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INHIBITION OF ALVEOLAR MACROPHAGE 5-LIPOXYGENASE METABOLISM BY AURANOFIN*

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Abstract—We have examined the effect of the oral gold compound auranofin (AF) on calcium ionophore A23187-induced arachidonic acid metabolism in the rat alveolar macrophage. Both reverse-phase high performance liquid chromatographic and radioimmunoassay analyses revealed that AF dose-dependently inhibited leukotriene B_4 and 5-hydroxyeicosatetraenoic acid synthesis in a parallel fashion with an $IC_{50} \sim 4.3\ \mu g/ml$. At the same time, AF augmented A23187-induced arachidonate release and cyclooxygenase metabolism. A possible mechanism for the inhibition of 5-lipoxygenase was suggested by the capacity of AF to dose-dependently deplete ATP ($IC_{50} \sim 5.9\ \mu g/ml$), a cofactor for 5-lipoxygenase. These data indicate that, at therapeutic concentrations, AF acts *in vitro* as a selective inhibitor of macrophage 5-lipoxygenase metabolism. This likely represents an important mechanism of action of AF in chronic inflammatory disorders.

Chrysotherapy has well-established efficacy in the treatment of rheumatoid arthritis [1]. In addition, gold salts have been shown to attenuate airway responsiveness in animals [2] as well as humans [3], and to decrease symptom scores and medication requirements in patients with asthma [3, 4]. It is generally recognized that chronic inflammation plays a prominent role in the pathogenesis of both rheumatoid arthritis [5] and asthma [6], and prominent among the many potential mediators of inflammation which have been suggested to be important in these conditions are the eicosanoids, a group of oxygenated derivatives of arachidonic acid (AA) [7]. Recent studies have demonstrated that auranofin (AF), an oral gold compound, inhibits the synthesis of leukotrienes, potent pro-inflammatory 5-lipoxygenase metabolites of AA, in human blood neutrophils [8, 9], basophils [10], and lung mast cells [10].

Although the macrophage is thought to play a central role in chronic inflammatory disorders [11], has the capacity to release relatively large amounts of cyclooxygenase and 5-lipoxygenase eicosanoids [12], and is a putative target cell for the anti-inflammatory effects of gold [13], we are aware of no information on the modulation by gold compounds of macrophage-derived eicosanoid synthesis. We therefore examined the effect of AF on calcium ionophore A23187-induced AA metabolism in the rat alveolar macrophage (AM), a cell which produces large amounts of 5-lipoxygenase metabolites including both leukotrienes B_4 (LTB_4) and C_4 (LTC_4) [14]. Our results indicated that AF inhibits AM 5-lipoxygenase metabolism at therapeutic concentrations, and they suggest that the mechanism of this inhibitory action may involve the depletion of cellular ATP, a cofactor for the 5-lipoxygenase enzyme [15, 16].

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METHODS

Macrophage isolation and culture. Respiratory disease-free 125–150 g female Wistar rats were obtained from Charles River (Portage, MI) and housed under specific pathogen-free conditions. Following anesthesia with intraperitoneal sodium pentobarbital, lungs were surgically excised and lavaged as previously described [17]. Lavage fluid, as well as Hanks' balanced salt solution (HBSS) (GIBCO, Grand Island, NY) and medium 199 with modified Earle's salts (M199) (GIBCO) all contained 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 mg/ml amphotericin B (Antibiotic-Antimycotic Solution, Sigma Chemical Co., St. Louis, MO). Cells (2×10^6) suspended in 1.5 ml of M199 were plated in 35 × 10 mm plastic culture dishes (Falcon Plastics, Oxnard, CA) and cultured at 37° in a humidified atmosphere of 5% CO_2 in air. After 1 hr, non-adherent cells were removed by washing twice with HBSS. The resultant adherent cell population has been found to contain 95% AM by morphologic criteria and esterase staining [17] with viability exceeding 90% as assessed by trypan blue exclusion. Macrophage monolayers were then cultured overnight (16 hr) in M199 containing 10% heat-inactivated newborn calf serum (NCS) (GIBCO) in the presence or absence of radiolabeled AA prior to experimental incubations. Following overnight culture, these monolayers have been found to contain approximately 8.5 μg DNA [17] and 100 μg protein [14].

Prelabeling of macrophage cultures. In selected experiments, cellular lipids were prelabeled by including 0.2 μCi of [1-^{14}C]AA (sp. act. 54–57 mCi/mmol, Dupont-New England Nuclear, Boston, MA) in the medium during overnight culture. To remove unincorporated label, cells were washed with HBSS, incubated for an additional hour with M199 containing 10% NCS, and washed again prior to experimental incubations. The uptake of radiolabel by macrophage cultures, determined as described previously [17], was routinely 35–40%.

Incubations with A23187 and AF. Auranofin, provided by Smith Kline & French Laboratories (Philadelphia, PA), was prepared as a stock solution at 20 mg/ml in DMSO, and diluted in M199 to final desired concentrations. Following overnight incubation, duplicate cultures of labeled or unlabeled AM were washed twice with HBSS and preincubated for 15 min with 1 ml of M199 alone or M199 containing AF at concentrations ranging from 0.5 to 100 μg/ml. Then calcium ionophore A23187 (Calbiochem-Behring Corp., LaJolla, CA), 10 μM, was added and the incubation continued for an additional 30 min. The final concentration of DMSO in all cultures was 0.5%, which did not affect cell viability or eicosanoid synthesis.

Eicosanoid extractions. Eicosanoids were extracted from culture medium using Sep-pak C_{18} cartridges (Waters Associates, Milford, MA) according to the method of Westcott *et al.* [18]. Methanol/water (80:20, v/v) eluates containing eicosanoids were dried under nitrogen and stored at −70°. Recoveries for this extraction procedure, assessed using tritiated standards (Dupont-New England Nuclear) added to M199, were 65–70% for LTB_4, LTC_4, and thromboxane B_2 (TxB_2, the stable breakdown product of TxA_2) [19].

Radioimmunoassays. LTB_4, LTC_4, and TxB_2 in medium from unlabeled AM cultures were quantitated by radioimmunoassays (RIA) performed by the Ligand Core Laboratory of the University of Michigan Diabetes Research and Training Center. Dried lipid extracts were dissolved in 1 ml of phosphate-buffered saline containing 0.1% gelatin (pH 7.4), and 100-μl aliquots were assayed in duplicate for each sample. The antibody sources and cross-reactivities, and the assay sensitivities for these RIA have been described previously [14]. The specificities of the RIA have been confirmed by analyses utilizing reverse-phase high performance liquid chromatography (RP-HPLC) [20, 21]. In all cases, quantities of immunoreactive eicosanoids reported were corrected for recovery.

Eicosanoid separation by reverse-phase high performance liquid chromatography. For separation of [^{14}C]AA metabolites produced by prelabeled AM, lipid extracts of pooled medium from duplicate cultures were dissolved in 500 μl of acetonitrile/water/trifluoroacetic acid (33:67:0.1, by vol.) and subjected to RP-HPLC using a Waters HPLC system equipped with a Waters 5 μm Bondapak C_{18} column (30 × 0.4 cm) eluted with acetonitrile/water/trifluoroacetic acid at 1 ml/min, as previously described [20]. Using this system, cyclooxygenase metabolites are eluted during an initial isocratic phase (33:67:0.1, by vol.), followed by lipoxygenase metabolites and free AA, which elute during a stepwise gradient increase of acetonitrile to 100:0:0.1 (by vol.). The eluate was monitored continuously for UV absorbance [210 nm for cyclooxygenase products and free AA, 280 nm for LTs, and 235 nm for monohydroxyeicosatetraenoic acids (HETEs)]. Authentic TxB_2, prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ were gifts of Dr. J. Pike (Upjohn Co., Kalamazoo, MI), and lipoxygenase standards LTB_4, LTC_4, 5-HETE, 12-HETE, and 15-HETE of Dr. J. Rokach (Merck Frosst, Inc., Quebec, Canada). Authentic 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) was obtained from the Cayman Chemical Co. (Ann Arbor, MI), and arachidonic acid from Nu-Chek Prep, Inc. (Elysian, MN). Eluate fractions of 1 ml were collected, and radioactivity was quantitated in 6 ml of ACS scintillant (Amersham, Arlington Heights, IL) using a Beckman LS1801 scintillation counter (Beckman Instruments, Inc., Fullerton, CA) with a counting efficiency for ^{14}C of approximately 75%. Radiolabeled eicosanoids were identified by their co-elution with authentic standards.

Adenosine triphosphate assay. Cellular ATP was determined by the luciferase–luciferin assay [22], as previously described [19]. Briefly, after experimental incubation, culture medium was removed and monolayers were scraped with a rubber policeman into iced 10 mM potassium phosphate, 4 mM $MgSO_4$ buffer, pH 7.4. Cell suspensions were placed in a 90–95° water bath for 4 min, and then on ice until assay, within 4 hr. At the time of assay, an aliquot of cell suspension was added to 50 mM Na_2HAsO_4, 20 mM $MgSO_4$ buffer, pH 7.4, in a glass scintillation vial. Fifty microliters of luciferase–luciferin (Sigma), reconstituted in sterile glycine buffer, was added to the assay mixture, and light emission was quantitated immediately in a Beckman LS1801 counter using the single photon monitor mode. Standard curves of log cpm versus log [ATP] were linear over the range 10^{-9} to 10^{-6} M ATP.

Glutathione assay. Monolayers were deproteinated with 0.7 M perchloric acid, neutralized with 2 M potassium hydroxide, and intracellular total (reduced plus oxidized) glutathione was quantitated by the glutathione reductase assay [23], recording the change in absorbance at 412 nm over time with a Beckman model 35 spectrophotometer. The details of the cellular processing and of the assay itself were as previously described [14].

Data analysis. All data are expressed as means ± SE. In all cases, immunoreactive eicosanoid levels in medium and cellular ATP and glutathione contents were determined in duplicate culture plates for each experimental condition and the average calculated to yield a single data point. The effect of AF on A23187-stimulated eicosanoid synthesis is expressed as the percent of the eicosanoid level found in cultures stimulated with A23187 alone, after correction for control levels of production. Similarly, the effects of AF on cellular content of ATP and glutathione are expressed as the percent of the content of cells incubated with A23187 alone.

RESULTS

A23187-induced AA metabolism. Figure 1A depicts a representative [^{14}C]eicosanoid RP-HPLC elution profile from AM cultures incubated for 30 min with 10 μM A23187 alone. The major peaks of radioactivity co-eluted with authentic standards

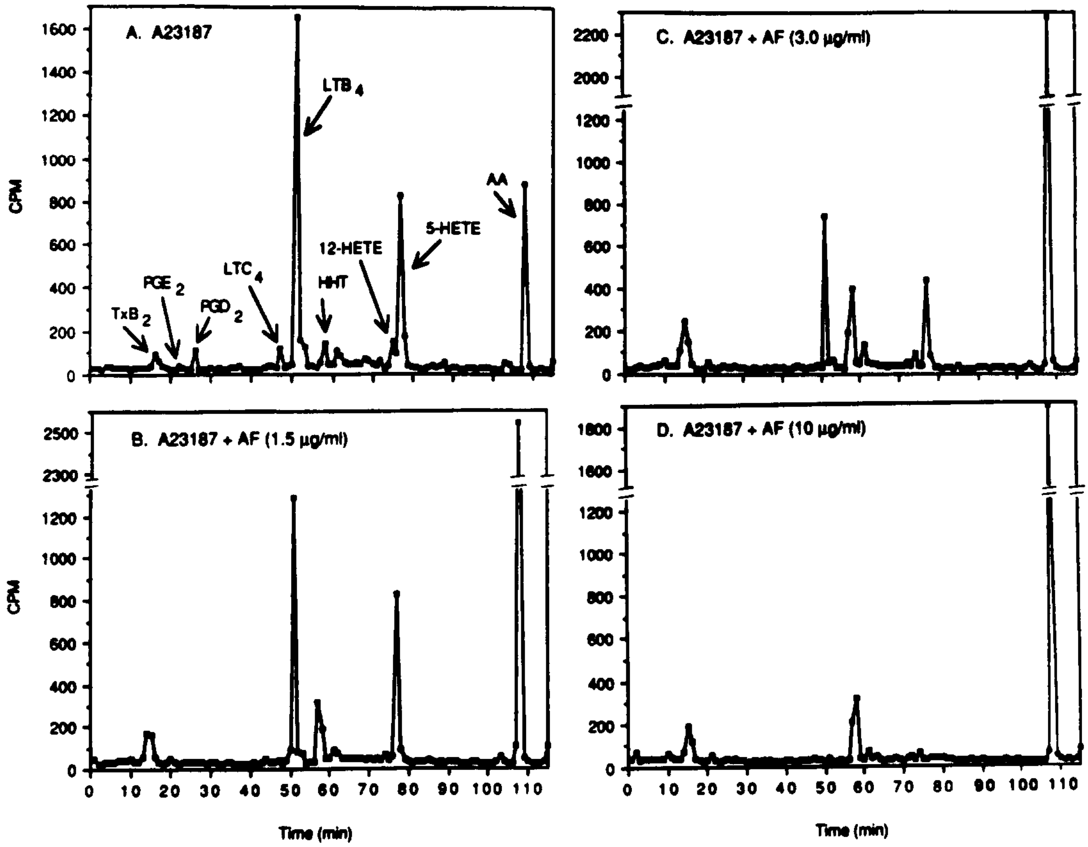

Fig. 1. RP-HPLC analysis of radiolabeled eicosanoids in culture medium from [^{14}C]AA-prelabeled AM. Macrophages were incubated for 30 min with 10 μM A23187 alone (A), or A23187 in the presence of AF at 1.5 μg/ml (B), 3 μg/ml (C), or 10 μg/ml (D). Retention times of authentic standards are indicated by arrows.

Table 1. Immunoreactive eicosanoid synthesis by unstimulated and A23187-stimulated alveolar macrophages in the absence of AF

	Eicosanoid (ng/plate)		
	LTC_4	LTB_4	TxB_2
Control	0.29 ± 0.01	0.10 ± 0.02	0.52 ± 0.09
A23187	11.30 ± 4.33	41.90 ± 7.02	1.95 ± 0.03

Alveolar macrophages (2 × 10^6) were incubated for 30 min in the presence or absence of 10 μM A23187, and eicosanoids were quantitated in medium by RIA. Each value is the mean ± SE of three individual experiments, each performed in duplicate.

for LTB_4, 5-HETE, and free AA. Additional smaller peaks co-eluted with the lipoxygenase products LTC_4 and 12-HETE, as well as the cyclooxygenase metabolites TxB_2, PGE_2, PGD_2, and HHT. The eicosanoids LTC_4, LTB_4, and TxB_2 were quantitated in unstimulated and A23187-stimulated cultures by RIA, and the mean values (N = 3) are summarized in Table 1.

Effects of AF on A23187-induced AA metabolism. The effects of 1.5, 3, and 10 μg/ml AF on A23187-induced macrophage AA metabolism are shown in the radioactivity RP-HPLC elution profiles depicted in Fig. 1, panels B, C, and D respectively. Ionophore-induced LTC_4 synthesis was eliminated at 1.5 μg/ml AF, while A23187-induced LTB_4 and 5-HETE synthesis were inhibited in parallel fashion by AF over the concentration range 1.5 to 10 μg/ml. In contrast, at all doses tested, AF augmented A23187-induced release of free AA as well as the cyclooxygenase products TxB_2 and HHT. That this increase in radioactivity in AA, TxB_2, and HHT represents augmented phospholipase activity and not merely a shunting of [^{14}C]arachidonate away from the 5-lipoxygenase pathway is suggested by the greater total release of radiolabeled AA plus metabolites in cultures containing AF.

To assess in a more quantitative fashion the effects of AF on AM arachidonate metabolism, unlabeled cultures were incubated with 10 μM A23187 in the presence and absence of various concentrations of AF and eicosanoids measured by RIA. As shown in Fig. 2, AF dose-dependently inhibited LTC_4 ($IC_{50} \sim 1$ μg/ml, >90% inhibition at 5 μg/ml) with greater potency than LTB_4 ($IC_{50} \sim 4.3$ μg/ml, >90% inhibition at 10 μg/ml). AF dose-dependently augmented A23187-induced TxB_2 synthesis by as much as 5-fold over the same concentration range at which it inhibited LT synthesis (Fig. 2). Thus, both RP-

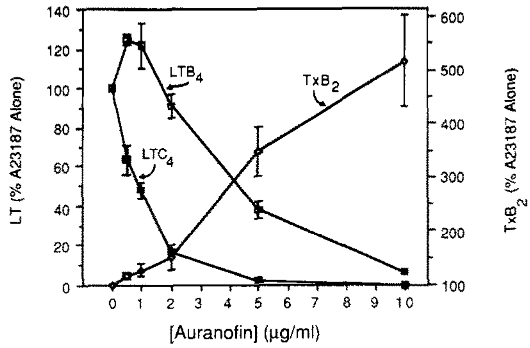

Fig. 2. Effect of AF concentration on A23187-induced synthesis of immunoreactive eicosanoids. Alveolar macrophages were incubated for 30 min with 10 μM A23187 either alone or in the presence of various concentrations of AF. Immunoreactive LTB_4, LTC_4, and TxB_2 were quantitated in culture medium, and the data are expressed as the percent of the quantities produced by A23187-stimulated cultures alone. Each point represents the mean ± SE from duplicate determinations in three independent experiments.

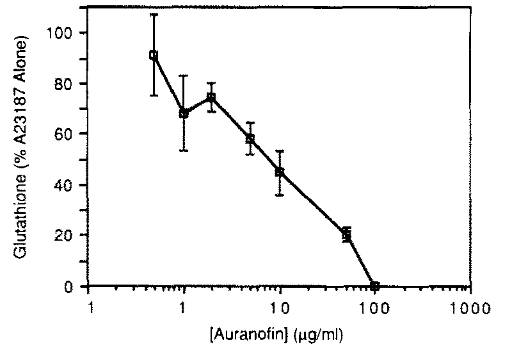

Fig. 4. Effect of AF concentration on intracellular glutathione content in AM incubated with A23187. Cultures were incubated for 30 min with 10 μM A23187 alone or in the presence of AF at the indicated concentrations. Total glutathione content was determined by the glutathione reductase method, and results are expressed as the percent of the value determined for cultures incubated with A23187 alone. Data represent the mean values ± SE from duplicate determinations in three independent experiments.

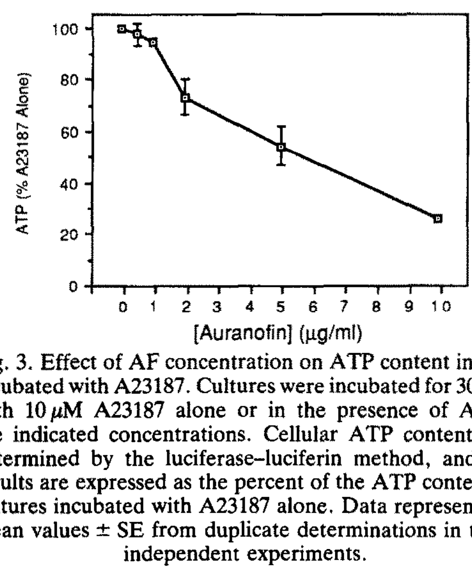

Fig. 3. Effect of AF concentration on ATP content in AM incubated with A23187. Cultures were incubated for 30 min with 10 μM A23187 alone or in the presence of AF at the indicated concentrations. Cellular ATP content was determined by the luciferase–luciferin method, and the results are expressed as the percent of the ATP content of cultures incubated with A23187 alone. Data represent the mean values ± SE from duplicate determinations in three independent experiments.

HPLC and RIA experiments demonstrated that AF: (1) inhibited LT synthesis while augmenting AA release and cyclooxygenase metabolism; and (2) inhibited the synthesis of LTC_4 with greater potency than other 5-lipoxygenase eicosanoids.

Effects of AF on macrophage ATP content. Using the luciferase/luciferin method, we determined the ATP content of cells following 30 min of incubation with 10 μM A23187 in the presence and absence of various concentrations of AF. The effects of AF on cellular ATP levels were determined relative to the ATP content of AM cultures containing A23187 alone both to duplicate the conditions under which eicosanoid levels were evaluated and because we have observed previously an independent ATP-depleting effect for A23187 [19]. In the current study, the ATP content of AM cultures incubated with A23187 alone in the absence of AF was 269 ± 77 pmol/plate (N = 3). As shown in Fig. 3, AF effected a dose-dependent depletion of cellular ATP, with an $IC_{50} \sim 5.9$ μg/ml. To evaluate the potential for AF to interfere with the luciferase/luciferin assay itself, different concentrations of AF were added directly to the assay buffer and ATP standard curves were generated. At final AF concentrations ≤ 10 μg/ml the gold compound had no effects on the ATP assay. Substantial interference with the measurement of ATP was observed at final AF concentrations > 10 μg/ml, however (data not shown).

Effects of AF on macrophage glutathione content. To examine the possibility that the preferential inhibition by AF of LTC_4 synthesis, as compared to LTB_4 synthesis, was related to its ability to conjugate reduced glutathione, thereby decreasing the amount available for LTC_4 synthesis [24], we measured total glutathione levels in AM cultures. The glutathione content following 30 min of incubation with A23187 in the absence of AF was 369 ± 80 pmol/plate (N = 3). Auranofin exposure depleted cellular glutathione in a dose-dependent fashion, albeit with a potency far less than that observed for the inhibition of LTC_4 synthesis by AF; the IC_{50} for glutathione was ~ 8 μg/ml, and 90% depletion was achieved at an AF concentration between 50 and 100 μg/ml (N = 3) (Fig. 4).

DISCUSSION

Although other investigators have examined the effects of AF on LT synthesis in several different cell types [8–10], no information is available regarding its effects on the synthesis of LT and other oxygenated metabolites of AA by macrophages. Yet this issue is particularly germane to understanding the anti-inflammatory actions of AF, for the following reasons: (1) macrophages are thought to play a key role in the pathogenesis of chronic inflammatory disorders such as rheumatoid arthritis and asthma

[11]; (2) the macrophage is generally regarded as an important target cell for the effects of chrysotherapy [13]; and (3) as compared to most other inflammatory cells, including the neutrophil, the macrophage has the capacity to synthesize larger quantities [25] as well as a more diverse spectrum of eicosanoids, including both LTC_4 and LTB_4 as well as a variety of cyclooxygenase metabolites [12]. We therefore undertook the present study to examine comprehensively the modulatory effects of AF on AA metabolism by the rat AM, a cell whose profile of eicosanoid synthesis appears to closely resemble that of the human AM [26]. Ionophore A23187 was selected as an agonist because it is a potent stimulus for arachidonate metabolism via both cyclooxygenase and lipoxygenase pathways which bypasses the need for specific receptor interactions; as such, it can be regarded as a maximal stimulus of AA metabolism [27].

Analysis by RP-HPLC of the eicosanoid products of prelabeled AM indicated that A23187 stimulated the release of free AA and its metabolism to 5-lipoxygenase products ($LTB_4 >$ 5-HETE $> LTC_4$) to a greater extent than cyclooxygenase products (HHT $= TxB_2 = PGD_2 > PGE_2$). Auranofin caused a parallel inhibition of both LTB_4 and 5-HETE synthesis by AM. This suggests that its inhibitory site of action was the 5-lipoxygenase enzyme itself, consistent with the findings of Honda and coworkers for intact neutrophils as well as cell-free homogenates [9]. Also in agreement with other reports [8, 9], AF inhibited A23187-induced LTC_4 synthesis ($IC_{50} \sim 1\ \mu g/ml$) with greater potency than that observed for LTB_4 synthesis ($IC_{50} \sim 4.3\ \mu g/ml$), as revealed by both RP-HPLC and RIA analyses. The concentrations of AF at which we observed inhibition of LT synthesis are within the ranges reported by other investigators in blood neutrophils [8, 9] as well as basophils and lung mast cells [10]. In addition, as therapeutic AF concentrations in blood are in the 2–3 $\mu g/ml$ range [28], and as gold has been shown to concentrate in macrophages at inflammatory sites [29], including alveolar macrophages [30], it is likely that the AF concentrations required for inhibition of LTC_4, as well as LTB_4, synthesis in the present study can be achieved locally at sites of inflammation *in vivo*.

It is important to note that the inhibitory effects of AF in our system were specific for the 5-lipoxygenase pathway; the cyclooxygenase pathway, as assessed by both RIA and RP-HPLC quantitation of TxB_2, the major cyclooxygenase metabolite of the rat AM [14], was spared. Such specificity is consistent with the findings of Parente *et al.* [8] that AF inhibits neutrophil synthesis of LT, but not PGE_2. In fact, our data indicated that A23187-induced TxB_2 synthesis was augmented over the same concentration range at which LT synthesis was inhibited. Analysis by RP-HPLC of the total products of prelabeled cells suggests that this reflects an actual augmentation of total phospholipase activity by AF, rather than mere shunting away from the 5-lipoxygenase pathway. In this regard, it is relevant that AF has been shown recently to stimulate phospholipase C activity in sonicates of a murine macrophage-like cell line [31].

ATP is a cofactor for 5-lipoxygenase [15, 16], and we have reported recently that ATP depletion accounts for the inhibition of AM 5-lipoxygenase caused by both unsaturated fatty acids [19] and hydrogen peroxide [32]. Because AF is thought to interfere with mitochondrial functions [33], we considered the possibility that an ATP-depleting effect may similarly play a role in its inhibition of 5-lipoxygenase. Our data suggest such a possibility, as the IC_{50} for ATP depletion was similar to that for LTB_4 synthesis inhibition. A definitive causal link between the capacities of AF to inhibit 5-lipoxygenase and deplete cellular ATP remains to be established, however. We speculate that ATP depletion may also play a role in the cytotoxicity and alterations in plasma membrane morphology attributed to AF [33].

We next considered the possibility that the preferential inhibition by AF of macrophage LTC_4, as opposed to LTB_4 and 5-HETE synthesis, may be related to the capacity to conjugate reduced glutathione based on its sulfhydryl reactivity [33]. We have demonstrated previously [14] that the sulfhydryl reactant *N*-ethylmaleimide depletes AM glutathione with the consequence that synthesis of LTC_4, which is formed by the glutathione transferase-catalyzed conjugation of reduced glutathione and LTA_4, is specifically inhibited. Our results demonstrated that AF did indeed deplete cellular glutathione, but the dose–response relationships for glutathione depletion and LTC_4 inhibition suggest that these two phenomena were not causally related. A likely alternative explanation for the preferential inhibition of LTC_4 synthesis is the higher K_m value for LTA_4 of glutathione transferase (catalyzing LTC_4 synthesis) than of LTA_4 hydrolase (catalyzing LTB_4 synthesis) [9]. The consequence of such a difference in K_m values would be that, as LTA_4 levels decline due to 5-lipoxygenase inhibition, LTC_4 synthesis would be more susceptible to substrate limitation than would LTB_4 synthesis. In accord with this possibility, we have observed a similar preferential inhibition of LTC_4 versus LTB_4 synthesis in the settings of 5-lipoxygenase inhibition caused by both unsaturated fatty acids [19] and hydrogen peroxide [32].

Leukotriene C_4 increases microvascular permeability, while LTB_4 is a leukocyte chemoattractant which also stimulates leukocyte adherence and activation as well as modulating lymphocyte proliferation, differentiation, and function [7]. In addition, of particular relevance to the pathogenesis of asthma are the capacities of LTB_4 to increase airway reactivity [34] and of LTC_4 to cause bronchoconstriction [35] and stimulate mucus secretion in airways [36]. Our results indicate that, at therapeutic concentrations, AF, an oral gold compound approved for use in the United States, acts *in vitro* as a selective inhibitor of macrophage 5-lipoxygenase metabolism. For all of these reasons, it is likely that inhibition of macrophage LT synthesis represents an important mechanism of action of AF in chronic inflammatory disorders such as rheumatoid arthritis and asthma.

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REFERENCES

1. Lipsky PE, Remission inducing therapy in rheumatoid arthritis. *Am J Med* **73** (Suppl): 40–49, 1983.
2. Yamauchi N, Suko M, Morita Y, Suzuki S, Ito K and Miyamoto T, Decreased airway responsiveness to histamine in gold salt-treated guinea pigs. *J Allergy Clin Immunol* **74**: 802–807, 1984.
3. Bernstein DI, Bernstein IL, Bodenheimer SS and Pietruska RG, An open study of auranofin in the treatment of steroid-dependent asthma. *J Allergy Clin Immunol* **81**: 6–16, 1988.
4. Muranaka M, Miyamoto T, Shida T, Kabe J, Makino S, Okumura H, Takeda K, Suzuki S and Horiuchi Y, Gold salt in the treatment of bronchial asthma—A double-blind study. *Ann Allergy* **40**: 132–137, 1978.
5. Silver RM and Zvaifler NJ, Pathogenesis; Immunologic considerations. In: *Rheumatoid Arthritis: Etiology, Diagnosis and Management* (Eds. Utsinger PD, Zvaifler NJ and Ehrlich GE), pp. 71–89. J. B. Lipincott, Philadelphia, 1985.
6. O'Byrne PM, Hargreave FE and Kirby JG, Airway inflammation and hyperresponsiveness. *Am Rev Respir Dis* **136** (Suppl): 35–37, 1987.
7. Samuelsson B, Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. *Science* **220**: 568–575, 1983.
8. Parente JE, Wong K, Davis P, Burka JF and Percy JS, Effects of gold compounds on leukotriene B_4, leukotriene C_4, and prostaglandin E_2 production by polymorphonuclear leukocytes. *J Rheumatol* **13**: 47–51, 1986.
9. Honda Z, Iizasa T, Morita Y, Matsuta K, Nishida Y and Miyamoto T, Differential inhibitory effects of auranofin on leukotriene B_4 and leukotriene C_4 formation by human polymorphonuclear leukocytes. *Biochem Pharmacol* **36**: 1475–1481, 1987.
10. Marone G, Columbo M, Galeone D, Guidi G, Kagey-Sobotka A and Lichtenstein LM, Modulation of the release of histamine and arachidonic acid metabolites from human basophils and mast cells by auranofin. *Agents Actions* **18**: 100–102, 1986.
11. Davies P, Bonney RJ, Humes JL and Kuehl FA Jr, The role of macrophage secretory products in chronic inflammatory processes. *J Invest Dermatol* **74**: 292–296, 1980.
12. MacDermot J, Kelsey CR, Waddell KA, Richmond R, Knight RK, Cole PJ, Collery CT, Landon DN and Blair IA, Synthesis of leukotriene B_4 and prostanoids by human alveolar macrophages: Analysis by gas chromatography/mass spectrometry. *Prostaglandins* **27**: 163–178, 1984.
13. Lipsky PE, Ugai K and Ziff M, Alterations in human monocyte structure and function induced by incubation with sodium thiomalate. *J Rheumatol* **6** (Suppl 5): 130–136, 1979.
14. Peters-Golden M and Shelly C, Modulation of alveolar macrophage-derived 5-lipoxygenase products by the sulfhydryl reactant, *N*-ethylmaleimide. *J Biol Chem* **262**: 10594–10600, 1987.
15. Ochi K, Yoshimoto T, Yamamoto S, Taniguchi K and Miyamoto T, Arachidonate 5-lipoxygenase of guinea pig peritoneal polymorphonuclear leukocytes: Activation by adenosine 5′-triphosphate. *J Biol Chem* **258**: 5754–5758, 1983.
16. Ahnfelt-Ronne I and Olsen UB, Leukotriene production in rat peritoneal leukocytes requires intact energy metabolism. *Biochem Pharmacol* **34**: 3095–3100, 1985.
17. Peters-Golden M, Bathon J, Flores R, Hirata F and Newcombe DS, Glucocorticoid inhibition of zymosan-induced arachidonic acid release by rat alveolar macrophages. *Am Rev Respir Dis* **130**: 803–809, 1984.
18. Westcott JY, Chang S, Balazy M, Stene DO, Pradelles P, Maclouf J, Voelkel NF and Murphy RC, Analysis of 6-keto $PGF_{1\alpha}$, 5-HETE, and LTC_4 in rat lung: Comparison of GC/MS, RIA, and EIA. *Prostaglandins* **32**: 857–873, 1986.
19. Peters-Golden M and Shelly C, Inhibitory effect of exogenous arachidonic acid on alveolar macrophage 5-lipoxygenase metabolism: Role of ATP depletion. *J Immunol* **140**: 1958–1966, 1988.
20. Peters-Golden M and Thebert P, Inhibition by methylprednisolone of zymosan-induced leukotriene synthesis in alveolar macrophages. *Am Rev Respir Dis* **135**: 1020–1026, 1987.
21. Sporn PHS, Peters-Golden M and Simon RH, Hydrogen peroxide-induced arachidonic acid metabolism in the rat alveolar macrophage. *Am Rev Respir Dis* **137**: 49–56, 1988.
22. Stanley PE and Williams SG, Use of the liquid scintillation spectrometer for determining adenosine triphosphate by the luciferase enzyme. *Anal Biochem* **29**: 381–392, 1969.
23. Anderson ME, Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol* **113**: 548–555, 1985.
24. Rouzer CA, Scott WA, Griffith OW, Hamill AL and Cohn ZA, Arachidonic acid metabolism in glutathione-deficient macrophages. *Proc Natl Acad Sci USA* **79**: 1621–1625, 1982.
25. Scott WA, Pawlowski NA, Murray HW, Andreach M, Zrike J and Cohn ZA, Regulation of arachidonic acid metabolism by macrophage activation. *J Exp Med* **155**: 1148–1160, 1982.
26. Balter MS, Eschenbacher WL and Peters-Golden M, Arachidonic acid metabolism in cultured alveolar macrophages from normal, atopic, and asthmatic subjects. *Am Rev Respir Dis* **138**: 1134–1142, 1988.
27. Tripp CS, Mahoney M and Needleman P, Calcium ionophore enables soluble agonists to stimulate macrophage 5-lipoxygenase. *J Biol Chem* **260**: 5895–5898, 1985.
28. Dahl SL, Coleman ML, Williams HJ, Altz-Smith M, Cay DR, Paulus HE, Weinstein A and Kaplan S, Lack of correlation between gold concentrations and clinical response in patients with definite or classic rheumatoid arthritis receiving auranofin or gold sodium thiomalate. *Arthritis Rheum* **28**: 1211–1218, 1985.
29. Vernon-Roberts B, Dore JL, Jessop JD and Henderson WJ, Selective concentration and localization of gold in macrophages of synovial and other tissues during and after chrystotherapy in rheumatoid patients. *Ann Rheum Dis* **35**: 477–486, 1976.
30. Garcia JGN, Munim A, Nugent KM, Bishop M, Hoie-Garcia P, Parhami N and Keogh BA, Alveolar macrophage gold retention in rheumatoid arthritis. *J Rheumatol* **14**: 435–438, 1987.
31. Snyder RM, Mirabelli CK, Clark MA, Ziegler JT and Crooke ST, Effect of auranofin and other gold complexes on the activity of phospholipase C. *Mol Pharmacol* **32**: 437–442, 1987.
32. Sporn PHS and Peters-Golden M, Hydrogen peroxide inhibits alveolar macrophage 5-lipoxygenase metabolism in association with depletion of ATP. *J Biol Chem* **263**: 14776–14783, 1988.
33. Snyder RM, Mirabelli CK and Crooke ST, The cellular pharmacology of auranofin. *Semin Arthritis Rheum* **17**: 71–80, 1987.
34. O'Byrne PM, Leikauf GD, Aizawa H, Bethel RA, Ueki IF, Holtzman MJ and Nadel JA, Leukotriene B_4 induces airway hyperresponsiveness in dogs. *J Appl Physiol* **59**: 1941–1946, 1985.
35. Holroyde MC, Altounyan REC, Cole M, Dixon M and Elliott EV, Bronchoconstriction produced in man by leukotrienes C and D. *Lancet* **2**: 17–18, 1981.

36. Shelhamer JH, Marom Z, Sun F, Bach MK and Kaliner M, The effects of arachinoids and leukotrienes on the release of mucus from human airways. *Chest* **81** (Suppl): 36–37, 1982.