dans laquelle X représente R_1 , OR_1 , ou SR_1 ;

où R_1 représente

- (i) un atome d'hydrogène ;
- (ii) un groupe alkyle contenant 1 à 8 atomes de carbone, inclus, qui peut être à chaîne linéaire ou ramifiée ;
- (iii) un groupe cycloalkyle contenant 3 à 10 atomes de carbone ;
- (iv) un groupe aralkyle contenant 7 à 12 atomes de carbone ;
- (v) un groupe phényle ;
- (vi) un groupe phényle substitué,



où Z_i , Z_{ii} , Z_{iii} , Z_{iv} et Z_v sont chacun indépendamment choisis parmi -NO₂, -CN, -C(=O)-R_i, -SO₃H, un atome d'hydrogène, un atome d'halogène, un groupe méthyle, -OR_x, où R_x contient de 1 à 8 atomes de carbone, inclus, qui peut être une chaîne linéaire ou ramifiée, et un groupe hydroxyle ;

(vii) une molécule marqueur détectable ; ou

(viii) un groupe alcényle à chaîne linéaire ou ramifiée contenant 2 à 8 atomes de carbone, inclus ;

où Q_1 représente (C=O), SO_2 ou (CN), sous réserve que lorsque Q_1 représente CN, alors X est absent ;

où Q_3 et Q_4 représentent chacun indépendamment O, S ou NH ;

où l'un parmi R_2 et R_3 représente un atome d'hydrogène, et l'autre représente

(a) H ;

(b) un groupe alkyle contenant 1 à 8 atomes de carbone, inclus, qui peut être une chaîne linéaire ou ramifiée ;

(c) un groupe cycloalkyle contenant 3 à 6 atomes de carbone, inclus ;

(d) un groupe alcényle contenant 2 à 8 atomes de carbone, inclus, qui peut être à chaîne linéaire ou ramifiée ;
ou

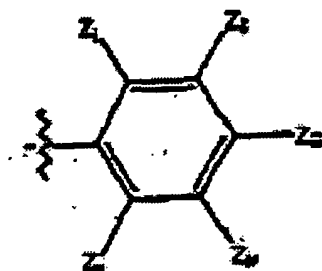
(e) $R_a Q_2 R_b$ où Q_2 représente -O- ou -S- ; où R_a représente un groupe alkyle contenant 0 à 6 atomes de carbone, inclus, qui peut être à chaîne linéaire ou ramifiée et R_b représente un groupe alkyle contenant 0 à 8 atomes de carbone, inclus, qui peut être à chaîne linéaire ou ramifiée, sous réserve que lorsque R_b vaut 0, alors R_b représente un atome d'hydrogène ;

où R_4 représente

(a) H ;

(b) un groupe alkyle contenant 1 à 6 atomes de carbone, inclus, qui peut être à chaîne linéaire ou ramifiée ;

où R_5 représente



où Z_i , Z_{iii} , Z_{iv} et Z_v , sont chacun indépendamment choisis parmi - NO_2 , -CN, -C(=O)- R_i , - SO_3H , un atome d'hydrogène, un atome d'halogène, un groupe méthyle, - OR_x , où R_x contient 1 à 8 atomes de carbone, inclus, et peut être à chaîne linéaire ou ramifiée, et un groupe hydroxyle ou un groupe alkyle substitué ou non substitué, ramifié ou non ramifié ;

où Y_1 représente -OH, un groupe méthyle, -SH, un groupe alkyle contenant de 2 à 4 atomes de carbone, inclus, à chaîne linéaire ou ramifié, un groupe alcoxy contenant 1 à 4 atomes de carbone, inclus, ou $CH_a Z_b$, où $a+b=3$, $a=0$ à 3, $b=0$ à 3 et Z représente un groupe cyano, nitro ou un atome d'halogène ;

où R_6 représente

(a) H ;

(b) un groupe alkyle contenant de 1 à 4 atomes de carbone, inclus, à chaîne linéaire ou ramifiée ;

où T représente O ou S, et ses sels pharmaceutiquement acceptables pour préparer un agent pharmaceutique destiné au traitement d'une dermatite séborrhéique, d'une dermatose pustuleuse ou des pellicules, associés à une infiltration neutrophiles polymorphonucléaires (PMN) initiée par $TNF\alpha$.

2. Utilisation selon la revendication 1, dans laquelle l'agent pharmaceutique est sous forme d'un shampoing ou d'un produit de nettoyage corporel.

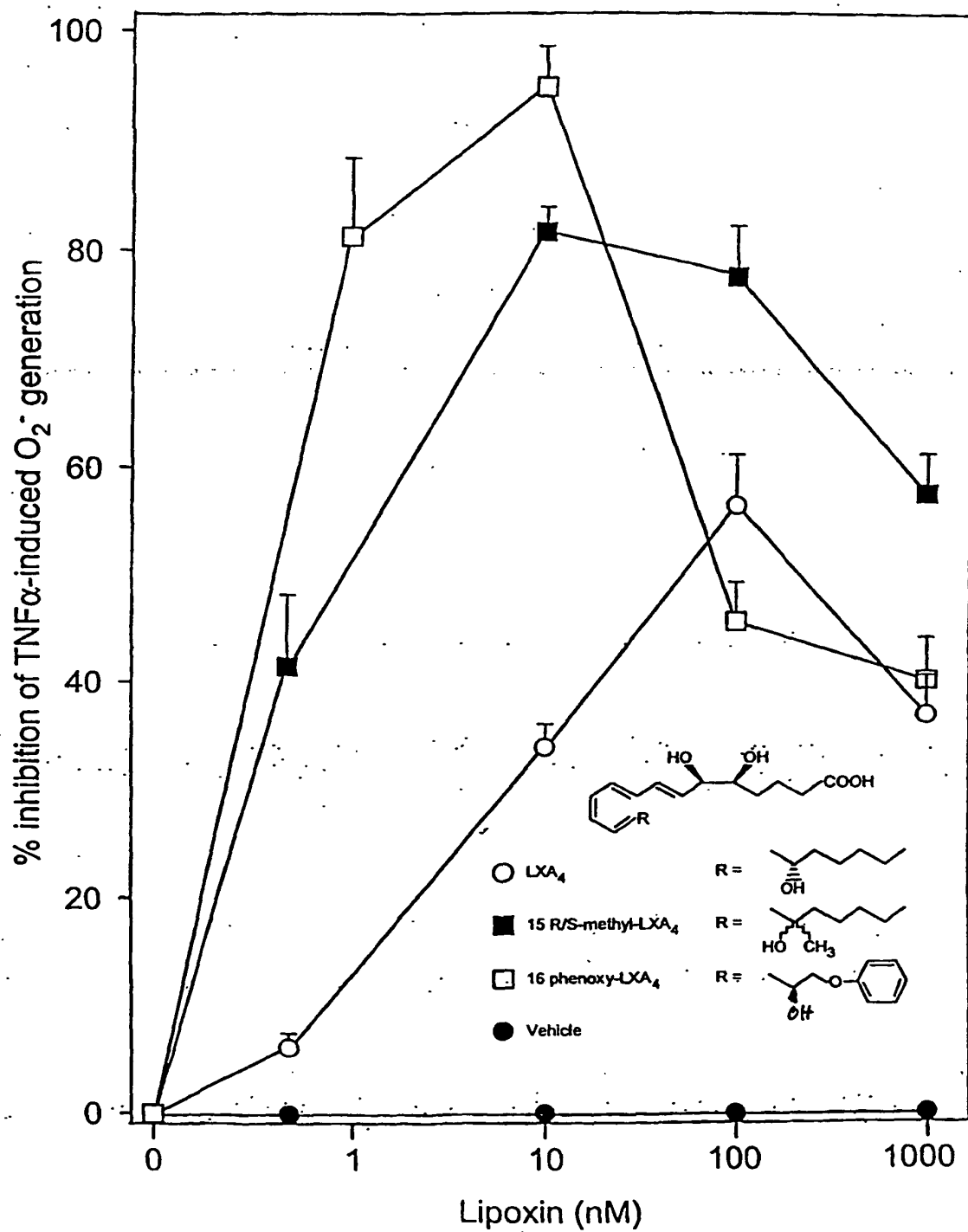


FIG. 1

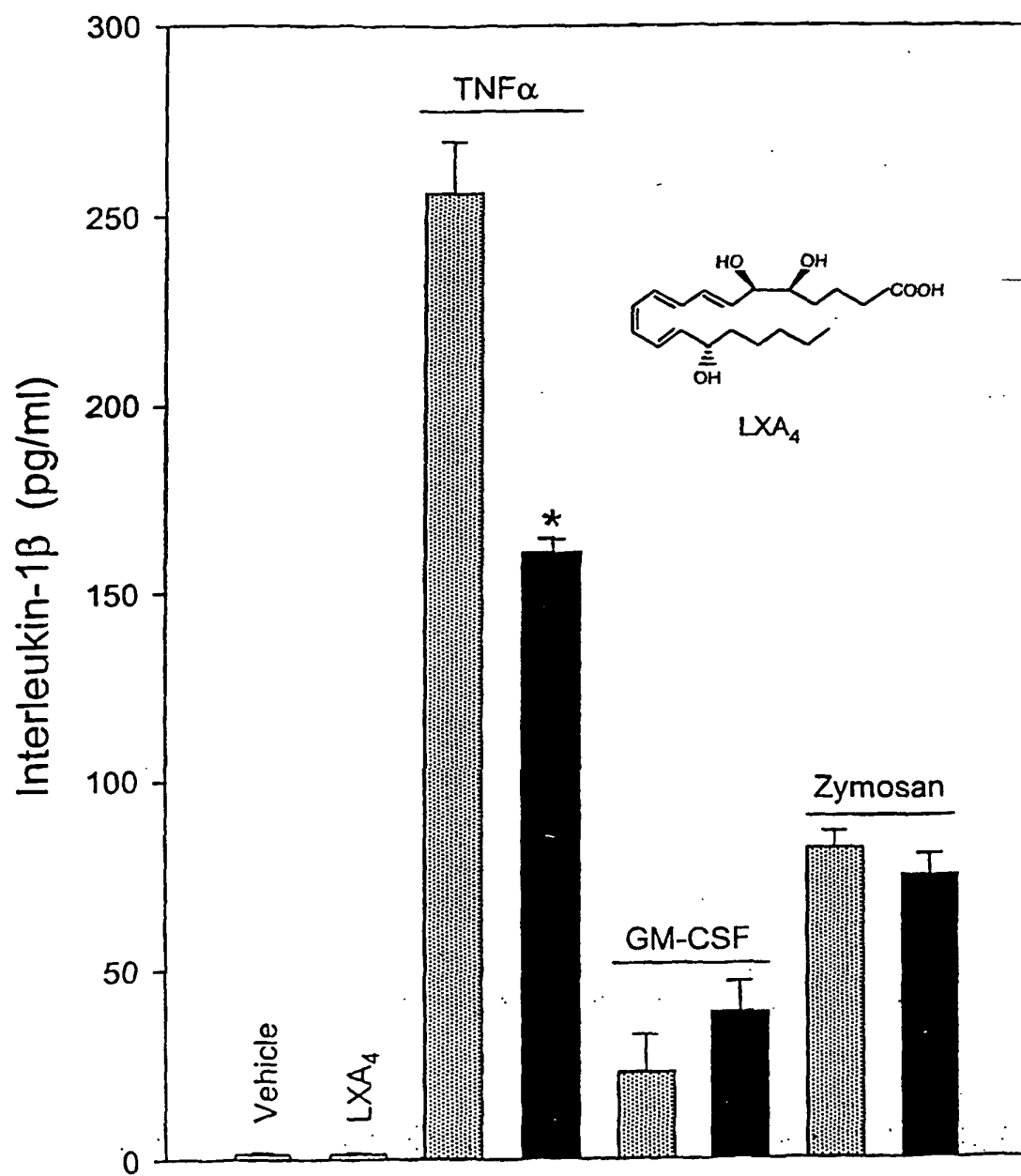


FIG. 2A

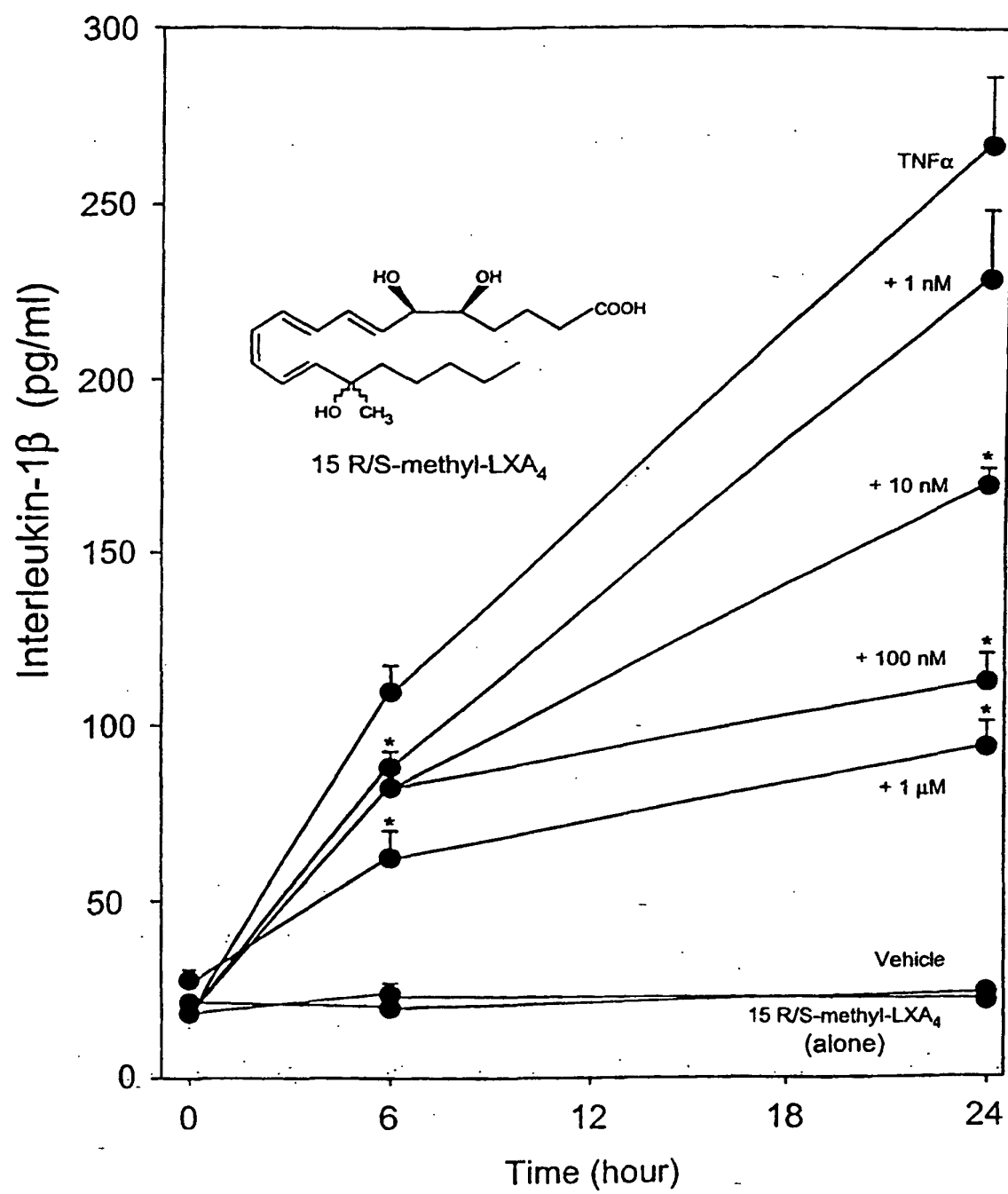


FIG. 2B

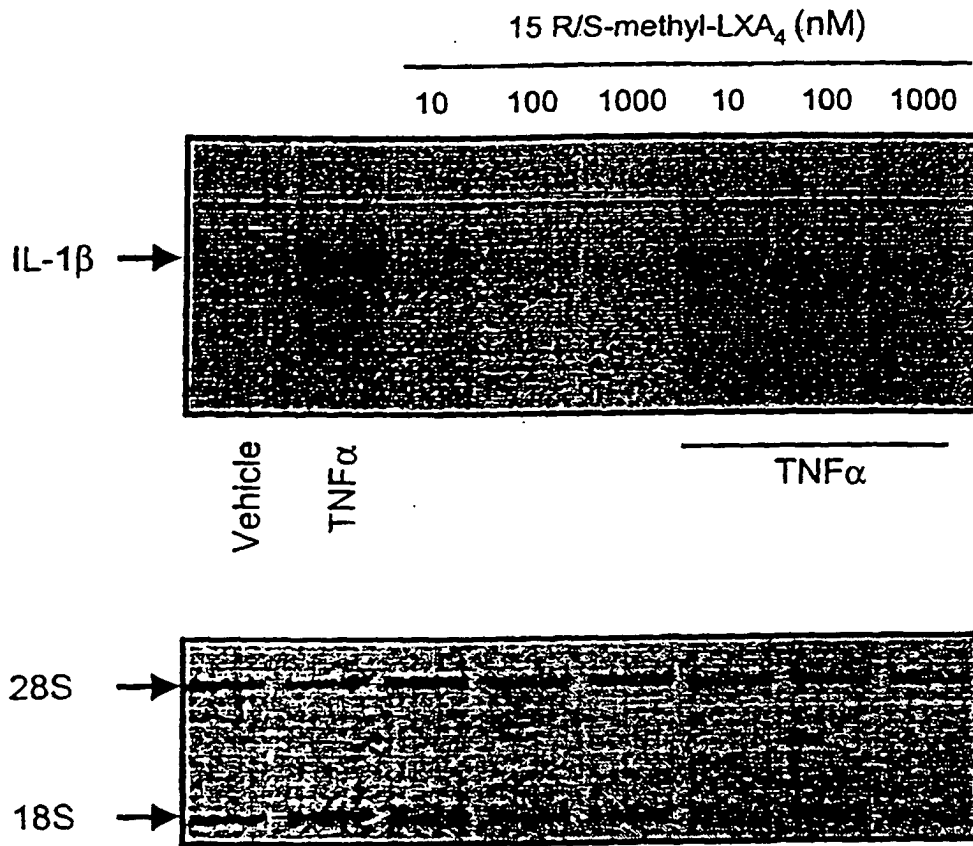


FIG. 3

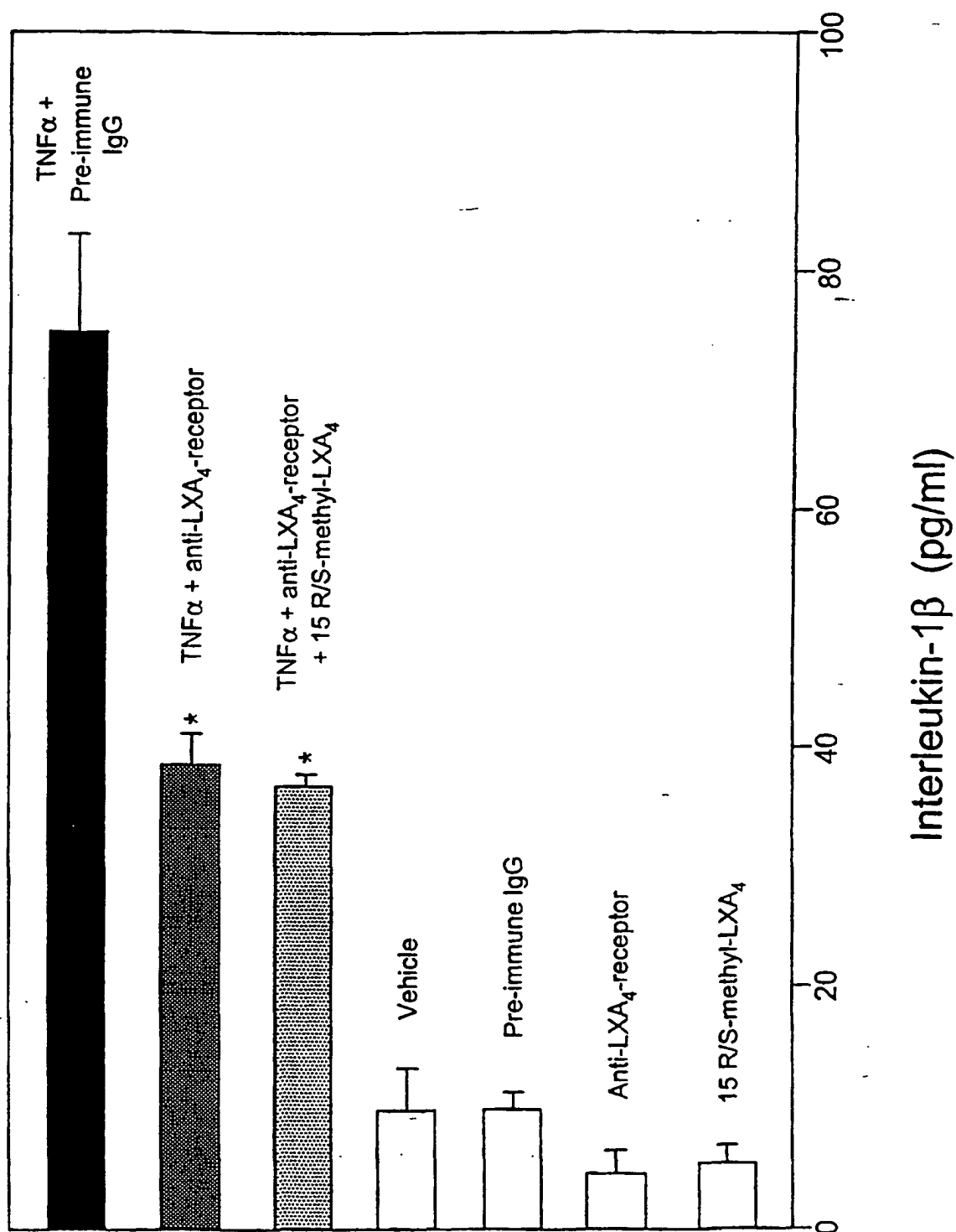


FIG. 4

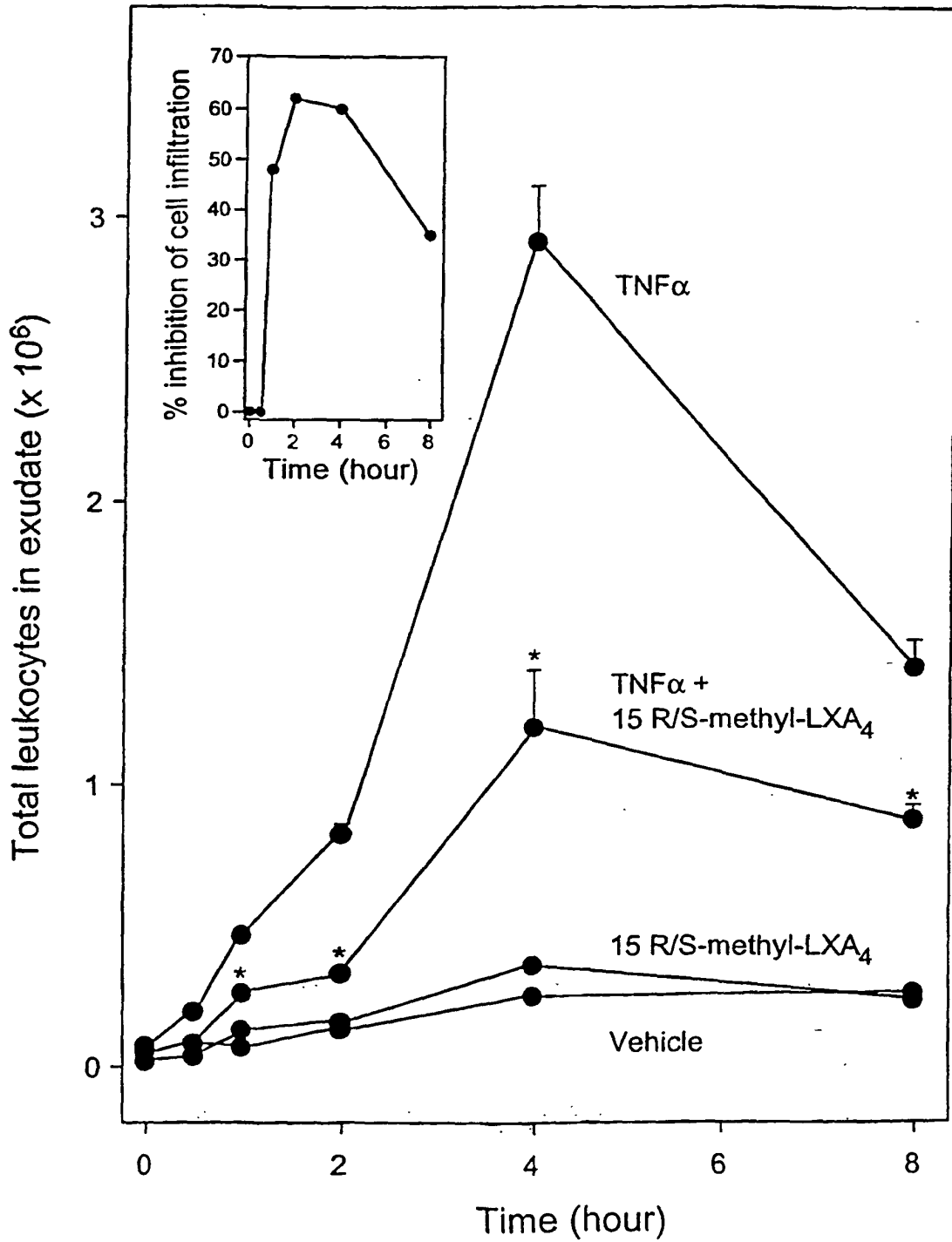


FIG. 5

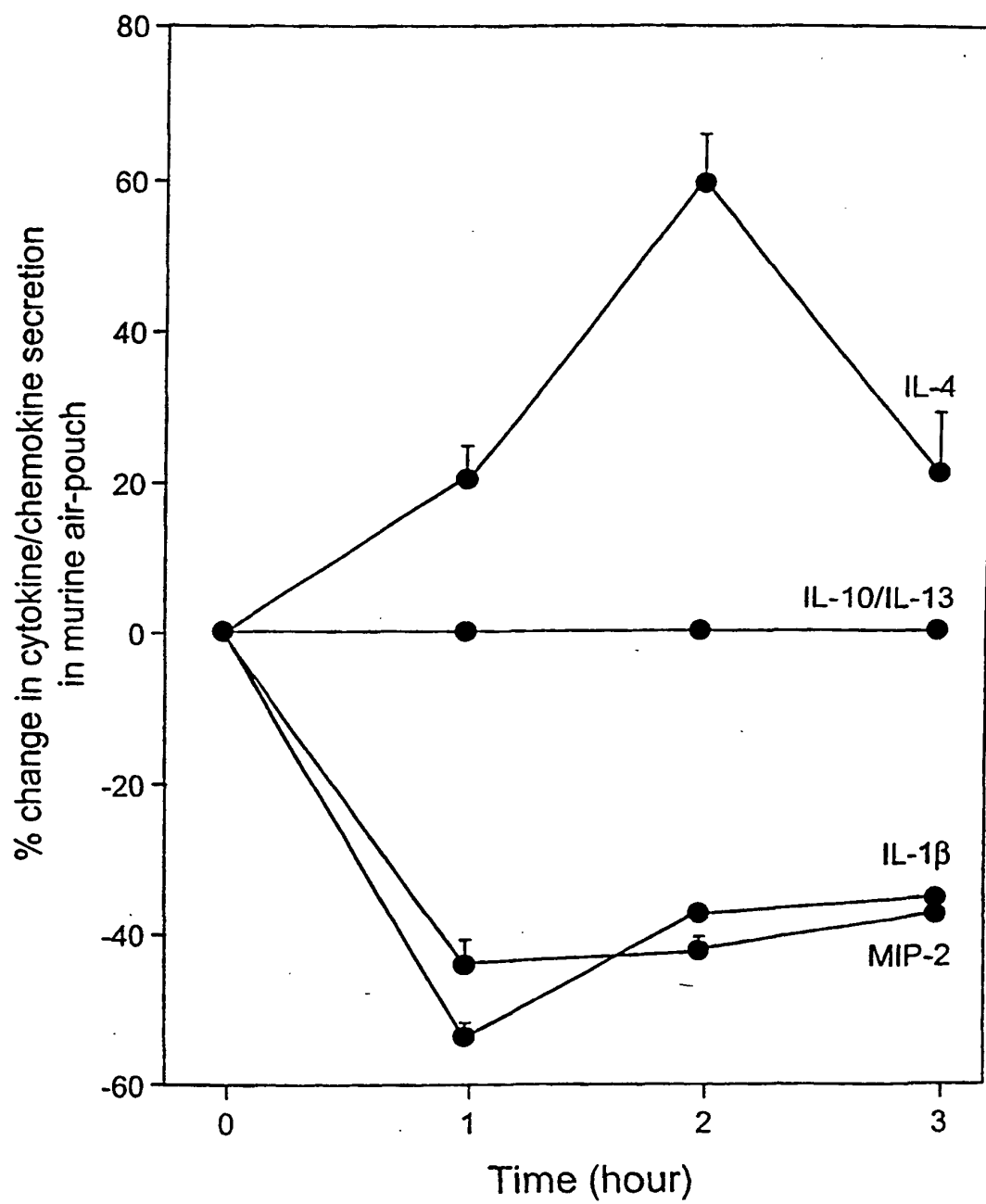


FIG. 6