THE ORAL GOLD COMPOUND AURANOFIN TRIGGERS ARACHIDONATE RELEASE AND CYCLOOXYGENASE METABOLISM IN THE ALVEOLAR MACROPHAGE

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ABSTRACT

We examined the effect of *in vitro* incubation with the oral gold compound auranofin (AF) on arachidonic acid (AA) release and metabolism by rat alveolar macrophages (AMs). AF stimulated dose- and time-dependent release of ^{14}C-AA from prelabeled AMs, which reached 4.7 ± 0.3% (mean ± SEM) of incorporated radioactivity at 10 μg/ml for 90 min, as compared to 0.5 ± 0.1% release following control incubation for 90 min ($p<0.001$). Similar dose- and time-dependent synthesis of thromboxane (Tx) A_2 (measured as TxB_2) and prostaglandin (PG) E_2 was demonstrated by radioimmunoassay of medium from unlabeled cultures, reaching 18-fold and 9-fold, respectively, of the control values at 10 μg/ml AF for 90 min ($p<0.001$ for both). AF-induced TxB_2 and PGE_2 synthesis was inhibited by indomethacin as well as by pretreatment with methylprednisolone. No increase in the synthesis of immunoreactive leukotrienes (LT) B_4 or C_4 was noted at any dose or time of AF. High performance liquid chromatographic separation of ^{14}C-eicosanoids synthesized by prelabeled AMs confirmed that AF induced the release of free AA and its metabolism to cyclooxygenase, but not 5-lipoxygenase, metabolites. The ability of AF to trigger macrophage AA metabolism may be relevant to the exacerbation of certain inflammatory processes which sometimes accompany gold therapy.

INTRODUCTION

Gold salts are efficacious in the treatment of rheumatoid arthritis (1) and, perhaps, asthma (2,3). However, they have been observed to exacerbate adjuvant arthritis in the rat (4), and are well-recognized to cause pneumonitis in humans (5). Because macrophage-derived metabolites of arachidonic acid (AA) are thought to be important in the pathogenesis of chronic inflammatory disorders (6) such as rheumatoid arthritis and asthma, and because the macrophage is a putative target cell for the anti-inflammatory effects of chrysotherapy (7), we have initiated studies to examine the effects of *in vitro* incubation with the oral gold compound auranofin (AF) on macrophage AA metabolism. In a previous study (8), we observed that therapeutic concentrations of AF inhibited ionophore A23187-induced 5-lipoxygenase metabolism in the rat alveolar macrophage (AM). However, thromboxane A_2 synthesis was concomitantly augmented by AF. Therefore, in the present study we have examined the effect of AF alone on AA metabolism in the same cell. Our results indeed indicate that AF triggers dose- and time-dependent release of AA and its cyclooxygenase metabolites. These findings may be relevant to the seemingly paradoxical potential of gold compounds to exacerbate inflammation.

METHODS

Macrophage isolation and culture. Alveolar macrophages were obtained by bronchoalveolar lavage from respiratory disease-free 125-150 g female Wistar rats (Charles River, Portage, MI), as previously described (9). Two x 10^6 cells suspended in medium 199 with modified Earle's salts (M199; GIBCO, Grand Island, NY) were plated in 35 x 10 mm plastic culture dishes (Falcon Plastics, Oxnard, CA) and cultured at 37° C in a humidified atmosphere of 5% CO_2 in air. After 1 h, non-adherent cells were removed by washing twice with Hanks' balanced salt solution (HBSS; GIBCO). The resultant adherent cell population has been found to contain 95% AMs by morphologic criteria and esterase staining (9) with viability as assessed by trypan blue exclusion exceeding 90%. Macrophage monolayers were then cultured overnight (16 h) in M199 containing 10% heat-inactivated newborn calf serum (NCS; GIBCO) in the presence or absence of radiolabeled AA prior to experimental incubations. In some experiments, overnight incubations were carried out in the presence of 1 μM methylprednisolone (10). Following overnight culture, these monolayers have been found to contain approximately 8.5 μg DNA (9) and 100 μg protein (11).

Prelabeling of macrophage cultures. In selected experiments, cellular lipids were prelabeled by including 0.2 μCi of [1-^{14}C] AA (specific activity 54-57 mCi/mmol, Dupont-New England Nuclear, Boston, MA) in the medium during overnight culture (11). The uptake of radiolabel by macrophage cultures, determined as described previously (9), was 31.6 ± 1.4% (mean ± SEM, n=4).

Incubations. Duplicate cultures of unlabeled or prelabeled AMs were washed twice with HBSS and incubated for 30, 60, or 90 min with 1 ml of M199 containing 0, 2, 5, or 10 μg/ml AF. Auranofin (generously provided by Smith Kline & French Laboratories, Philadelphia, PA) was prepared as a stock solution at 20 mg/ml in DMSO, and diluted directly into M199 to reach the desired final concentrations. All cultures contained DMSO at a final concentration of 0.5%. In some experiments, indomethacin (Sigma), 5 μM in 0.05% ethanol (final concentration) was added to cultures simultaneous with AF. Neither viability nor eicosanoid synthesis was affected by DMSO or ethanol in the concentrations utilized. In selected experiments, prelabeled cultures were incubated for 30 min with the agonist ionophore A23187 (Calbiochem-Behring Corp., La Jolla, CA), 1 μM in 0.5% DMSO, or the detergent Triton-X 100 (Sigma), 0.01-0.1%.

Release of total radioactivity and free ^{14}C-AA. Total radioactivity released from prelabeled cells was determined by counting duplicate 50 μl aliquots of medium in 9 ml of ACS scintillant (Amersham Corp., Arlington Heights, IL). To assess deacylation of free ^{14}C-AA from prelabeled cells, lipids were extracted from cells plus medium with chloroform/methanol (2:1, vv), and separated by thin layer chromatography (TLC) on Silica Gel 60 plates developed with hexane/diethyl ether/acetic acid (70:30:1, v/v/v), as described (12). Spots migrating with authentic AA standard (Nu-chek Prep, Inc., Elysian, MN) were visualized by exposure to iodine vapor, cut out, eluted with methanol, and radioactivity quantitated by liquid scintillation spectrometry. Both total counts and free ^{14}C-AA counts were expressed as a percentage of total incorporated radioactivity.

Extraction of eicosanoids. Eicosanoids were extracted from culture media of prelabeled or unlabeled cells using C_{18} Sep-Pak cartridges (Waters Associates, Milford, MA), and

a modification (11) of the method of Westcott, et. al. (13). Recoveries for this extraction procedure, assessed using tritiated standards (Dupont-New England Nuclear) added to M199, were 65-70% for thromboxane B_2 (TxB_2, the stable metabolite of TxA_2), leukotriene C_4 (LTC_4), and leukotriene B_4 (LTB_4) (11).

Reverse-phase high performance liquid chromatography (RP-HPLC). Radiolabeled eicosanoids were separated by RP-HPLC with a Waters HPLC system and a Waters 5 μm Bondapak C_{18} column, as previously described (10). The mobile phase, acetonitrile/water/trifluoroacetic acid, was used at a 1 ml/min flow rate. Cyclooxygenase metabolites were eluted during an initial isocratic phase (33:67:0.1, v/v/v) and lipoxygenase metabolites and free AA during a stepwise gradient increase of acetonitrile to 100:0:0.1 (v/v/v). The eluate was continuously monitored for UV absorbance (210 nm for cyclooxygenase products and free AA, 280 nm for leukotrienes, and 235 nm for monohydroxyeicosatetraenoic acids). Eicosanoids were identified by their co-elution with authentic standards. One ml eluate fractions were collected and products quantitated by liquid scintillation spectrometry in 6 ml scintillant. Authentic cyclooxygenase metabolites were the generous gift of Dr. J. Pike (Upjohn Co., Kalamazoo, MI) and lipoxygenase metabolites, of Dr. J. Rokach (Merck Frosst, Inc., Quebec, Canada).

Radioimmunoassays. Thromboxane B_2, prostaglandin E_2 (PGE_2), LTB_4, and LTC_4 were quantitated by radioimmunoassays (RIA). Sep-Pak extracts were dried, dissolved in 1 ml of phosphate-buffered saline containing 0.1% gelatin, and 0.1 ml aliquots assayed in duplicate. The sources, sensitivities, and cross-reactivities of the antibodies employed have been described previously (14). In all cases, concentrations of immunoreactive eicosanoids were corrected for recovery.

Assessment of viability by exclusion of trypan blue. Following control or experimental incubation, medium was removed and replaced with 0.05% trypan blue (Sigma) in normal saline. After 10 sec, dye was removed by aspiration and cultures immediately examined under light microscopy. The percentage of cells excluding trypan blue was determined as the average value from 200 cells counted in each of the duplicate cultures.

Data analysis. All data are expressed as mean values ± SEM. Viability, total radioactivity release, free ^{14}C-AA release, and immunoreactive eicosanoids were determined in duplicate culture plates for each condition and the average calculated to yield a single data point. Unless otherwise indicated, all data represent the results from 3 individual experiments. The significance of differences between group means was assessed by one-way analysis of variance and the Newman-Keuls Multiple Range Test. In all cases, a p value <0.05 was considered significant.

RESULTS

Effect of AF on release of total radioactivity and ^{14}C-AA. In unstimulated cultures, approximately 1.5% of incorporated radioactivity was released into culture medium as free AA plus eicosanoids at all time points (Fig. 1A). No increases in release of radioactivity were observed with AF at 2 μg/ml. Five μg/ml AF induced time-dependent release of radioactivity, which approached statistical significance as compared to control at 60 min ($0.05<p<0.1$) and was highly significant ($p<0.001$) at 90 min. The highest dose of AF (10 μg/ml) also stimulated time-dependent release of radioactivity, which reached 10.3 ± 1.5% at 90 min.

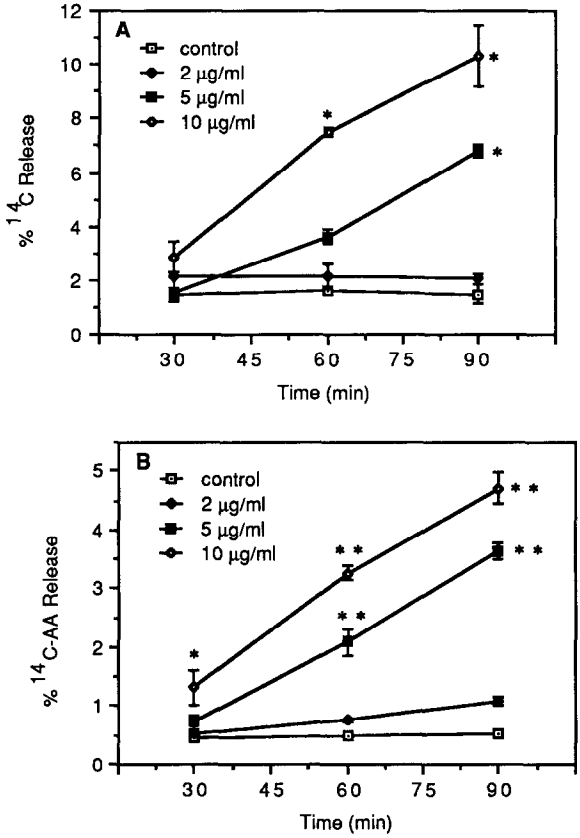

Fig. 1. Effect of AF on release of total radioactivity and radiolabeled AA from prelabeled cells. ^{14}C-AA prelabeled AMs were incubated for the indicated times in the presence or absence of AF at the concentrations shown. Release of total ^{14}C radioactivity (A) and of free ^{14}C-AA (B) were determined and are plotted as the percent of total incorporated radioactivity measured prior to the experimental incubations. Each point represents the mean ± SEM of 3 independent experiments, each performed in duplicate.

Since fatty acid deacylation is the initial and probably the rate-limiting step in the metabolism of AA (15), we quantitated free ^{14}C-AA in cells plus medium of cultures incubated for various times with and without AF in various concentrations (Fig. 1B). Approximately 0.5% of the initial incorporated radioactivity was constitutively released as free AA at all time points. At 2 µg/ml AF, AA release tended to increase over time, reaching approximately 1% at 90 min ($0.05<p<0.1$). Significant increases in AA re-

lease occurred with 5 μg/ml at 60 min and 90 min ($p<0.001$ vs control at both time points), and with 10 μg/ml AF at all time points, reaching 4.7 ± 0.3% at 90 min.

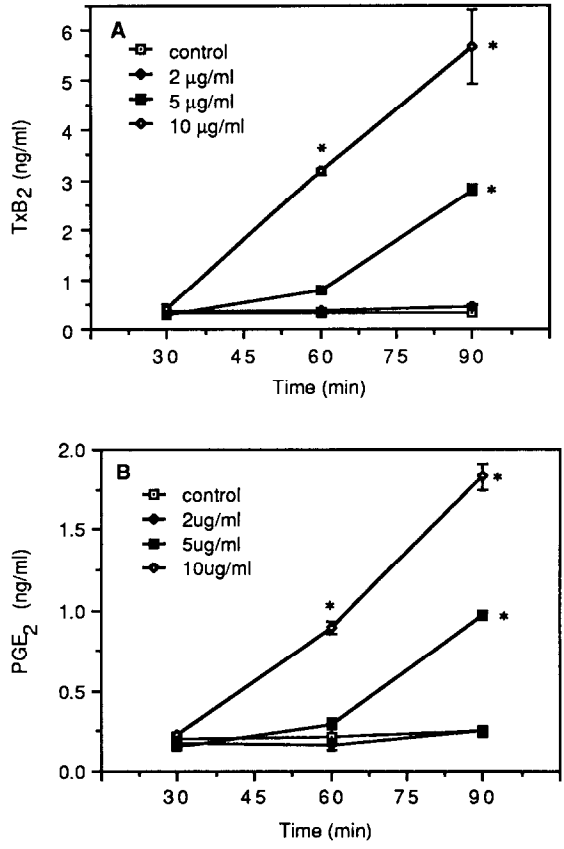

Fig. 2. Effect of AF on macrophage synthesis of immunoreactive TxB_2 (A) and PGE_2 (B). Unlabeled AMs were incubated for the indicated times in the presence or absence of AF at the concentrations shown and eicosanoids quantitated by RIA. Data are expressed in ng/ml (ng/plate) and represent the mean ± SEM of 3 independent experiments, each performed in duplicate.

Effect of AF on macrophage eicosanoid synthesis. Radioimmunoassay of media from unlabeled cultures similarly demonstrated dose- and time-dependent synthesis of TxB_2 (Fig. 2A) and PGE_2 (Fig. 2B). As compared to control cultures incubated for comparable times, these differences were highly significant ($p<0.001$) for 5 μg/ml AF at 90 min and 10 μg/ml AF at both 60 and 90 min. At 10 μg/ml AF incubation for 90 min,

the maximal levels of immunoreactive TxB_2 and PGE_2 were 5.66 ± 0.93 ng/ml and 1.83 ± 0.10 ng/ml, respectively. Of note, the TxB_2:PGE_2 ratio also increased with AF stimulation from a value of approximately 1.5 in unstimulated cultures to a value of > 3 in AMs incubated with 10 μg/ml AF for 90 min. This reflected a 9-fold increase in PGE_2 synthesis and a 18-fold increase in TxB_2 synthesis under maximal conditions as compared to control conditions.

In contrast to the marked stimulatory effects of AF on AM synthesis of TxB_2 and PGE_2, no such stimulation of immunoreactive LTB_4 or LTC_4 synthesis was observed at any time or dose of AF. Table 1 depicts the results of experiments utilizing 90 min incubations, but no increase in LT synthesis was seen at shorter time points either.

The effects of AF on arachidonate release and metabolism were also investigated by RP-HPLC analysis of radiolabeled eicosanoids released by prelabeled cells. Fig. 3 shows RP-HPLC radioactivity elution profiles from a representative experiment in which AMs were incubated for 60 min with medium alone (A) or AF, 5 μg/ml (B). Each chromatogram represents the elution profile of eicosanoids pooled from duplicate culture plates. As shown, unstimulated cultures released a single peak of radioactivity corresponding to a small amount of free AA. In contrast, cultures stimulated with AF demonstrated an increased release of free AA, as well as peaks corresponding to the cyclooxygenase products TxB_2, PGE_2, and HHT. Although small peaks corresponding to 15-hydroxyeicosateraenoic acid (15-HETE) and 12-HETE were present, no peaks corresponding to the 5-lipoxygenase products LTC_4, LTB_4, or 5-HETE were detected.

Table 1. Effects of AF on Macrophage LTB_4 and LTC_4 synthesis[a]

AF concentration[b]	LTB_4[c]	LTC_4[c]
control	0.22 ± 0.04	0.24 ± 0.12
2 μg/ml	0.17 ± 0.09	0.17 ± 0.01
5 μg/ml	0.17 ± 0.06	0.13 ± 0.06
10 μg/ml	0.18 ± 0.08	0.10 ± 0.01

[a]Results are the mean ± SEM from 3 independent experiments, each performed in duplicate.

[b]All incubations shown were for 90 min.

[c]Immunoreactive LT values expressed in ng/ml.

Effects of indomethacin and methylprednisolone on AF-induced eicosanoid synthesis. The effects of indomethacin (5 μM) on immunoreactive eicosanoid synthesis induced by 5 μg/ml AF for 60 min were examined. Indomethacin completely inhibited AF-induced synthesis of TxB_2 (AF, 0.84 ± 0.15 ng/ml; AF plus indomethacin, 0.11 ± 0.01 ng/ml) and PGE_2 (AF, 0.33 ± 0.03; AF plus indomethacin, 0.15 ± 0.01) (mean ± one-half the range, n=2). At the same time, indomethacin failed to augment synthesis of LTB_4 (AF, 0.17 ± 0.02 ng/ml; AF plus indomethacin, 0.16 ± 0.04) (mean ± one-half the range, n=2) in cultures incubated with AF.

In one experiment, the effect of 16 h pretreatment with the glucocorticoid methylprednisolone (1 μM) on AF-induced TxB_2 synthesis was evaluated. When im-

munoreactive TxB_2 levels were corrected for control amounts, methylprednisolone pretreatment resulted in a 53% inhibition of TxB_2 synthesis induced by 5 μg/ml AF for 60 min (data not shown).

Effect of AF on macrophage viability. Macrophage viability was preserved in both control cultures and cultures exposed to 2 μg/ml AF for up to 90 min (Fig. 4). At 5 μg/ml AF, viability was reduced only at the 90 min incubation time ($p<0.001$ vs 90 min control). At 10 μg/ml AF, time-related cytotoxicity was observed at incubation times of

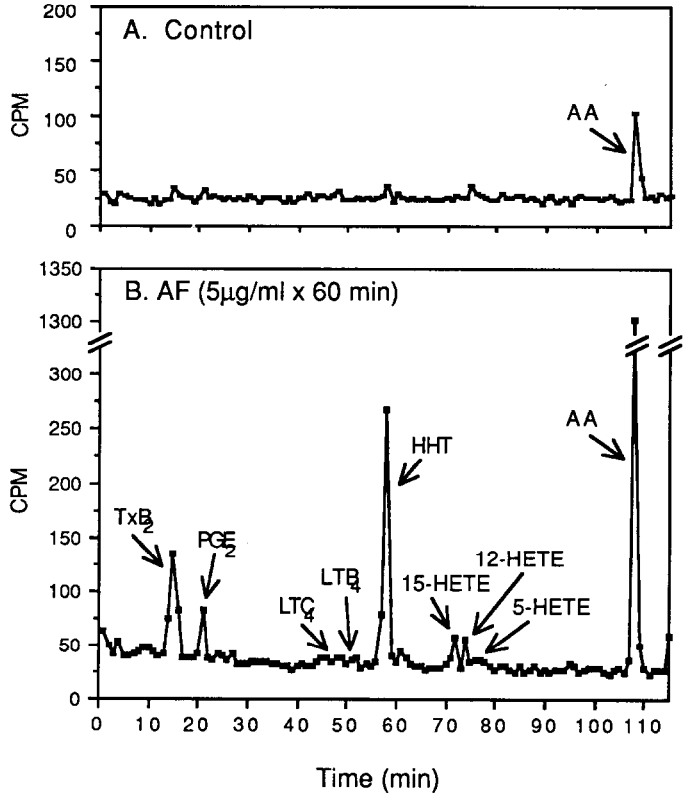

Fig. 3 RP-HPLC analysis of radiolabeled eicosanoids induced by AF. Prelabeled cells were incubated for 60 min in the absence (A) or presence (B) of 5 μg/ml AF, after which eicosanoids were extracted and separated by RP-HPLC. Data represent results from pooled duplicate cultures. The retention times of authentic standards are indicated by arrows.

both 60 min ($p<0.005$ vs 60 min control) and 90 min ($p<0.001$ vs 90 min control), at which time only 33% of cells excluded trypan blue.

Relationship between AA release and metabolism and cytotoxicity induced by AF. It was apparent from the foregoing results that AF had the capacity to both trigger AA release and cyclooxygenase metabolism, as well as to cause cytotoxicity, in AM cultures. In order to investigate the relationship between cell injury and AA release and metabolism in the AM, we related cell viability (as assessed by trypan blue exclusion), total release of radioactivity, and the release of free arachidonate and metabolites (by RP-HPLC analysis) in prelabeled cultures incubated under a variety of conditions. For this purpose, we examined AMs exposed to: 1) AF under both non-toxic (5 μg/ml for 60 min) and toxic (50 μg/ml for 90 min) conditions; 2) calcium ionophore A23187, a maximal stimulus for macrophage AA metabolism; and 3) the detergent Triton-X 100 at various concentrations. For each condition, the total amount of radiolabeled AA plus metabolites released into culture medium and eluted by RP-HPLC was expressed relative to the amount of ^{14}C-AA incorporated into cells, as well as to the amount of total radioactivity released into medium.

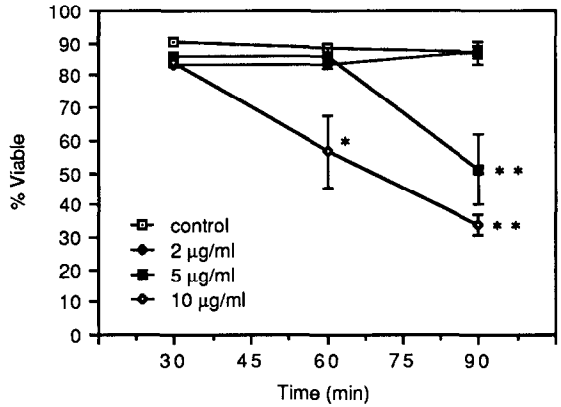

Fig 4. Effect of AF on macrophage viability. AM cultures were incubated for the indicated times with either medium alone or medium containing AF at the concentration shown. The percent of 200 cells counted which excluded trypan blue is plotted. Each point represents the mean ± SEM of 3 independent experiments, each performed in duplicate.

As demonstrated in Table 2, 5 μg/ml AF and A23187 were respectively weak and potent agonists of AA metabolism in the absence of cytotoxicity. In contrast, an inverse relationship between viability and total release of radioactivity was observed in cultures exposed to high-dose AF, 0.01% Triton-X, and 0.1% Triton-X. At the high dose of Triton-X, virtually all cells were injured and all incorporated radioactivity released. However, separation of radiolabeled eicosanoids by RP-HPLC revealed that under these conditions of increasing toxicity, progressively smaller proportions of incorporated radioactivity were released into media as eicosanoids. 0.1% Triton-X, which injured 10-fold as many cells and released 10-fold as much total radioactivity as did A23187, released only one-tenth the quantity of eicosanoids as did A23187, and

Table 2. Relationship between Macrophage Cytotoxicity and AA Metabolism

	control x 60 min	AF (5μg/ml) x 60 min	A23187 (1μM) x 30 min	AF (50μg/ml) x 90 min	Triton-X (0.01%) x 30 min	Triton-X (0.1%) x 30 min
viability	90	86	85	44	28	9
% total release[a]	1.6	3.6	11.8	15.0	28.4	99.3
% eicosanoid release[b]	0.3	1.8	10.4	3.3	3.2	1.1
% total release due to eicosanoids[c]	20.0	48.7	88.6	22.5	11.2	1.1
% total release due to:						
cyclooxygenase[d]	5.6	16.4	9.6	9.4	2.9	0.3
5-lipoxygenase[e]	1.3	0	41.2	0	0	0
12- + 15-HETE	1.5	1.8	20.0	1.2	3.5	0.1
free AA	11.6	30.5	17.6	11.9	4.8	0.7

[a] cpm in medium/cpm incorporated
[b] cpm in AA + metabolites/cpm incorporated
[c] cpm in AA + metabolites/cpm in medium
[d] sum of TxB_2 + PGE_2 + HHT
[e] sum of LTC_4 + LTB_4 + 5-HETE

even less than the weaker agonist AF (5 μg/ml). When radioactive eicosanoids were expressed relative to the total counts released into medium, it was apparent that with increasing toxicity, only a small proportion of these total counts were accounted for by free AA or its metabolites; presumably, the majority of these counts represent intact ^{14}C-AA-containing phospholipids. Therefore, these data fail to support a simple relationship between membrane injury *per se* and the activities of phospholipase or either of the oxygenation enzymes.

DISCUSSION

The major finding of this study was that the oral gold compound AF triggered dose- and time-dependent release of arachidonate and its cyclooxygenase metabolites from cultures of rat AMs. This was demonstrated by a variety of techniques: 1) quantitation of total radioactivity (AA plus metabolites) released from ^{14}C-AA prelabeled cells; 2) separation and quantitation of free ^{14}C-AA released from prelabeled cells; and 3) quantitation by RIA of TxB_2 and PGE_2, the major cyclooxygenase metabolites synthesized by these cells (11). Furthermore, RP-HPLC analysis of eicosanoids synthesized by prelabeled cells confirmed that AF stimulated the release of AA and its metabolism via the cyclooxygenase pathway to HHT ~ TxB_2 >PGE_2. Of note, both RIAs and RP-HPLC analysis failed to demonstrate the synthesis of 5-lipoxygenase metabolites by AMs incubated with AF. Inhibition of prostaglandin synthesis by methylprednisolone pretreatment and by indomethacin supports the assumption that AF-induced AA metabolism proceeds via the actions of phospholipase and cyclooxygenase, as expected. While Snyder and associates (16) have demonstrated the capacity of AF to stimulate phospholipase C activity in sonicates of a macrophage-like cell line, to our knowledge ours is the first report to document activation of AA metabolism by a gold compound in intact cells.

The rat AM has the capacity to synthesize large amounts of the 5-lipoxygenase products LTB_4, LTC_4, and 5-HETE along with cyclooxygenase products in response to classical agonists such as zymosan particles (10) or the calcium ionophore A23187 (14). However, AF failed to trigger 5-lipoxygenase metabolism despite the fact that it mobilized free AA and stimulated cyclooxygenase metabolism. This is reminiscent of our previous observations (14) with the sulfhydryl reactant N-ethylmaleimide. Because in some experimental systems cyclooxygenase inhibition can shunt free AA to lipoxygenase products (17), we examined the effect of indomethacin on AF-induced eicosanoid synthesis. Despite the complete inhibition of AF-induced TxB_2 and PGE_2 synthesis to levels below control values, indomethacin did not augment LT synthesis, indicating the lack of substrate shunting to the 5-lipoxygenase pathway under these conditions. It is conceivable that AF itself could complex with or catabolize 5-lipoxygenase eicosanoids, thereby interfering with our ability to detect them even if synthesized. However, other investigators (18, 19) have previously excluded such an effect of AF on exogenously added LT standards, and we have similarly shown that N-ethylmaleimide did not influence recovery of exogenous LTs added to cultures of AMs (14). We therefore conclude that the lack of detectable 5-lipoxygenase eicosanoids in AF-treated cultures reflects a lack of synthesis. In a previous study we demonstrated that AF inhibited AM 5-lipoxygenase metabolism stimulated by A23187 (8); this inhibition appears to be mediated, at least in part, by the depletion of intracellular ATP (unpublished results), a co-factor for the 5-lipoxygenase enzyme (20). We propose that the inability of AA mobilized by AF to be metabolized via the 5-lipoxygenase pathway is due to the dual capacities of AF to both trigger AA release and inhibit the 5-lipoxygenase enzyme.

The increase in the ratio of TxB_2: PGE_2 with increasing concentrations of AF may reflect the facts that synthesis of PGE_2 (21), but not TxB_2 (22), is dependent on reduced glutathione, and that AF dose-dependently depletes cellular glutathione (unpublished results). This is further suggested by the fact that levels of LTC_4, whose synthesis is directly dependent on the conjugation of reduced glutathione to LTA_4, but not levels of LTB_4, declined progressively with increasing concentrations of AF. Since TxA_2 has primarily pro-inflammatory actions (23) while PGE_2 has prominent down-regulatory effects on leukocyte activation (23), cellular immune function (24), and fibrogenesis (25), the increased TxB_2:PGE_2 ratio observed with AF treatment might result in a net augmentation of the inflammatory process.

Auranofin has previously been shown to be cytotoxic for a variety of cell types at concentrations ranging from 1-10 μg/ml (26). Our data demonstrated similar dose- and time-dependent cytotoxicity for AMs, as judged by the inability to exclude the vital dye trypan blue. In order to ascertain the possible role of cytotoxicity in the ability of AF to trigger AA release and mertabolism, we conducted studies of arachidonate metabolism and viability using a broad range of agonists and toxins. If membrane injury alone were capable of activating the release and subsequent metabolism of AA, increases in eicosanoid synthesis would have been expected to parallel the increasing cytotoxicity caused by 50 μg/ml AF, 0.01% Triton-X, and 0.1% Triton-X. However, the data in Table 2 demonstrate that this was not the case; indeed, an inverse relationship was observed between cytotoxicity and AA release and metabolism. The detergent Triton-X at 0.1%, which released nearly all of the incorporated ^{14}C-AA into the medium, resulted in the synthesis of only 60% the quantity of eicosanoids as did the weak agonist AF and 10% as much as the potent agonist A23187. Since gold compounds cause lung injury in humans (5), any role that cytotoxicity might play in the capacity of AF to trigger AA release and metabolism may in fact be germane. Nonetheless, the results described above, as well as the fact that clear stimulation of AA release and/or metabolism was observed under AF incubation conditions unassociated with cytotoxicity (e.g., 5 μg/ml AF for 60 min and 10 μg/ml AF for 30 min), leads us to conclude that the agonist activity of AF is independent of its cytotoxic potential. Rather, it is likely that the capacity of AF to trigger arachidonate metabolism is a consequence of its sulfhydryl or thiol reactivity (26), as this is a property shared by a variety of metallic (27-29) and non-metallic (14, 30) sulfhydryl-reactive compounds.

Because macrophages are relatively enriched in their AA content as compared to neutrophils (31), and because they produce a diverse spectrum of cyclooxygenase and lipoxygenase eicosanoids (32), macrophage-derived eicosanoids have been proposed to play a prominent role in chronic inflammation. In the case of the AM, its significance is further emphasized by its strategic position as the resident effector cell of the lower respiratory tract (33). For these reasons, and because gold has been shown to accumulate in macrophages at inflammatory sites (34), including the lung (35), AA metabolism induced by AF at therapeutic concentrations may be relevant to the pathogenesis of pneumonitis which sometimes complicates the therapeutic use of gold (5), as well as the exacerbation of other inflammatory processes such as adjuvant arthritis (4).

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