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DIFFERENTIAL INHIBITORY EFFECTS OF AURANOFIN ON LEUKOTRIENE B_4 AND LEUKOTRIENE C_4 FORMATION BY HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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Abstract—Auranofin (AF) is a newly introduced oral gold compound having antirheumatic properties, and its efficacy in the treatment of bronchial asthma is now under investigation. In this study, we examined the effects of AF on leukotriene (LT) formation by human polymorphonuclear leukocytes (PMNs) stimulated with the calcium ionophore A23187. AF inhibited LTC_4 formation in a dose-dependent manner with an IC_{50} (concentration required to produce 50% inhibition of control) of 3.2 μM. In contrast, LTB_4 formation was not prevented by AF at concentrations up to 6 μM, but it was reduced to 59 ± 4% (mean ± SE, N = 3) of control by an 8 μM concentration. As a next step, we explored the mechanisms of the differential inhibitory effects of AF using cell-free systems. When arachidonic acid (AA) and reduced glutathione (GSH) were used as substrates, AF inhibited LTC_4 synthesis more effectively (IC_{50} = 14 μM) than LTB_4 synthesis (IC_{50} = 100 μM). However, LTB_4 and LTC_4 syntheses from LTA_4 were affected only slightly by AF within the concentrations tested (3–100 μM). These results in the cell-free systems indicate that the inhibition of LT formation was caused by a reduction of LTA_4 synthesis and that the differential inhibitory effects can be ascribed to the higher K_m value of glutathione *S*-transferase for LTA_4 than that of LTA_4 hydrolase in PMNs. In accordance with this hypothesis, LTC_4 synthesis was more dependent than LTB_4 synthesis on LTA_4 concentrations within 25–100 μM, and AA-861, a 5-lipoxygenase inhibitor, caused similar differential inhibitory effects on the formation of LTs by intact PMNs. The inhibitory effect of AF on LT formation at physiological concentrations may play some role in the efficacy of this drug.

Leukotrienes (LTs†) are newly recognized potent mediators derived from arachidonic acid (AA), and the mechanisms of pharmacological modulation of their synthesis have been widely studied in view of their relevance to bronchial asthma and inflammatory diseases such as rheumatoid arthritis [1, 2].

Chrysotherapy has been shown to be effective in the treatment of these disorders [3, 4]. Almost a decade agao, auranofin (AF), an oral gold compound, was made available and proven to be effective in the treatment of rheumatoid arthritis [5, 6]; its efficacy in bronchial asthma is now under investigation.

AF has been reported to modulate in an *in vitro* system, various functions of the polymorphonuclear leukocytes (PMNs). It inhibits chemotaxis [7], lysosomal enzyme release [8], histamine release [9], and generation of superoxide anion [10]. The exact site of action, however, is, obscure although some early and common events of activation have been known to be inhibited by AF [9, 11]. Recent studies have shown that the products of the 5-lipoxygenase pathway directly induce or augment these functions [12–14]. Very recently, AF was found to inhibit the release of LTC_4 and histamine from human basophils and mast cells [15].

These findings *in vivo* and *in vitro* raised the possibility that AF may affect LT formation. Thus, we examined the effects of AF on LTB_4 and LTC_4 formation by PMNs and found the preferential inhibitory effect of this compound on LTC_4 synthesis. We further studied the mechanisms of this action of AF using cell-free systems.

MATERIALS AND METHODS

Materials. Synthetic LTs and LTA_4-methyl ester were obtained from Paesel GmbH & Co., Frankfurt, West Germany. LTA_4-methyl ester was hydrolyzed in tetrahydrofuran (THF)/0.1 M lithium hydroxide, (4/1, v/v) overnight at room temperature under stirring. Immediately before use, THF was evaporated under a N_2 stream, and LTA_4-lithium salt was resuspended in ethanol. AA, reduced glutathione (GSH), prostaglandin B_2 (PGB_2) and piperazine-*N*,*N*′-bis [2-ethane-sulfonic acid] (Pipes) were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. Ficoll/Paque and Dextran T-500 were from Phar-

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† Abbreviations: LTs, leukotrienes; AF, auranofin; AA, arachidonic acid; GSH, reduced glutathione; PG, prostaglandin; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; Pipes, piperazin-*N*,*N*′-bis[2-ethane-sulfonic acid]; γ-GTP, gamma-glutamyl transpeptidase; PMNs, polymorphonuclear leukocytes; AA-861, 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone.

macia Fine Chemicals, Uppsala, Sweden. The calcium ionophore A23187 was from the Calbiochem-Behring Corp., La Jolla, CA, U.S.A. Auranofin (AF) (2,3,4,6-tetra-*O*-acethyl-1-thio-β-D-glucopyranosato-*S*-[triethyl phosphine]gold) was a gift from the Fujisawa Pharmaceutical Co., Osaka, Japan. AA-861 [2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone], a 5-lipoxygenase inhibitor [16], was donated by the Takeda Pharmaceutical Co., Osaka, Japan. Ionophore A23187 was dissolved in dimethyl sulfoxide (DMSO) at 1 mg/ml and stored at −20°. AF and AA-861 were freshly dissolved before use in ethanol at 20 mg/ml and in DMSO at 10 mg/ml respectively. [14,15-^{3}H]LTC_4 was from the New England Nuclear Corp., Boston, MA, U.S.A. Sep-Pak C18 cartridges were from Water Associates, Milford, MA, U.S.A. Gamma-glutamyl transpeptidase (γ-GTP) was from the Oriental East Co., Ltd., Tokyo, Japan. A Nucleosil C18 column (250 × 4.6 mm) and a TSK-ODS-80TM column (150 × 4.6 mm) (Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan were used as stationary phases of reverse phase high performance liquid chromatography (HPLC). All solvents used for mobile phases were of HPLC-grade purity.

Preparation of PMNs and homogenate of PMNs. PMNs were separated from heparinized peripheral blood by Ficoll/Paque centrifugation [17] and dextran sedimentation followed by hypotonic lysis of erythrocytes. These preparations were composed of neutrophils (93–96%), eosinophils (3–7%)and mononuclear cells (less than 2%). In the cellular system, PMNs were resuspended in Pipes buffer (119 mM NaCl, 5 mM KCl, 25 mM Pipes, 40 mM NaOH, 2 mM Ca^{2+} and 0.5 mM Mg^{2+}, pH 7.4) at 2×10^7/ml. In the cell-free systems, PMNs were resuspended in 20 mM potassium-phosphate buffer, pH 7.4, containing 0.15 M NaCl, 1 mM EDTA and bovine serum albumin (5 mg/ml) at 10^8/ml and sonicated at an output of 4 for 10 sec, ten times at 4° (ultrasonic vibrator UR-200P, Tomy Seiko Co., Tokyo, Japan). Then the suspension was centrifuged at 1000 *g* for 10 min at 4°. The supernatant fraction termed PMN homogenate, was used as the source of enzymes. In experiments where LTA_4 was used as a substrate, the pH of the PMN homogenates was adjusted to around pH 8 with 0.2 vol. of 50 mM Tris–HCl buffer, pH 8.0, to avoid rapid decomposition of LTA_4 in acidic solution [18].

Leukotriene formation in cellular and cell-free systems. In the cellular system, 500 μl of the cell suspension was preincubated with various concentrations of AF (1–8 μM) or AA-861 (1–4 μM) for 15 min at 37°. These treatments did not affect cell viability as evidenced by the trypan blue exclusion test. Then PMNs were stimulated with ionophore A23187 (0.5 μg/ml) at 37° under gentle shaking. The final concentrations of DMSO and ethanol were below 0.1%. After indicated periods, the cell suspensions were rapidly cooled on an ice bath and centrifuged at 500 *g* for 10 min at 4°. The supernatant fractions were transferred to another set of tubes containing 2 ml of methanol and a known amount of PGB_2 (internal standard) and stored at −20° until extraction. The cell pellets were resuspended in 500 μl of methanol with PGB_2, sonicated at an output of 4 for 10 sec at 4°, and stored at −20° overnight. In some experiments, [14,15-^{3}H]LTC_4 was added to the cell suspensions at the start of the stimulation to examine the effect of AF itself on the recovery of LTC_4.

In the cell-free systems, 100 μl of the standard reaction mixture contained 90 μl of PMN homogenate, 100 μM AA, 3 mM GSH, and 2 mM Ca^{2+} or 90 μl of PMN homogenate mixed with 0.2 vol. of Tris–HCl buffer, pH 8.0, 25–100 μM LTA_4 and 3 mM GSH. In the inhibition study, PMN homogenate was preincubated with various concentrations of AF (3–100 μM) for 15 min at 37°. Ca^{2+} and GSH were added 1 min prior to the start of the reaction. Then the reaction was initiated by the addition of 100 μM AA or 25 μM LTA_4, proceeded at 37° and terminated after indicated periods by the addition of 500 μl of methanol containing a known amount of PGB_2. The methanol solution was stored at −20° until extraction. The final concentrations of ethanol in the reaction mixture were below 0.5% in cases where AA was used and 2 to 2.5% in cases where LTA_4 was used.

Analysis of the products. Precipitated materials in the methanol solution were removed by centrifugation. Then the supernatant fractions were dried under N_2 stream, reconstituted in 200 μl of 30% (v/v) methanol, and centrifuged at 3000 *g* for 5 min at 4°. The clear supernatant fractions were analyzed by HPLC.

HPLC stationary phases were eluted with a solvent system of acetonitrile/methanol/0.5% (w/v) EDTA in water/acetic acid (3:1:3:0.006, by vol., pH 5.6) or acetonitrile/methanol/0.5% EDTA in water/acetic acid (3:1:4.7:0.008, by vol., pH 5.9) at 1 ml/min according to the method reported by Izumi *et al.* [19] or a slight modification. Eluted compounds were monitored continuously at 280 nm by a u.v. detector. Quantification of LTs was based on the comparison of their peak heights to those of synthetic standards, and the values were corrected for the recovery of PGB_2. When tritiated LTC_4 was involved in the reaction mixture, sequential aliquots of 1 ml were collected and assayed for radioactivity.

Identification of the products. Eluted peaks were collected and extracted using Sep-Pak C18 cartridges [20] mainly to remove EDTA. Eluted materials with methanol from Sep-pak were dried under a N_2 stream and resuspended in a small amount of phosphate-buffered saline. This solution was assayed for the contractile activity on guinea pig ileum [21] and for the aggregation of PMNs [12]. Conversion of LTC_4 to LTD_4 by γ-GTP was performed as described previously [22].

RESULTS

Time course of LT synthesis. Within 15 min after stimulation with ionophore A23187, the amount of LTC_4 reached the maximum level and that of LTB_4 decreased to 80 ± 4% (mean ± SE, N = 3) probably due to ω-oxidation [23]. The reaction period of 15 min was chosen to maximize LTC_4 synthesis. In the cell-free systems, the reaction periods employed were 1 min when LTA_4 was used and 5 min in the case of AA, during which periods the syntheses of

Table 1. Control values of LTs formed in cellular and cell-free systems without AF

	Cellular system		Cell-free system	
	LTs (pmol formed by 10^7 PMNs)		LTs (pmol/0.1 ml PMN homogenate)	
	Released	Retained	AA + GSH	LTA_4 + GSH
LTB_4	197 ± 4	53 ± 8	130 ± 20	82 ± 4
LTC_4	177 ± 14	41 ± 6	80 ± 5	79 ± 7

In the cellular system, PMNs were stimulated with ionophore A23187 (0.5 μg/ml) for 15 min, and both extracellularly released and intracellularly retained LTs were quantified by HPLC. In the cell-free system, PMN homogenates were incubated with 100 μM AA and 3 mM GSH for 5 min or with 25 μM LTA_4 and 3 mM GSH for 1 min. Produced LTs were quantified by HPLC. Each value is the mean ± SE, N = 3.

LTB_4 and LTC_4 were both linear functions of time (data not shown). Control values of LTs formed without AF are summarized in Table 1. In the cellular system, about 20% of LTs were retained in cells. Both LTD_4 and LTE_4 were below measurable limits under the present assay conditions.

Identification of the products. Eluted compounds were first identified by co-elution with synthetic standards. Then peaks corresponding to LTs were collected, extracted, and assayed biologically. The peak corresponding to LTB_4 contained materials showing aggregating activity on PMNs (data not shown) and that corresponding to LTC_4 contained materials possessing FPL55712-antagonized contractile activity on guinea pig ileum (Fig. 1a). Furthermore, the peak corresponding to LTC_4 was totally converted to that corresponding to LTD_4 by γ-GTP (Fig. 1b).

Differential inhibitory effects of AF and AA-861

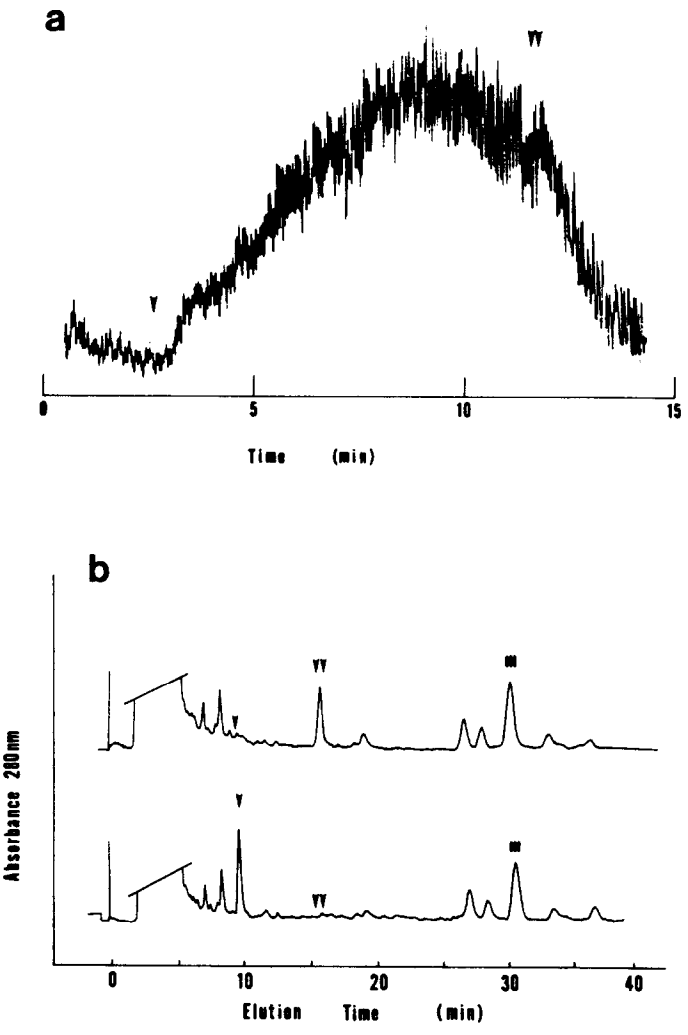

Fig. 1. (a) FPL55712-antagonized contraction of guinea pig ileum by the extract of the peak that corresponded to LTC_4. The additions of the extract and FPL55712 are indicated by ▼ and ▼▼ respectively. (b) Peak-shift by γ-GTP. The peak corresponding to LTC_4 (▼, in the lower chart) was totally converted to that corresponding to LTD_4 (▼▼, in the upper chart). Peak III indicates the peak corresponding to LTB_4.

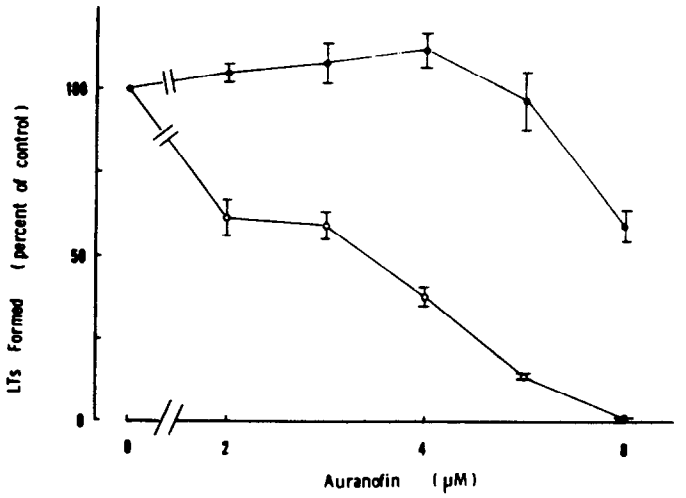

Fig. 2. Inhibitory effects of AF on LT formation by intact PMNs. PMNs were preincubated with various concentrations of AF for 15 min at 37°. Then ionophore A23187 (0.5 μg/ml) was added, and the incubation was continued for another 15 min. LTB_4 (●) and LTC_4 (○) released from PMNs were quantified by HPLC. Data are expressed as percent of control without AF (mean ± SE, N = 3).

on LTB_4 and LTC_4 formation by intact PMNs. Figure 2 shows dose-dependent effects of AF on LT formation by ionophore A23187-stimulated PMNs. AF reduced the amount of extracellularly released LTC_4 in a dose-dependent manner with an IC_{50} of 3.2 μM. On the other hand, the amount of LTB_4 was not affected by AF at concentrations up to 6 μM, and it was reduced to 59 ± 4% (mean ± SE, N = 3) of control at 8 μM. The amounts of LTs retained in cells were not influenced by AF (data not shown). AA-861; a 5-lipoxygenase inhibitor, produced similar differential inhibitory effects on the formation of LTs by ionophore A23187-stimulated PMNs. The IC_{50} values for LTC_4 synthesis and LTB_4 synthesis were 1.2 and 4.4 μM respectively (Fig. 3).

In aqueous solution without EDTA, LTC_4 has been reported to suffer from decomposition by metal [24] and to act as a chelator for itself [25]. Both decomposition and chelating action of LTC_4 may result in a reduction of measurable LTC_4 and mimic reduced LTC_4 formation. However, this explanation seems unlikely, for the recovery of tritiated LTC_4 from samples treated with AF was almost the same as that from samples not treated with this gold compound (Fig. 4).

Effects of AF on LTB_4 and LTC_4 synthesis by homogenate of PMNs. AF inhibited LTC_4 synthesis from AA and GSH more effectively than it did LTB_4 synthesis in the cell-free system. The IC_{50} for LTB_4 synthesis was seven times as high as that for LTC_4 synthesis (100 μM vs 14 μM) (Fig. 5). When LTA_4 and GSH were used as substrates, AF at concentrations below 30 μM affected LT synthesis only

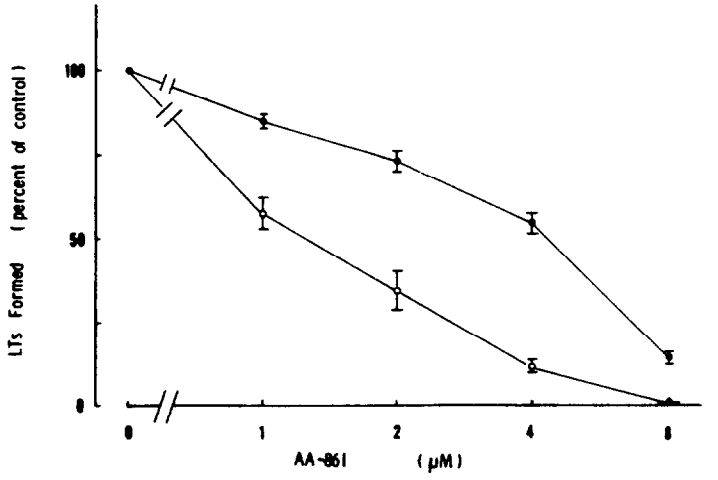

Fig. 3. Inhibitory effects of AA-861 on LT formation by intact PMNs. Experimental conditions were essentially identical to those stated in the legend of Fig. 2 except that AA-861 was used instead of AF. Key: (●) LTB_4 and (○) LTC_4. Data are expressed as percent of control without AA-861 (mean ± SE, N = 3).

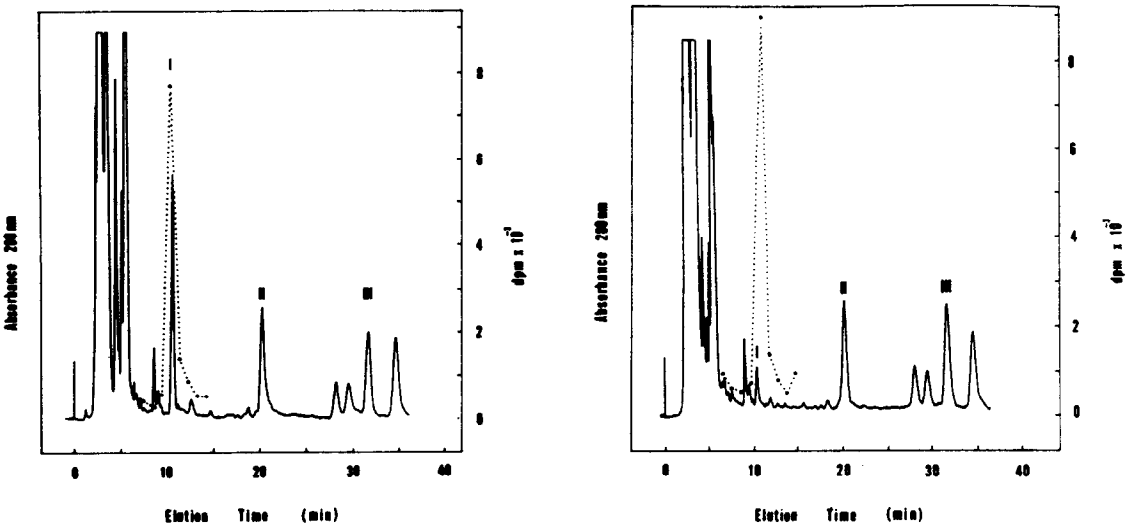

Fig. 4. Lack of significant decomposition of LTC_4 by AF. PMNs not treated (left panel) or treated (right panel) with 6 μM AF were stimulated wtih ionophore A23187 (0.5 μg/ml) for 15 min at 37°. Tritiated LTC_4 was added at the start of the stimulation. Released LTs were analyzed by HPLC (Nucleosil C18 column, 250 × 4.6 mm), using a solvent system of acetonitrile/methanol/0.5% (w/v) EDTA in water/acetic acid, 3:1:4.7:0.008, pH 5.9, at 1 ml/min. Sequential aliquots of 1 ml were assayed for radioactivity. The solid line represents u.v. absorbance at 280 nm (peak I, LTC_4; peak II, PGB_2; and peak III, LTB_4). The dotted line represents radioactivity (dpm). Generated LTC_4 was reduced significantly by AF, whereas the recoveries of tritiated LTC_4 were almost the same.

slightly. AF at 100 μM reduced LTC_4 synthesis to 82 ± 8% (mean ± SE, N = 3) of control, whereas it increased LTB_4 synthesis to 113 ± 9% (mean ± SE, N = 3) of control (Fig. 6).

Dependency of LTB_4 and LTC_4 syntheses on LTA_4 concentrations. Dependency of LTB_4 and LTC_4 syntheses on LTA_4 concentrations was examined. As shown in Fig. 7, LTC_4 synthesis was more dependent on LTA_4 concentration than LTB_4 synthesis within the concentrations tested (25–100 μM). LTC_4 formed from 25 μM LTA_4 was 38 ± 2% (mean ± SE, N = 3) of that from 100 μM LTA_4, whereas LTB_4 from 25 μM LTA_4 was 71 ± 2% (mean ± SE, N = 3) of that from 100 μM LTA_4.

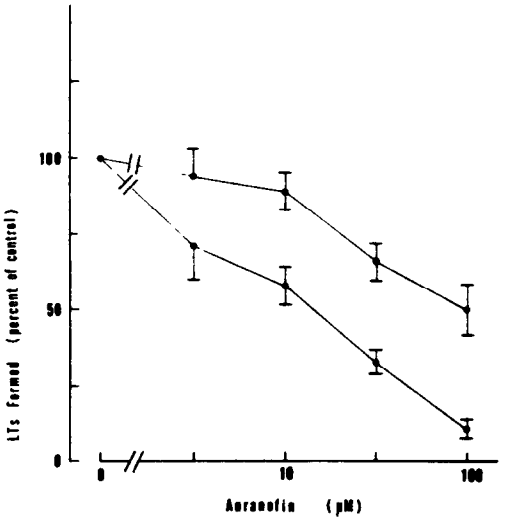

Fig. 5.Inhibitory effects of AF on LT synthesis from AA and GSH by PMN homogenate. PMN homogenates were preincubated with various concentrations of AF for 15 min at 37°. Substrates were added, and the reaction was continued for another 5 min. LTB_4 (●) and LTC_4 (○) were quantified by HPLC. Data are expressed as percent of control without AF (means ± SE, N = 4).

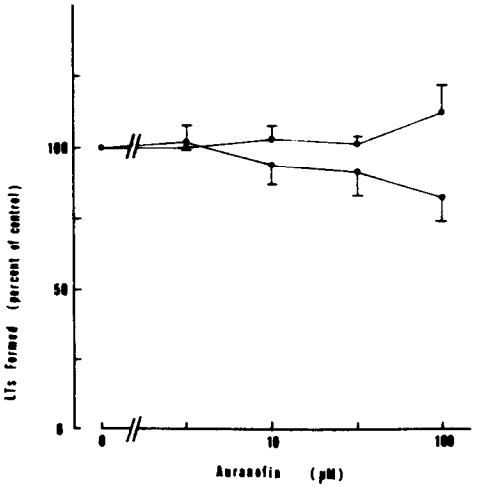

Fig. 6. Effects of AF on LT synthesis from LTA_4 and GSH by PMN homogenate. PMN homogenates were preincubated with various concentrations of AF for 15 min at 37°. Substrates were added, and the reaction was continued for another 1 min. LTB_4 (●) and LTC_4 (○) were quantified by HPLC. Data are expressed as percent of control without AF (mean ± SE, N = 3).

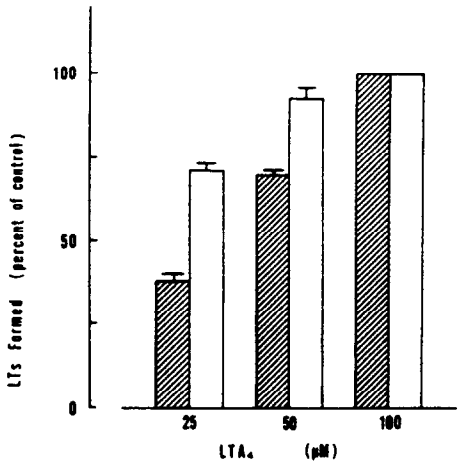

Fig. 7. Dependency of LT synthesis on LTA_4 concentrations. PMN homogenates were incubated with LTA_4 (25–100 μM) and 3 mM GSH for 1 min at 37°. LTB_4 (open bars) and LTC_4 (hatched bars) were quantified by HPLC. LTs derived from 100 μM LTA_4 were defined as control. Data are expressed as percent of control (mean ± SE, N = 3).

DISCUSSION

AF exerted differential effects on the formation of LTB_4 and LTC_4 by ionophore A23187-stimulated PMNs. The amount of released LTC_4 was reduced to 50% of control by AF at 3.2 μM. This concentration of gold can be achieved in the plasma of patients receiving AF at a recommended dose (6 mg daily) for 21 weeks [26]. On the other hand, AF inhibited LTB_4 formation less effectively than it did LTC_4 formation (Fig. 2). The lack of the inhibitory effect of AF on LTB_4 formation at physiological concentrations was observed previously by Hafström *et al.* [27]. In their study, however, LTC_4 was not measured. Very recently, AF was found to inhibit the release of immunoreactive LTC_4 from human basophils and mast cells stimulated with anti-IgE with an IC_{50} of around 1 μM [15]. These findings are almost consistent with our observations.

Williams *et al.* [28] reported that some portion of LTB_4 was retained in cells and that the proportion of LTB_4 released was dependent on the nature of the stimuli. Therefore, the reduced LT release could be due to the increased retention of LTs. This possibility, however, is, unlikely because the amounts of LTs retained in cells were not influenced by AF. Decomposition by AF or chelating action of LTC_4 did not occur under the experimental conditions. Thus, the reduction of LT release from AF-treated cells was thought to be attributable to the inhibition of LT synthesis.

Next we studied the mechanisms of action of AF in inhibiting LT synthesis using cell-free systems. AF inhibited LTC_4 formation more effectively than LTB_4 formation from AA and GSH (Fig. 5). This result is very similar to that obtained in the cellular system, although the IC_{50} value for LTC_4 formation in the cell-free system was higher than that in the cellular system (14 μM vs 3.2 μM). This discrepancy was thought to be due to different conditions between the two systems, that is, in the cell-free system AA was supplied exogenously, whereas in the cellular system AA was supplied endogenously by means of phospholipases. Furthermore, in the cell-free system, EDTA and GSH were contained in the reaction mixture and these compounds could possibly bind to AF, modifying its effects [29]. On the other hand, AF scarcely affected LT synthesis from LTA_4 and GSH (Fig. 6).

These results in the cell-free systems indicated that AF inhibited LTB_4 and LTC_4 formation by blocking LTA_4 synthesis from AA by 5-lipoxygenase [30] and that the preferential reduction of LTC_4 was ascribed to a higher K_m value of glutathione *S*-transferase for LTA_4 than that of LTA_4 hydrolase in PMNs. Söderström *et al.* have reported that three types of human liver cytosolic glutathione *S*-transferases are able to catalyze LTA_4 conversion and that their K_m values for LTA_4 range from 130 to 190 μM. Bach *et al.* [32] have also demonstrated that the apparent K_m of particulate glutathione *S*-transferase in crude homogenates of rat basophilic leukemia cells is 210 μM. In either case, these values are about six to nine times as high as that of LTA_4 hydrolase in human PMNs [18]. As shown in Fig. 7, LTC_4 formation was actually more dependent on LTA_4 concentrations than LTB_4 formation within the concentrations tested (25–100 μM). Furthermore, AA-861, a known 5-lipoxygenase inhibitor [16], caused similar differential inhibitory effects on the synthesis of LTs (Fig. 3). These results strongly support the explanation. Alternatively, AF may decrease the stability of LTA_4 or it may act on phospholipases as well as on 5-lipoxygenase. These possibilities could not be excluded in this study. In addition, calcium ionophore bypasses physiological transmembranous events that lead to calcium mobilization and subsequent cell activation. Thus, we could not evaluate the effects of AF on those events. These problems are now under investigation in our laboratory.

In clinical aspects, pharmacological prevention of LT synthesis is considered to be desirable in treating allergic and inflammatory disorders in view of a diversity of actions of these compounds [2]. LTC_4 has a potent contracting activity on smooth muscles and has a stimulating effect on mucous secretion [1]. These potencies directly link to the pathogenesis of bronchial asthma. Furthermore, LTC_4 increases vascular permeability and subsequently causes exudation of macromolecules into extravascular space [8], which is essential to local inflammation. Therefore, the inhibitory effect of AF on LTC_4 synthesis at physiological concentrations may contribute to its clinical efficacy.

In conclusion, we demonstrated that AF, a clinically useful oral gold compound, had differential inhibitory effects on the formation of LTB_4 and LTC_4 by PMNs stimulated with ionophore A23187. LTC_4 formation was inhibited by physiological concentrations of AF, whereas the inhibition of LTB_4 formation required rather high concentrations of this compound. The mode of action was thought to be reduced LTA_4 formation and subsequent preferential decrease of LTC_4 formation. This inhibitory effect of AF may play some role in the clinical

efficacy of this drug, and it is worthwhile to note that a reduction of LTA_4, under some conditions, brings about differential effects on the synthesis of biologically active LTs.

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REFERENCES

1. B. Samuelsson, *Science* **220**, 568 (1983).
2. M. K. Bach, *Biochem. Pharmac.* **33**, 515 (1984).
3. The Cooperative Clinics Committee of the ARA, *Arthritis Rheum.* **16**, 353 (1973).
4. M. Muranaka, T. Miyamoto, T. Shida, J. Kabe, S. Makino, H. Okudaira, T. Takeda, S. Suzuki and Y. Horiuchi, *Ann. Allergy* **40**, 132 (1978).
5. F. Roismann and A. Misher, *Ann. rheum. Dis.* **35**, 251 (1976).
6. F. E. Berglöf, K. Berglöf and D. T. Walz, *J. Rheumatol.* **5**, 68 (1978).
7. I. Hafström, A. M. Uden and J. Palmblad, *Scand. J. Rheumatol.* **12**, 97 (1983).
8. M. J. Dimartino and D. T. Walz, *Inflammation* **2**, 131 (1977).
9. T. Takaishi, Y. Morita, K. Kudo and T. Miyamoto, *J. Allergy* **74**, 296 (1984).
10. P. Davis, C. Johnston, C. L. Miller and K. Wong. *Arthritis Rheum.* **26**, 82 (1983).
11. I. Hafström, B. E. Seligmann, M. M. Friedman and J. I. Gallin, *J. Immun.* **132**, 2007 (1984).
12. C. N. Serhan, A. Radin, J. E. Smolen, H. Korchak, B. Samuelsson and G. Weissmann, *Biochem, biophys, Res. Commun.* **107**, 1006 (1982).
13. S. P. Peters, A. Kagey-Sobotka, D. W. Macglashan, Jr., M. I. Siegel and L. M. Lichtenstein, *J. Immun.* **129**, 797 (1982).
14. J. C. Gay, J. K. Beckmann, A. R. Brash, J. A. Oates and J. N. Lukens, *Blood* **64**, 780 (1984).
15. G. Marone, M. Columbo, D. Galeone, G. Guidi, A. Kagey-Sobotka and L. M. L. Lichtenstein, *Agents Actions* **18**, 101 (1986).
16. T. Yoshimoto, C. Yokoyama, K. Ochi, S. Yamamoto, Y. Maki, Y. Ashida, S. Terao and M. Shiraishi, *Biochim. biophys. Acta* **713**, 470 (1982).
17. A. Böyum, *Scand. J. clin. lab. Invest.* **21** (Suppl. 97), 77 (1968).
18 O. Rådmark, T. Shimizu, H. Jörnvall and B. Samuelsson, *J. biol. Chem.* **259**, 12339 (1984).
19. T. Izumi, T. Shimizu, Y. Seyama, N. Oishi and F. Takaku, *Biochem. biophys. Res. Commun.* **135**, 139 (1986).
20. S. A. Metz, M. E. Hall, T. W. Harper and R. C. Murphy, *J. Chromat.* **233**, 193 (1982).
21. R. P. Orange and K. F. Austen, *Adv. Immunol.* **10**, 105 (1968).
22. L. Örning and S. Hammarström, *J. biol. Chem.* **255**, 8023 (1980).
23. G. Hansson, J. Å. Lindgren, S.-E. Dahlén, P. Hedqvist and B. Samuelsson, *Fedn Eur. Biochem. Soc. Lett.* **130**, 107 (1981).
24. J. Y. Westcott, K. L. Klay and R. C. Murphy, *J. Allergy* **74** (Suppl.), 363 (1983).
25. F. S. Anderson, J. Y. Westcott, J. A. Zirrolli and R. C. Murphy, *Analyt. Chem.* **55**, 1837 (1983).
26. S. L. Dahl, M. L. Coleman, H. J. Williams, M. Altz-Smith, D. R. Cay, H. E. Paulus, A. Weinstein and S. Kaplan, *Arthritis Rheum.* **28**, 1211 (1985).
27. I. Hafström, B. Ringertz, J. Palmblad and C. Malmsten, *Agents Actions* **15**, 551 (1984).
28. J. D. Williams, T. H. Lee, R. A. Lewis and F. Austen, *J. Immun.* **134**, 2624 (1985).
29. K. L. N. Bloka and E. M. Landaw, in *Current Clinical Practice* Series 7 (Eds. H. A. Capell, D. S. Cole, K. K. Manghaim and R. W. Morris), p. 32. Excerpta Medica, Amsterdam (1982).
30. T. Shimizu, T. Izumi, Y. Seyama, K. Tadokoro, O. Rådmark and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **83**, 4175 (1986).
31. M. Söderström, B. Mannervik, L. Örning and S. Hammarström, *Biochem. biophys. Res. Commun.* **128**, 265 (1985).
32. M. K. Bach, J. R. Brashler and D. R. Mortonnn, Jr., *Archs Biochem. Biophys.* **230**, 455 (1984).