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AURANOFIN INHIBITS HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS

MEIR SHALIT* and FRANCESCA LEVI-SCHAFFER†‡

*Allergy Unit, Department of Medicine A and †Department of Pharmacology, Hadassah Medical School, The Hebrew University, Jerusalem

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Abstract — The effect of auranofin on histamine release from immunologic and non-immunologic activated rat peritoneal mast cells cocultured with 3T3 fibroblasts (MC/3T3) was investigated. When MC/3T3 were preincubated with 2×10^{-5} M auranofin and thereafter challenged with anti-IgE antibodies, a maximal inhibition of histamine release (86.2%) was obtained. Non-immunological histamine release induced by compound 48/80, substance P and bradykinin was inhibited to a lesser degree, i.e. 36.0%, 37.6% and 24.0% respectively. Simultaneous incubation of auranofin and the stimulating agents resulted in a higher inhibition of histamine release: anti-IgE antibodies — 92.0%; compound 48/80 — 73.5%; substance P — 46.1%. We conclude that auranofin effectively reduces histamine release from immunologic and non-immunologic activated mast cells. This may be relevant to the control of allergic reactions.

The use of gold salts as anti-inflammatory agents in the treatment of rheumatoid arthritis and bronchial asthma has been widely reported (Bluhm, 1975; Klaustermeyer, Noritake & Kwong, 1987). Recently a new orally absorbable compound, auranofin (triethylphosphine gold) has been introduced as an effective anti-inflammatory drug in these diseases, although its mechanism of action is not fully understood as yet (Weisman & Hannifin 1979; Bernstein, Bernstein, Bodenheimer & Pietrusko, 1988). It has been suggested that auranofin inhibits various neutrophil functions such as chemotaxis, LTC_4 and LTB_4 synthesis as well as superoxide generation (Hafstrom, Uden & Palmblad, 1983). In addition, it affects other inflammatory cell types. At pharmacological concentrations it inhibits human lymphocyte ADCC and PHA stimulation (Russel, Davis & Miller 1982; Lorber, Simon, Leeb, Peter & Wilcox, 1979) and decreases PWM-induced immunoglobulin secretion (Salmeron & Lipsky, 1982).

The role of auranofin in allergic reactions has not been extensively investigated. However, few studies have shown that this gold compound exhibits inhibitory effects on isolated activated basophils and mast cells (Takaishi, Morita, Kudo & Miyamoto, 1984; Wojtecka-Lukasik, Sopata & Maslinski, 1986; Marone, Columbo, Guidi, Kagey-Sobotka & Lichtenstein, 1986).

We have developed an in-vitro system in which rat peritoneal and human lung mast cells are maintained viable and functionally active on a 3T3-fibroblast monolayer (MC/3T3) (Levi-Schaffer, Austen, Caulfield, Hein, Bloes & Stevens, 1985; Levi-Schaffer, Austen, Caulfield, Hein, Gravallese & Stevens, 1987). This model mimicks the environment *in vivo* in which mast cells are in close contact with fibroblasts in connective tissue locations. Moreover we have shown that under these in-vitro coculture conditions the rat peritoneal mast cells respond to immunologic and non-immunologic stimuli by releasing higher percentages of histamine compared to freshly isolated mast cells (Levi-Schaffer & Shalit, 1989).

In the present study we have investigated the effects of auranofin on immunologic and non-immunologic activation of these connective tissue type mast cells.

EXPERIMENTAL PROCEDURES

Materials

Substance P, bradykinin, compound 48/80, histamine dihydrochloride, were purchased from

‡To whom correspondence should be addressed at: Department of Pharmacology, Hadassah Medical School, The Hebrew University of Jerusalem, 91010 Jerusalem, Israel.

Sigma (St Louis, MO). Goat anti-rat IgE antibodies were obtained from Bethyl Laboratories (Montgomery, TX). Auranofin was kindly supplied by Smith, Kleine & French Laboratories. ^{14}C-labeled methyl-*S*-adenosyl-L-methionine was purchased from New England Nuclear (Boston, MA). RPMI-1640, penicillin, streptomycin, L-glutamine, non-essential aminoacids were obtained from Gibco (Grand Island, N.Y.). The contact inhibited 3T3 Swiss albino mouse skin fibroblast cell line was obtained from ATCC (Rockville, MD). Sterile tissue culture plasticware was purchased from Falcon (Oxnard, CA).

Preparation of cocultures of rat peritoneal mast cells with 3T3 fibroblasts (MC/3T3).

Mast cells were obtained from Sabra male rats (an outbred strain of the Hebrew University) weighing 250 – 300 g by peritoneal lavage and purified as previously described (Levi-Schaffer *et al.*, 1985). The freshly isolated and purified mast cells (>95%) were resuspended at a density of 0.8×10^5/2 ml in enriched medium (E.M.: RPMI-1640 containing 10% heat inactivated fetal calf serum (v/v), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, pH 7.2) and seeded on a confluent layer of 3T3 fibroblast cell line in 35 mm culture dishes. After seeding, the mast cells adhere to the fibroblast monolayer in about 10 min. The MC/3T3 were incubated in a 5% CO_2 humidified incubator at 37°C.

Activation of mast cells and inhibition of histamine release

Activation of MC/3T3 was performed 48 h after mast cells seeding on the fibroblasts. Culture medium was aspirated, the co-cultures were washed twice with 1 ml Tyrode's buffer containing 1.8 mM $CaCl_2$, 0.9 mM $MgCl_2$ and 0.1% gelatin (TG+ +), and incubated for 15 min at 37°C with either 1 ml TG+ + or 1 ml TG+ + containing optimal concentrations of the various secretagogues. Segretagogues and their concentrations were the following: compound 48/80 — 0.5 μg/ml; substance P — 10^{-4} M; bradykinin — 10^{-5} M; anti-IgE antibodies — 1:40. Auranofin was freshly dissolved at 10^{-2} M in ethanol. Further dilutions were in TG+ +. To test the effect of auranofin on histamine release MC/3T3 were preincubated with various concentrations of the drug for 15 min at 37°C prior to activation. In few experiments the preincubation period was extended to 30 and 60 min. In other experiments the secretagogues and auranofin were added simultaneously for an incubation period of 15 min. After incubation, supernatants were collected by aspiration, and the fibroblast-adherent mast cells were scraped from the tissue culture plates with a Teflon policeman in 1 ml TG+ + and disrupted by continuous sonication for 30 s. (output 5, 50% duty cycle, Heat System Ultrasonics W380).

All supernatants and cell sonicates were stored at −80°C until assayed for histamine content.

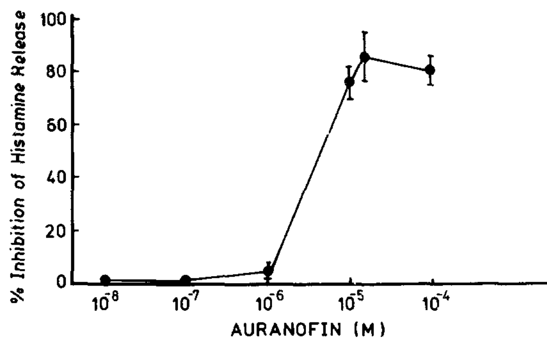

Fig. 1. Effect of different concentrations of auranofin on anti-IgE antibodies induced histamine release from MC/3T3. Cultures were preincubated for 15 min with auranofin before anti-IgE addition. The data represent the mean ± S.E. of two experiments performed in duplicate.

Histamine assay

Histamine was determined in the supernatants and in the cell sonicates by a radioenzymatic assay using ^{14}C-labeled-methyl-*S*-adenosyl-L-methionine (41 mCi/mmol), and a crude preparation of rat kidney methyl transferase (Shaff & Beaven, 1979). The assay was calibrated with solutions containing 0 – 10 ng histamine. The percentage of histamine released from the mast cells incubated with the various stimulating agents was calculated by dividing the amount of histamine in the supernatant by the sum of that in the supernatant and the respective sonicated cells.

Percentages of inhibition of histamine release were calculated according to the following formula:

$$100 - \frac{\text{\% histamine released in the presence of auranofin}}{\text{\% histamine released in the absence of auranofin}} \times 100$$

RESULTS

Effect of auranofin preincubation on immunologic activation of MC/3T3

Preincubation of auranofin for 15 min with MC/3T3 caused a dose-related inhibition of anti-IgE

Table 1. Effect of auranofin preincubation on non-immunologic induced histamine release from MC/3T3

Auranofin concentration (M)	Compound 48/80 0.5 μg/ml	Substance P 10^{-4} M	Bradykinin 10^{-5} M
	% Inhibition*		
10^{-6}	2.3 ± 1.9	nd	nd
10^{-5}	12.1 ± 3.5	10.5 ± 6.1	9.8 ± 5.2
2×10^{-5}	36.0 ± 1.4	37.6 ± 8.7	24.0 ± 5.1

*Data represent the mean ± S.E. of four experiments performed in duplicate. Per cent inhibition was calculated as described in Materials and Methods.

Table 2. Effect of auranofin simultaneously added with various secretagogues on histamine released from MC/3T3

Auranofin concentration (M)	Compound 48/80 0.5 μg/ml	Anti-IgE 1:40	Substance P 10^{-4} M
	% Inhibition*		
10^{-6}	5.0 ± 1.6	36.3 ± 3.2	nd
10^{-5}	60.2 ± 13.5	60.7 ± 13.2	35.0 ± 14.9
2×10^{-5}	73.5 ± 15.5	92.0 ± 7.1	56.1 ± 7.4

*Data represent the mean ± S.E. of three experiments performed in duplicate.

induced histamine release from activated MC/3T3 (Fig. 1). MC/3T3 activated with anti-IgE antibodies released 25.8 ± 2.4% of histamine while in the control cultures, incubated with buffer alone, histamine release was always lower than 5%. The highest inhibition of histamine release (86.2 ± 7.4%) was at a drug concentration of 2×10^{-5} M. An increase to 10^{-4} M in the auranofin concentration did not result in a parallel augmentation of histamine release inhibition. At a concentration of 10^{-6} M and lower, the drug was found inactive. In experiments in which MC/3T3 were preincubated with the effective concentrations of auranofin (10^{-5} M and 2×10^{-5} M) for 30 and 60 min and challenged with anti-IgE the percentages of inhibition were comparable to those observed with 15 min preincubation (data not shown).

Mast cells preincubated either with auranofin alone up to 60 min (10^{-4} M and lower concentrations) or with auranofin followed by anti-IgE antibodies retained their viability as demonstrated by trypan blue exclusion.

The inhibitory effects of auranofin on mast cell histamine release could also be demonstrated morphologically at the inverted microscope level. Most of the mast cells activated with anti-IgE antibodies lost the refractiveness and brightness of their membrane, while most of those preincubated with an optimal concentration of the drug kept this morphological property (not shown).

Effect of auranofin preincubation on non-immunologic activation of MC/3T3

Preincubation for 15 min of MC/3T3 with different auranofin concentrations displayed inhibitory effects on non-immunologic stimulation as well. MC/3T3 cultures activated with compound 48/80 (0.5 μg/ml) released 66.8 ± 16.7% histamine. By preincubating these cultures, before the challenge with the segretagogue, with 2×10^{-5} M auranofin a maximal decrease in histamine release (36.0 ± 1.4%) was obtained. Similar percentages of inhibition were obtained when the MC/3T3 were challenged with substance P (10^{-4} M) and bradykinin (10^{-5} M), i.e. 37.6 ± 8.7% and 24.0 ± 5.1% respectively. Stimulation of MC/3T3 cultures in the absence of auranofin with substance P resulted in 71.4 ± 3.9% and with bradykinin 67.8 ± 17.2% of histamine release.

Effect of auranofin simultaneously added with the segretagogues

Auranofin added simultaneously with anti-IgE antibodies inhibited histamine release from MC/3T3

in a dose-dependent fashion. As shown in Table 2 at concentrations of 10^{-6} M, 10^{-5} M and 2×10^{-5} M, inhibition was 36.3%, 60.7% and 92.0% respectively. When the cocultures were activated with compound 48/80 and the same concentrations of auranofin were added, percentages of inhibition were 5.0, 60.2 and 73.5 respectively.

DISCUSSION

In this study we have shown that immunologically and non-immunologically activated rat peritoneal mast cells cocultured with 3T3 fibroblasts (MC/3T3) are susceptible to inhibition by the gold compound auranofin. The MC/3T3 system is a mimick *in vitro* of the situation *in vivo* in which connective tissue-type mast cells are in close contact with fibroblasts. We have previously shown that under these tissue culture conditions, rat peritoneal mast cells are kept viable and preserve their functional activity and unique biochemical characteristics for more than a month (Levi-Schaffer *et al.*, 1985). Moreover these connective tissue-type mast cells display an enhanced response to immunologic and non-immunologic activation compared to freshly isolated mast cells (Levi-Schaffer & Shalit, 1989). Thus, this co-culture model is also a suitable system for studying inhibitory effects of drugs. When MC/3T3 were preincubated with various concentrations of auranofin and challenged with anti-IgE antibodies, the drug inhibited histamine release in a dose-dependent fashion (Fig. 1). Comparable dose-dependent inhibition, although with lower auranofin concentrations ($10^{-5} - 10^{-7}$ M) has been previously reported in human basophils and dispersed human lung mast cells challenged with anti-IgE antibodies (Takishi *et al.*, 1984; Marone *et al.*, 1986). The fact that to inhibit immunologic-induced histamine release from rat mast cells, we had to employ concentrations higher than those used with human cells, can be due to interspecies differences or to the different conditions employed *in vitro*. Molar concentrations similar to the ones used in our study are achieved in sera of patients treated for prolonged periods with auranofin (Blocka, Furst, Landaw, Dromgoole, Blomberg & Paulus, 1982; Finkelstein, Waltz, Batista, Mizraji, Roisman & Misher, 1976). Nevertheless even higher concentrations are obtained in areas of inflammation like inflamed synovia (Grahame, Billings, Laurence, Marks & Wood, 1974). We have also demonstrated that auranofin inhibits histamine release in MC/3T3 stimulated with optimal concentrations of compound 48/80, substance P and bradykinin (Table 1). However, in this case, the inhibitory effect was lower than that observed in immunologic activation. This difference in the inhibitory capacity of auranofin may be related either to the degree of mast cell activation and the subsequent higher percentage of histamine release or to a different mechanism of inhibition when immunologic or non-immunologic mast cell activation is undergone.

Similarly, it has been shown that the inhibitory effect of cromolyn sodium on histamine release from mast cells was stronger when the stimulus was weaker (Pearce & Tabar, 1983).

Moreover, auranofin, like cromolyn sodium, exhibited some tachyphylactic properties (Sung, Saunders, Krell and Chakrin, 1977; Church & Hiroi, 1987). In fact, when mast cells were simultaneously incubated with the drug and the activating agent, auranofin inhibited histamine release to a higher extent than when the drug was preincubated with the mast cells prior to the addition of the segretagogue. This phenomenon of tachyphylaxis was not observed with immunologic activation of the mast cells even when preincubation was extended up to 60 min, probably since the cells were already maximally inhibited.

Our observation that auranofin inhibits both immunologic and compound 48/80 activated mast cells is in accordance with the data obtained by Wojtecka-Lukasik *et al.* (1986) and Marone *et al.* (1986) who reported similar data on freshly isolated rat peritoneal mast cells and human lung mast cells. In the present report we have shown that auranofin also effectively affects histamine release induced by bradykinin and substance P, two mediators which have a central role in modulating a number of inflammatory processes.

Thus, inhibition of the stimulatory effects of substance P and bradykinin as well as of IgE mediated activation of mast cells may have relevance *in vivo* for using auranofin in the treatment of inflammatory and allergic diseases.

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