Sodium aurothiomalate does not block interleukin 1 production in rabbits

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SUMMARY Gold salts may attenuate inflammatory processes by inhibiting interleukin 1 production. It is shown that sodium aurothiomalate does not act as an antipyretic after intravenous injection of two pyrogens into rabbits. Consequently, it cannot suppress interleukin 1 production, a conclusion confirmed by an in vitro lymphocyte activating factor assay. Chronic sodium aurothiomalate treatment, however, did depress the blood leucocyte count significantly.

Key words: fever, inflammation, gold salts.

Gold treatment has been used in the management of rheumatic disease for most of this century, and yet its mechanism of action has not been established.[1][2] In rheumatoid arthritis gold alters the function of phagocytes at the site of inflammation.[3–5] Phagocytes play a major part in chronic inflammatory reactions through their capacity to ingest and degrade materials[6] and to secrete a number of biologically active substances,[7][8] which appear to mediate the inflammation. Gold particles accumulate in synovial macrophages[1][3] and inhibit lysosomal enzyme activity.[5] Mononuclear phagocytic activity is suppressed in patients with rheumatoid arthritis who receive gold treatment.[9] Also, prolonged incubation with sodium aurothiomalate reduces the function of human mononuclear phagocytes as accessory cells in the induction of cellular immune responses in vitro.[4]

We do not know whether gold compounds also interfere with the capacity of phagocytes to secrete mediators of inflammation, including the plenipotentiary mediator interleukin 1 (IL1).[10] Apart from its role in inflammation, IL1 is an essential intermediate in the genesis of fever.[10] If gold salts indeed inhibit IL1 synthesis or release, as has been suggested for the salt auranofin,[11] then they should act as antipyretics. We have investigated whether sodium aurothiomalate, a salt commonly used in gold treatment, acts as an antipyretic after intravenous

injection of pyrogens in conscious rabbits. We have also investigated the effect of aurothiomalate in vitro on rabbit monocytes stimulated with endotoxin.

Materials and methods

In vivo investigations were carried out on New Zealand White rabbits of either sex (mass 2–5 kg), conscious and restrained in conventional rabbit stocks. Rectal temperature was measured with indwelling copper-constantan thermocouples, and was monitored for at least an hour before injection of pyrogens or placebo solutions into an ear marginal vein. Temperature was measured for four hours after injections.

All experiments took place at an ambient temperature of between 21°C and 24°C, and at the same time of day, with pyrogen injections taking place at about 1100.

Results

Fig. 1 shows results for rabbits given daily intramuscular injections of 2 mg sodium aurothiomalate (Maybaker, SA) for four weeks. These rabbits therefore received a total of 56 mg of aurothiomalate, a dose comparable with the human therapeutic dose, per kilogram of body mass.[1] Immediately after the four weeks of aurothiomalate treatment the rabbits were given an injection of a pyrogen; either endotoxin (lipopolysaccharide (LPS) extracted from *Salmonella typhosa*, Difco, 0·08 µg/kg) or
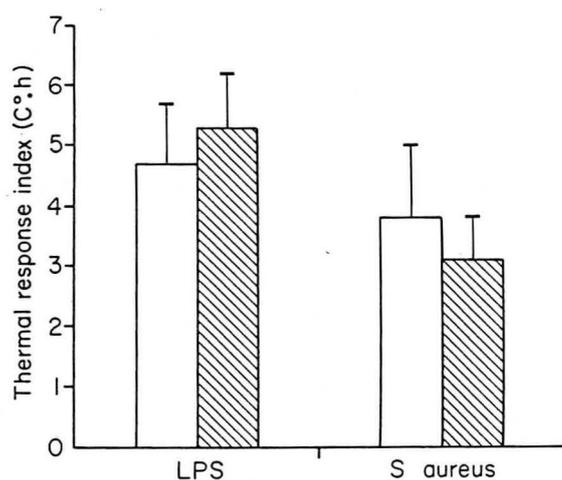
Fig 1 *Thermal response index (°C.h) of rabbits after intravenous injection of 0·08 µg/kg endotoxin (lipopolysaccharide) and of 5×10⁷ organisms of killed Staphylococcus aureus with (hatched) and without (open) daily treatment of 2 mg sodium aurothiomalate for four weeks before pyrogen injection. The mean of the four hour thermal response index (SD) is shown, with n=5 for lipopolysaccharide and n=3 for S aureus.*

Staphylococcus aureus (Calbiochem, 5×10^7 killed organisms). Control animals received the pyrogens without aurothiomalate pretreatment. The four hour thermal response index, calculated as described by Clark and Cumby,[12] was used as an index of fever. The thermal response index is the time integral (°C.h) of the rise in rectal temperature after injection of pyrogen.

For both pyrogens the thermal response index in rabbits treated with aurothiomalate was not significantly different from that for control rabbits ($p>0·05$, unpaired t test). Also, the fevers did not differ significantly in either peak amplitude or latency of onset. Chronic aurothiomalate treatment, therefore, had no significant antipyretic effect.

Acute aurothiomalate treatment also had no antipyretic effect. In six other rabbits we injected 10 mg of sodium aurothiomalate directly into the ear vein together with 1 µg of endotoxin. The fever which ensued had the same latency and peak amplitude as the fever evident when the endotoxin was injected alone. A potential antipyretic action of aurothiomalate could have been masked if intravenous aurothiomalate itself were pyrogenic. Intravenous aurothiomalate (10 mg) on its own, however, had no significant effect on rectal temperature.

As aurothiomalate, in two dosage regimens, had no significant antipyretic effect against two different pyrogens we concluded tentatively that it did not inhibit IL1 production. To confirm our conclusions we measured IL1 production by rabbit monocytes in vitro. Monocytes were isolated with Hypaque-Ficole (Pharmacia). Cell viability was determined by the trypan blue method. Then about 10^6 viable cells were incubated with each of three concentrations of sodium aurothiomalate (2·5, 25, and 100 µg/ml) in 1 ml of RPMI 1640–10% fetal calf serum for 96 hours at 37°C in an atmosphere of 95% air, 5% CO_2, and 100% humidity. All incubations were carried out in triplicate. After the incubation period the cells were harvested and cell viability again determined. The harvested cells then were stimulated for one hour with endotoxin (0·5–1 µg/10^5 cells), washed, and resuspended in fresh medium. The suspension was centrifuged 24 hours later, and the supernatants were collected and tested for IL1 activity by the lymphocyte activating factor assay.[13]

The lymphocyte activating factor assay showed IL1 activity only in supernatants from control monocytes and those incubated with the low concentration of sodium aurothiomalate (2·5 µg/ml), in which IL1 activity was 20% lower than control. The Table shows why there was an apparent reduction of IL1 at 2·5 µg/ml concentration and an absence of activity at higher concentrations: even at low concentration aurothiomalate killed almost all the monocytes. Indeed, when IL1 production was expressed per viable monocyte, production was enhanced in the presence of aurothiomalate.

Was the production of IL1 per viable leucocyte also enhanced in vivo? We measured the total leucocyte count in the rabbits before (mean (SE) 7·2 $(1·5) \times 10^9$/l) and after $(3·7 (1·6) \times 10^9$/l) treatment with 2 mg of intramuscular aurothiomalate a day for four weeks. The decline was highly significant ($p<0·01$, n=5, unpaired t test). The finding that fever was unaffected despite a halving in leucocyte count leads us to believe that, if anything, IL1 production per leucocyte was enhanced in vivo too.

Table *Changes in number of viable rabbit monocytes (mean of three runs each) after incubation for 96 hours with varying concentrations of sodium aurothiomalate*

Aurothiomalate concentration (µg/ml)	No of viable cells/µl		
	Before incubation	After incubation	Change (%)
0	690	650	6
2·5	880	87	90
25	1120	43	96
100	820	22	97

Discussion

Sodium aurothiomalate inhibits phagocytic activity in inflammation.[14-16] In vivo studies have shown that sodium aurothiomalate inhibits phagocytosis by both polymorphonuclear leucocytes and macrophages,[17] and subsequent in vitro studies have confirmed the inhibition of phagocytic activity of peripheral blood monocytes and tissue macrophages.[18 19] Phagocytic cells release IL1, which is a mediator of inflammation, so one may reasonably suppose that sodium aurothiomalate would inhibit IL1 release. If so, it should act as an antipyretic because IL1 also is an essential intermediate in fever.[10] Our results do not support this supposition. We showed that the rabbit's response to intravenous infection of the pyrogens endotoxin and *S aureus* was the same with or without sodium aurothiomalate treatment, whether aurothiomalate was given simultaneously with pyrogen or daily for four weeks before the pyrogen. In vitro, rabbit monocytes still produced IL1 after incubation with a concentration of sodium aurothiomalate that killed about 90% of the cells.

In vivo, sodium aurothiomalate treatment for four weeks reduced the number of circulating leucocytes by about 50%. In addition to inhibiting some of the inflammatory effects of leucocytes, gold therefore appears to cause a net decrease in the total number of circulating leucocytes. Leucopenia in humans after sodium aurothiomalate treatment has been noted previously.[20] The reduction in leucocyte count did not affect the animals' ability to develop fever, an observation which we have made in other circumstances.[21] It may be that aurothiomalate enhanced the release of IL1 from the surviving leucocytes, as indicated by our in vitro results.

We conclude that sodium aurothiomalate treatment, although it affects leucocyte count, does not affect fever, and therefore cannot inhibit IL1 production. This conclusion was supported by direct measurements of IL1 production.

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