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PREFERENTIAL INHIBITION OF 5-LIPOXYGENASE ACTIVITY BY MANOALIDE

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Abstract—Treatment of human polymorphonuclear leukocytes (PMNLs) with micromolar concentrations of the anti-inflammatory drug manoalide inhibited production of leukotriene B_4 (LTB_4) and LTC_4/LTD_4 in response to the calcium ionophore A23187. In an attempt to further define the mechanism(s) of action of this agent, we have examined its interaction with several lipoxygenase enzymes. In RBL-1 cells, manoalide inhibited 5-lipoxygenase (5-LO) activity with an approximate IC_{50} of 0.3 μM. This was equipotent in our system with the known lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA). Manoalide was virtually inactive, however, against 12-lipoxygenase activity in both human platelets and mouse epidermis, with little inhibition seen at concentrations up to 100 μM. Manoalide showed some activity against soybean lipoxygenase, although it was 30- to 50-fold less potent than as an inhibitor of the 5-lipoxygenase enzyme. These data indicate that manoalide is a selective 5-LO inhibitor and suggest the possibility that its anti-inflammatory actions may be due, at least in part, to inhibition of leukotriene synthesis.

Manoalide is a marine natural product isolated from the sponge *Lufferiella variabilis* (Fig. 1). Blankemeier and Jacobs [1] have identified it as a potent anti-inflammatory agent, demonstrating an inhibitory effect on phorbol ester induced inflammation in the mouse ear. Manoalide was shown to be more potent than indomethacin in this assay, although less potent than hydrocortisone. The fact that arachidonic acid-induced inflammation was not inhibited by manoalide suggests that the drug may be acting at an early point in the inflammatory response. *In vitro* studies, in fact, have shown manoalide to be a potent inhibitor of the neurotoxic action of β-bungarotoxin and both bee venom and cobra venom phospholipase A_2 (PLA_2) activities [2, 3], so manoalide may possibly be acting at the level of PLA_2 in the inflammation models just described.

The lipoxygenation of arachidonic acid leads to the production of a number of mediators of hypersensitivity and inflammation. These mediators include leukotrienes C_4, D_4 and E_4, LTB_4, 5-HETE, 12-HETE, and 15-HETE.† Each of these agents has been shown to affect one or more of a number of cellular functions, including proliferation, migration, secretion and contraction [4]. Modulation of lipoxygenase activity, therefore, might be expected to have a significant impact on hypersensitivity and/or inflammatory responses. We have examined the effect of manoalide on several enzymes within the arachidonic acid cascade in an attempt to further define the pharmacological profile of this compound and to assess its therapeutic potential.

MATERIALS AND METHODS

Materials.

[^{3}H]Arachidonic acid (83–87 Ci/mmol), [^{3}H]5-HETE (153 Ci/mmol), [^{3}H]12-HETE (225 Ci/mmol), [^{3}H]15-HETE (183 Ci/mmol) and [^{3}H]LTB_4 (59 Ci/mmol) were obtained from New England Nuclear. PGB_2, LTB_4 and all *trans*-LTB_4 were purchased from Bio Mol. Arachidonic acid, NDGA, soybean lipoxidase type I and Histopaque were obtained from Sigma. The radioimmunoassay (RIA) kit for LTC_4 was from New England Nuclear. All

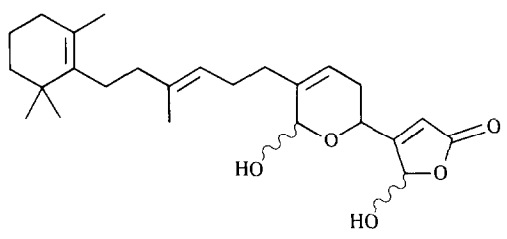

Fig. 1. Chemical structure of manoalide [3,7-Di-(hydroxymethyl) - 4 - hydroxy - 11 - methyl - 13 - (2,6,6 - trimethylcyclohexenyl)-2,6,10-tridecatrianoic acid-lactone].

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† Abbreviations: 5-HETE, 5-hydroxy-6,8,11,14-eicosatetraenoic acid; 12-HETE, 12-hydroxy-5,8,10,14-eicosatetraenoic acid; 15-HETE, 15-hydroxy-5,8,11,13-eicosatetraenoic acid; 15-HPETE, 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; NDGA, nordihydroguaiaretic acid; PGB_2, prostaglandin B_2; PMNL, polymorphonuclear leukocytes; RP-HPLC, reverse-phase high-performance liquid chromatography; RBL-1, rat basophilic leukemia cells; BW755C, 3-amino-1-[3-(trifluoromethyl) phenyl]-2-pyrazoline; GSH, glutathione; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

inorganic reagents were obtained from Mallinckrodt or Sigma. Manoalide was supplied by Drs. J. Faulkner (University of California, San Diego), and R. Jacobs (University of California, Santa Barbara). RBL-1 cells (CRL 1378) were from the American Type Culture Collection.

Leukotriene production by PMNLs

Polymorphonuclear leukocytes (PMNLs) from heparinized human whole blood were isolated using a double Histopaque gradient. PMNLs were separated from monocytes and red blood cells (RBCs) by centrifugation at 700 *g* for 30 min at room temperature. Contaminating RBCs were lysed by treating with 144 mM NH_4Cl, 17 mM Tris (pH 7.2) at 37°. The PMNLs were washed with phosphate-buffered saline (PBS) and resuspended at a concentration of 1×10^6 cells/ml. This mixed granulocyte preparation consisted of 95 ± 0.4% neutrophils, 4 ± 0.1% eosinophils and 1 ± 0.4% contaminating monocytes as determined by Giemsa–Jenner stain. Cell viability was determined with fluorescein diacetate. Suspensions (1 ml) of PMNLs were incubated with vehicle or test drug for 5 min at 37°. Following preincubation, the calcium ionophore A23187 (0.2 μM) was added to the cell suspensions, and the incubation continued for an additional 10 min. The reaction was stopped by the addition of an equal volume of cold 10 mM phosphate buffer (pH 7.4), containing 0.9% NaCl, 0.1% gelatin, 0.1% sodium azide and 0.01 M EDTA. The samples were centrifuged to remove the PMNLs, and the supernatant fractions were assayed for the presence of LTB_4 and LTC_4.

For the analysis of LTB_4, the samples were added to an equal volume of cold acetonitrile. The precipitated proteins were removed by centrifugation at 2000 *g* for 10 min, and the supernatant fractions were then diluted with 1.5 vol. of cold 1% formic acid, 1% triethylamine (TEA). The samples were loaded onto J.T. Baker C_{18} disposable columns, which had been pre-conditioned with 6 ml of 1% TEA in methanol and 6 ml of 1% formic acid, 1% TEA. The columns were then washed with 3 ml of 1% formic acid, 1% TEA, 3 ml of petroleum ether and 3 ml of 10% CH_3CN, 1% TEA. Leukotrienes were eluted with 2 ml of 70% CH_3CN, 1% TEA. The samples were dried and then reconstituted in 50% methanol, 30 mM ammonium acetate, pH 5.8. LTB_4 was assayed by RP-HPLC, using a Waters Nova-Pak C_{18} cartridge (5 mm × 10 cm) and a Kratos UV detector set at 280 nm. The mobile phase consisted of the following: Solvent A, 10% CH_3CN; 0.2% $NH_4CH_3CO_2$; Solvent B, 90% CH_3CN; 0.08% $NH_4CH_3CO_2$. Samples were eluted using a gradient system with an initial condition of 90% Solvent A and 10% Solvent B. The percentage of A was decreased linearly to 73% by 4.5 min. From 4.5 to 13 min, A was decreased to 65% using a non-linear gradient. The flow rate was 2.5 ml/min. Authentic LTB_4, all *trans*-LTB_4, LTC_4 and PGB_2 were used as standards. Recoveries using this assay procedure were 70 ± 2, 78 ± 2, 57 ± 2 and 70 ± 4% for LTB_4, all *trans*-LTB_4, LTC_4 and PGB_2 respectively. PGB_2 was added to the samples prior to extraction as an internal standard for measuring percent recovery.

Levels of LTC_4 in individual samples were too low to be detected by UV absorbance; therefore, unextracted sample supernatants were analyzed by radioimmunoassay. Since the antiserum to LTC_4 cross-reacted significantly (50%) with LTD_4, the RIA determinations are given as amounts of LTC_4/LTD_4. HPLC analysis of pooled samples, however, demonstrated LTC_4 to be the major peptido-leukotriene formed.

5-LO activity in RBL-1 cells

RBL-1 cells were grown in Earle's Minimum Essential Medium with 10% fetal calf serum, 1% L-glutamine, and 1% penicillin-streptomycin. 5-Lipoxygenase activity was determined by modification of the method of Jakschik *et al.* [5]. At the time of assay, the cells were washed and resuspended in 35 mM NaH_2PO_4 buffer (pH 7.0) with 1 mM EDTA. The cells were homogenized using a Kontes micro cell-disrupter, and the homogenate was spun at 100,000 *g* for 45 min. The Ca^{2+} concentration was adjusted to 2.0 mM. Vehicle, or test drug, was preincubated with 500 μl of the 100,000 *g* supernatant (equivalent to 2×10^7 cells) for 15 min on ice. The assay was started by the addition of supernatant to tubes containing [^{3}H]arachidonic acid (200 mCi/mmol; 10 μM). The enzyme reaction was allowed to proceed for 12 min at 4°. Under these conditions, the conversion of arachidonic acid was linear for both time and enzyme content. The reaction was stopped by the addition of 1 ml of cold acetone.

The reaction mixture was centrifuged, washed with petroleum ether, acidified, and extracted twice with chloroform. The organic fractions were dried under N_2 and the residue was dissolved in ethanol. The arachidonic acid metabolites were assayed by RP-HPLC [6], using an Ultra-sphere ODS column and a Radiomatic flow detector. The samples were eluted with 55% CH_3CN:45% H_2O, pH 3.0, at a flow rate of 1 ml/min. Authentic 5-HETE, 12-HETE, 15-HETE and LTB_4 standards were used to correlate with HPLC retention times of the products formed in the incubation mixture. Recoveries of the monohydroxy-eicosatetraenoic acids using this extraction procedure were 90 ± 2, 87 ± 3 and 83 ± 3% for 5-HETE, 12-HETE and 15-HETE respectively. 5-HETE production was taken as a measure of 5-LO activity.

12-Lipoxygenase activity

Human platelets. 12-Lipoxygenase activity was determined using a modification of the method of Lagarde *et al.* [7]. Human blood was collected with acid citrate dextrose and centrifuged at 200 *g* for 20 min. The platelet-rich plasma was adjusted to pH 6.4 with citric acid and centrifuged at 1200 *g* for 20 min. The pellet was resuspended in 0.15 M NaCl, 4 mM KCl, 3 mM EDTA, 10 mM HEPES buffer, pH 7.2, and then centrifuged at 1200 *g* for 20 min. The final pellet was suspended in 10 mM Tris–HCl, pH 7.7, with 1 mM GSH, and was homogenized with a Kontes micro cell-disrupter. The sample was centrifuged at 100,000 *g* for 45 min. A 500-μl aliquot of the high speed supernatant fraction (equivalent to 5–6 × 10^6 cells) was preincubated with vehicle or test drug for 5 min at 37°. The reaction was started by

the addition of the supernatant to tubes containing [^{3}H]arachidonic acid (100 mCi/mmol; 20 μM). The incubation was carried out for 2 min at 37°, and then the reaction was stopped by lowering the pH to 3.0 with 0.05 N HCl. The reaction mixture was extracted with ethyl acetate, the samples were dried under N_2, and the residue was redissolved in ethanol. The arachidonic acid metabolites were assayed by RP-HPLC as described above.

Mouse epidermis. Skin was obtained from hairless mice killed by cervical dislocation. The epidermis was heat-separated from the underlying dermis and placed in cold 50 mM Tris–HCl, pH 7.5, with 250 mM sucrose. The epidermis was homogenized using a "Tissumizer" and centrifuged at 1200 *g* for 20 min. The supernatant fraction was then centrifuged at 100,000 *g* for 45 min. The Ca^{2+} concentration of the high speed supernatant was adjusted to 2 mM. The samples were then preincubated with vehicle or test drug at 4° for 15 min. The assay was started by the addition of the high speed supernatant to tubes containing [^{3}H]arachidonic acid (200 mCi/mmol, 10 μM) and continued for 10 min at 30°. The reaction was stopped by the addition of 2 vol. of cold acetone. The reaction mixture was then extracted and analyzed by RP-HPLC as described for RBL-1 cells.

Soybean lipoxygenase activity

Soybean lipoxygenase activity was determined in a reaction mixture containing 0.2 M borate buffer (pH 9.0), arachidonic acid (300 μM), lipoxidase (800 units) and vehicle or test drug in a final volume of 3 ml. Under these conditions, the reaction was linear for both time and enzyme concentrations. Enzyme and vehicle or drug were preincubated for 30 min at room temperature. The enzyme reaction was initiated by the addition of arachidonic acid, and the ΔA at 234 nm was monitored for 5 min at room temperature.

RESULTS

Effect of manoalide on human PMNLs

Incubation of a human mixed granulocyte preparation with 0.2 μM A23187 led to the release of approximately 30–35 ng LTB_4 per 10^6 cells into the medium (Fig. 2). Preincubation of the cells with manoalide for 5 min before the addition of A23187 produced a dose-dependent inhibition of this response (Fig. 3), together with a reduction in the levels of the isomer all *trans*-LTB_4. The approximate IC_{50} value for manoalide in this preparation was 8×10^{-7} M. The lipoxygenase inhibitor NDGA also inhibited LTB_4 release, with a 90% reduction in LTB_4 levels observed at 1 μM (data not shown). Stimulation of this PMNL preparation with A23187 also led to the release of 2–4 ng LTC_4/LTD_4 per 10^6 cells. Again, manoalide produced a dose-dependent inhibition of the response, with an approximate IC_{50} value of 3×10^{-7} M (Fig. 4). NDGA was equipotent with manoalide in this preparation. Mepacrine, a reported inhibitor of PLA_2, was much less effective than either manoalide or NDGA, with significant inhibition of LTC_4/LTD_4 production occurring only at concentrations greater than 10 μM. High concentrations of manoalide (> 10 μM) were cytotoxic, leading to loss of viable cells as measured by fluorescein diacetate. Inhibition of both LTB_4 and LTC_4/LTD_4 production, however, occurred at drug concentrations 50–100 times less than that affecting cell viability. NDGA was not cytotoxic at any of the concentrations tested.

RBL-1 5-lipoxygenase activity

In the 100,000 *g* supernatant fractions of RBL-1 cells, [^{3}H]arachidonic acid was converted principally into 5-HETE, 12-HETE and small amounts of 15-HETE (Fig. 5). 5-HETE production was taken as a measure of 5-LO activity and represented approximately a 4–6% conversion of substrate. Initial studies in which the enzyme reaction was run at 37° resulted

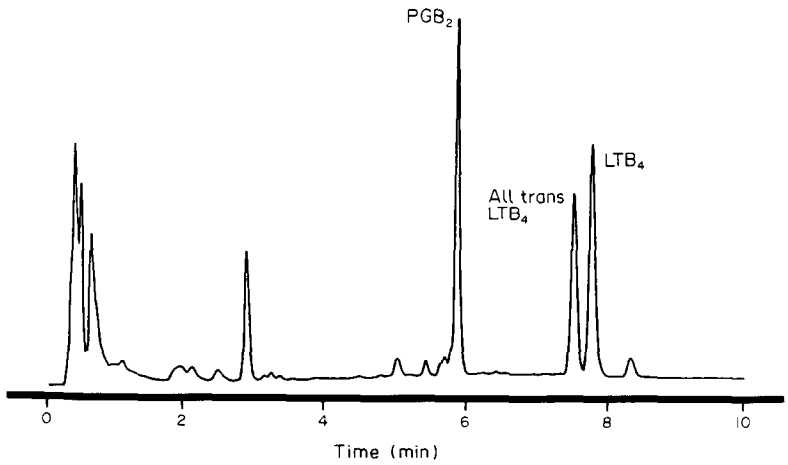

Fig. 2. RP-HPLC analysis of leukotrienes released by human PMNLs stimulated with 0.2 μM A23187. The chromatogram demonstrates the absorbance at 280 nm under conditions described in Materials and Methods. PGB_2 was added to the sample as an internal standard.

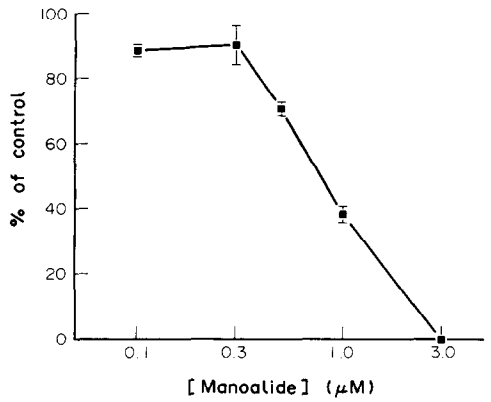

Fig. 3. Inhibition of LTB_4 synthesis by manoalide. Human PMNLs were pretreated for 5 min at 37° with increasing concentrations of manoalide prior to stimulation with 0.2 μM A23187. Results are given as percent of the control levels of 30–35 ng LTB_4 per 10^6 cells. Values represent the means ± SEM of three experiments.

in a rapid, non-linear production of 5-HETE over time. Decreasing the reaction temperature to 4° resulted in an assay linear for both time and enzyme concentrations. Preincubation of the enzyme with either manoalide or NDGA produced a dose-dependent inhibition of 5-HETE production (Fig. 6). The approximate IC_{50} for manoalide was 3×10^{-7} M, while NDGA was only slightly more potent. An apparent time-dependency of the effect of manoalide on 5-HETE production was observed when addition of manoalide with the substrate at the beginning of the enzyme reaction led to a marked reduction in its potency. Although a significant amount of 12-HETE was also produced in the reaction mixture, pre-

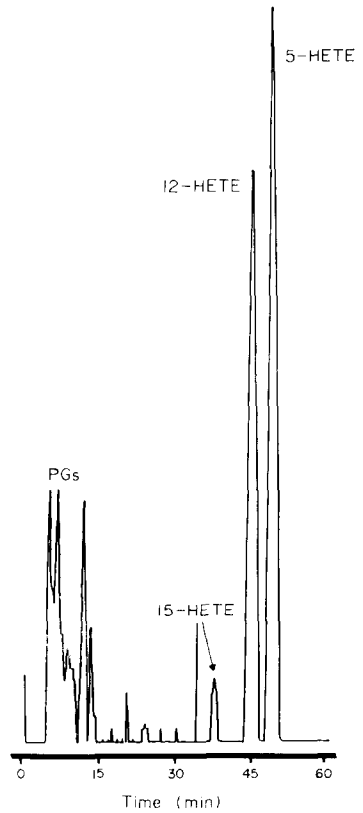

Fig. 5. Reverse-phase HPLC separation of lipid extract of 100,000 *g* supernatant fraction from RBL-1 cells incubated with [^{3}H]arachidonic acid. The chromatogram demonstrates elution of radioactivity under conditions described in Materials and Methods.

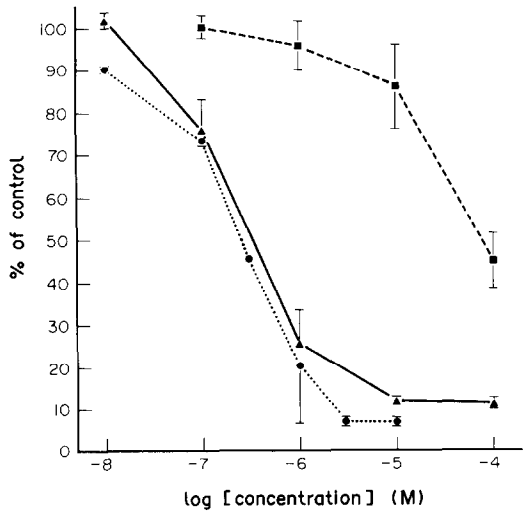

Fig. 4. Effects of manoalide, NDGA, and mepacrine on LTC_4/LTD_4 synthesis by human PMNLs. Cells were preincubated with vehicle or test drug for 5 min prior to the addition of 0.2 μM A23187. Under control conditions, PMNLs released 2–4 ng LTC_4/LTD_4 per 10^6 cells. Key: manoalide (▲), NDGA (●), and mepacrine (■). Values represent the means ± SEM of three to four experiments.

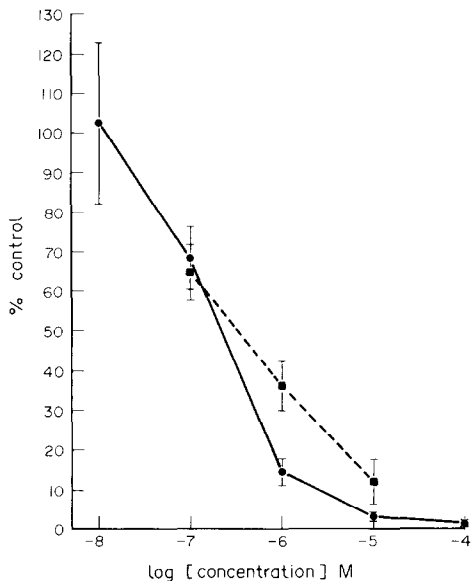

Fig. 6. Inhibition of 5-HETE production in RBL-1 cells by NDGA and manoalide. Under control conditions, incubation of 100,000 *g* supernatant fraction of RBL-1 cells with [^{3}H]arachidonic acid led to the production of approximately 10.0 μg 5-HETE/10^6 cells. Enzyme was precinubated with NDGA (●–●) or manoalide (■–■) for 15 min at 4°. Values represent the means ± SEM of four experiments.

treatment with manoalide did not lead to a change in 12-HETE levels relative to control at any concentration tested (84 ± 18, 113 ± 18 and 89 ± 16% of control for 0.1, 1.0 and 10.0 μM manoalide respectively).

12-Lipoxygenase activity

The conversion of arachidonic acid to lipoxygenase products was measured both in human platelets and in mouse epidermal homogenates. In platelets, 12-HETE was the principal metabolite formed. The addition of NDGA led to a dose-dependent inhibition of 12-lipoxygenase activity, whereas manoalide had little effect at concentrations up to 100 μM (Fig. 7). In epidermal samples, 12-HETE also was the major product formed. Again, manoalide had very little effect on 12-lipoxygenase activity in this tissue at concentrations up to 100 μM (Fig. 8). This was in marked contrast to NDGA which produced a dose-dependent inhibition of 12-HETE production with an IC_{50} of 2–3 μM. Small amounts of 15-HETE and 5-HETE were detected in epidermal homogenates, but the levels were quite variable and no clear drug effects could be observed.

Effect of manoalide on soybean lipoxidase

Soybean lipoxygenase activity was examined in the presence of increasing concentrations of manoalide, BW755C and NDGA (Fig. 9). Preincubation time had a significant effect on these agents, with maximum responses seen only after exposure of the enzyme to the drugs for periods of 10 min or longer. BW755C was a very potent inhibitor of soybean lipoxygenase activity with an IC_{50} of 3×10^{-8} M. Both manoalide and NDGA, on the other hand, were much less active, showing 50% inhibition of the enzyme only at concentrations greater than 10 μM. In an attempt to determine the reversibility of the effect of manoalide, drug and enzyme were incubated for 30 min and then dialyzed for 24 hr at 4° before addition to the reaction mixture. Under these conditions, the inhibitory effect of manoalide on soybean lipoxygenase activity was not reversible.

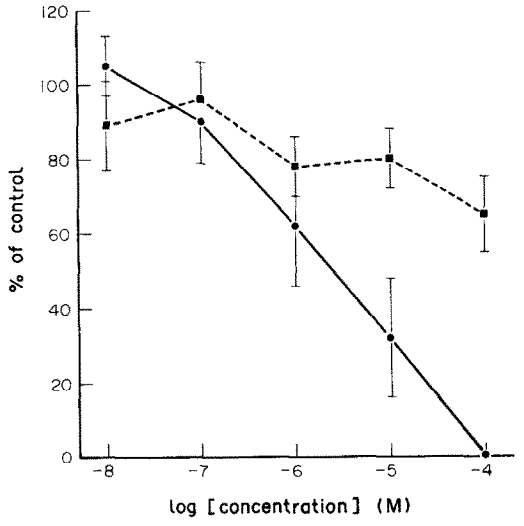

Fig. 7. Effects of manoalide and NDGA on 12-lipoxygenase activity in human platelets. Incubation of 100,000 *g* supernatant fraction of platelet homogenate with [^{3}H]arachidonic acid led to the production of approximately 4 μg 12-HETE/mg protein. Enzyme activity was determined after preincubation with manoalide (■—■) or NDGA (●—●) for 5 min at 37°. Values represent the means ± SEM of four experiments.

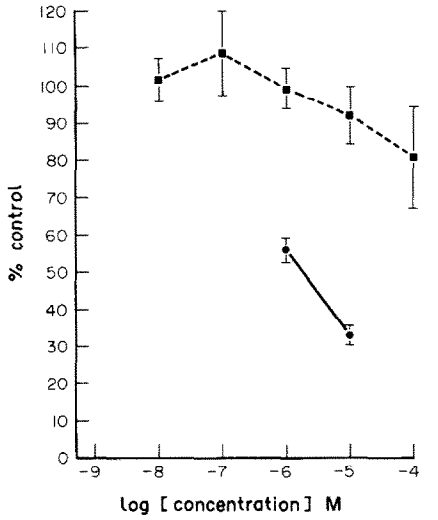

Fig. 8. Effects of manoalide and NDGA on 12-lipoxygenase activity in mouse epidermis. 12-HETE production by the cytosolic fraction of mouse epidermal homogenates was approximately 70 ng/mg protein. Enzyme activity was determined after preincubation of supernatant fractions with NDGA (●—●) or manoalide (■---■) for 15 min at 4°. Values represent the means ± SEM of four experiments.

DISCUSSION

These studies demonstrate that manoalide can inhibit the production of leukotrienes in human pro-inflammatory cells, which correlates with its

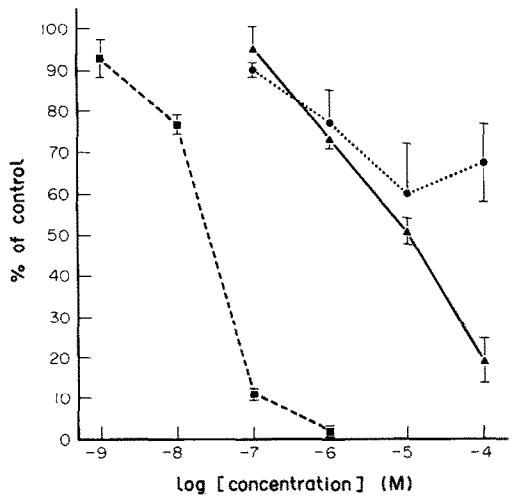

Fig. 9. Effects of manoalide, NDGA, and BW755C on soybean lipoxidase activity. Enzyme activity was determined after preincubation with vehicle or manoalide (▲–▲), NDGA (●—●) or BW 755C (■–■) for 30 min at room temperature. Values represent the means ± SEM of three to four experiments.

observed anti-inflammatory activity *in vivo*. It has long been recognized that PMNL and mononuclear leukocyte infiltration is a major contributor to the inflammatory response. Stimulation of PMNLs leads to the release of preformed products (e.g. proteolytic enzymes) and newly formed small molecules, including arachidonic acid metabolites formed via cyclooxygenase and lipoxygenases. Examination of the effect of manoalide on activation of these cells, therefore, could give some insight into its mode of action. In our studies, stimulation of a mixed granulocyte preparation with A23187 led to the release of both LTB_4 and LTC_4/LTD_4, and manoalide was shown to be a potent inhibitor of this response. NDGA also was a potent inhibitor, and only slightly more active than reported previously for inhibition of LTB_4 synthesis in ionophore-stimulated rat neutrophils [8]. Recent studies have shown that LTB_4 is produced mainly by neutrophils, whereas eosinophils are responsible for the generation of LTC_4 [9, 10]. The importance of LTB_4 as a leukocyte chemotactic agent, together with the demonstrated roles of eosinophils in immediate hypersensitivity reactions [11] and of peptidoleukotrienes in inflammation [12], suggest that the inhibition of LTB_4 and LTC_4/LTD_4 production seen in our studies points to a therapeutic potential for manoalide.

The mechanism of action for inhibition of LTB_4 and LTC_4/LTD_4 production in these whole cell preparations, however, is not clear. Although manoalide has been shown to be a potent PLA_2 inhibitor [2, 3, 13], there are multiple points in the arachidonic acid cascade where interruption of leukotriene biosynthesis could occur. One such step is at the level of oxygenation of arachidonic acid. Since manoalide inhibited both LTB_4 and the non-enzymatically formed all *trans*-LTB_4, it is likely to be acting at a step prior to the synthesis of the unstable intermediate LTA_4. We, therefore, examined the effect of the drug on several lipoxygenase enzymes. In cytosolic fractions of RBL-1 cells, the 5-lipoxygenase pathway has been shown to be a major route for arachidonic acid metabolism, although both 12- and 15-lipoxygenase activities have been reported by other investigators [14]. In our preparation, we observed a production of 5-HETE comparable to that reported previously, with lesser amounts of 12- and 15-HETE formed. Reduction of 5-HETE synthesis by manoalide demonstrated it to be a potent 5-lipoxygenase inhibitor. It was, in fact, just as active as NDGA, the prototypical inhibitor of this enzyme. Manoalide had little, if any, effect on 12-HETE synthesis. It has been reported, however, that the 12-lipoxygenase enzyme in RBL-1 cells may be different from that in platelets, as suggested by varying effects of Ca^{2+} on enzyme activity [15]. For this reason, we examined the activity of manoalide in more detail in human platelets and mouse epidermis.

In addition to the production of prostaglandins, arachidonic acid is oxygenated by platelet lipoxygenase to 12-HPETE, which is then reduced to the 12-hydroxy compound 12-HETE. In our studies, 12-HETE was the only monohydroxy compound formed by the cytosolic fraction from human platelets. Manoalide had no effect on 12-HETE production at concentrations up to 100 μM, although NDGA was shown to inhibit the platelet lipoxygenase activity at levels similar to that reported previously [8]. Since selectivity and potency of lipoxygenase inhibitors have been shown to vary with species or type of tissue or cell, we also examined the effect of manoalide on 12-lipoxygenase activity in mouse epidermis. Similar to results reported previously for both keratinocyte cell culture and epidermal homogenates [16–18], we observed that the major arachidonic acid products formed were the mono-HETES, with 12-HETE >> 15-HETE > 5-HETE. Again, manoalide had no effect on 12-HETE production at concentrations up to 100 μM. These data demonstrate that manoalide had no inhibitory activity against 12-lipoxygenase in a number of different cell types.

Soybean lipoxygenase has been studied extensively, and it has been shown that incubation of the enzyme with arachidonic acid leads to the production of the 15-hydroperoxy compound exclusively [19]. In our studies, NDGA was shown to be a weak inhibitor of this enzyme, with significant effects occurring only at concentrations greater than 10 μM. This is in keeping with previous reports in which NDGA inhibited the enzyme only at high concentrations (IC_{50} = 70 μM) [20]. BW755C, on the other hand, was found to be a very potent inhibitor of soybean lipoxidase, while manoalide was approximately 500-fold less active than BW755C in this system. The differences in potency of these lipoxygenase inhibitors may be related to their abilities to bind to multiple sites postulated to be present on the enzyme(s) [20]. The time-dependency and irreversibility of the effect of manoalide is consistent with a non-competitive form of inhibition—perhaps similar to that already reported for PLA_2 [3, 13]. Further study will be needed to clarify this issue.

Our data indicate that manoalide is a potent and selective 5-lipoxygenase inhibitor, with much less activity seen against the 12- or 15-lipoxygenase enzymes. Since 5-lipoxygenase products are known to be important mediators of inflammation, inhibition of this enzyme might be expected to have significant anti-inflammatory effects. Together with its known anti-PLA_2 activity, these studies suggest that the usefulness of manoalide as a potential therapeutic agent may be related to its ability to block the synthesis of pro-inflammatory mediators at multiple points.

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