Effect of sodium aurothiomalate on carrageenin induced inflammation of the air pouch in mice

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Abstract
Acute inflammation was induced by injecting carrageenin into a 6 day old air pouch in mice. Sodium aurothiomalate was then given twice to each of three groups of mice via different routes. It was found that the mice injected intravenously with sodium aurothiomalate showed the most striking reduction in the number of exudate leucocytes in the inflammatory cavity, although the amount of gold found in their inflamed pouch lining tissue was the least. The amount of gold in plasma was highest in the mice injected intravenously with sodium aurothiomalate and the least amount of gold was found in the mice injected directly into the air pouch with sodium aurothiomalate. The amount of gold in the inflamed pouch lining tissue reached its peak at 24 hours after injection and a significant decrease of exudate leucocytes was only seen 24 and 72 hours after injection. The amount of gold in the exudate fluid was negligible at all the times studied. No significant difference was noted in the degree of inflammatory suppression when increasing doses of sodium aurothiomalate were injected into the air pouch.

These findings show that there is no direct correlation between the gold concentration in the inflamed tissue and suppression of the inflammatory reactions in the cavity. Cytotoxic and phagocytic analysis of leucocytes in the exudate showed that there was a significant suppression of the neutrophil activities in all the mice treated with sodium aurothiomalate. It is therefore concluded that the significant reduction in the number of exudate leucocytes at the carrageenin induced inflammatory site after treatment with sodium aurothiomalate is most likely due to the direct action of gold on the functional activities of circulating neutrophils.

Gold compounds have been proved to be effective in treating rheumatoid arthritis which is characterised by an active synovitis of the joints. However, the mechanism of action in the suppression of acute inflammation is still not clearly understood. In vitro studies show that gold compounds inhibit various functions of circulating leucocytes. This leads to the suggestion that the efficacy of the gold compounds in treatment may relate to the suppression of immune processes. A fall in the number of circulating lymphocytes in patients with rheumatoid arthritis appears to be independent of the clinical efficacy of treatment with gold. Studies on the correlation between gold concentrations in whole blood and synovial tissues and clinical efficacy have also resulted in conflicting conclusions.

This study was undertaken to determine the effect of gold in the early phase of acute inflammation. It is hoped this may help to explain the mechanism of action of gold on active synovitis in patients with rheumatoid arthritis. A six day old air pouch in the mouse was chosen as a bioassay because the pouch can easily be used for studying inflammatory responses and shows many characteristic features of synovium, reacting to various stimuli in a similar way.

Materials and methods
MICE
Female Swiss albino mice, each weighing about 20–25 g, were used throughout the experiments. The mice were fed freely with mouse pellets and water.

Air pouches were induced by injecting 5 ml of air into the subcutaneous tissue on the back of the mice. Three days later the air pouch was reinforced with 2.5 ml of air. Carrageenin powder (kindly provided by Marine Colloid) was dissolved in 0.9% saline to a concentration of 10 mg/ml. The solution was sterilised and homogenised by storing in an oven at 90°C for about one hour. Carrageenin solution (1 ml), maintained at 37°C, was injected into the 6 day old air pouch to induce inflammation.

Sodium aurothiomalate solution (May and Baker) for injection was prepared at a dose of 0.7 µg/g body weight in 0.9% saline. The volume used ranged from 0.10 to 0.15 ml. All injected solutions were sterilised by passage through Millipore filter chambers before use. Unless otherwise stated, the mice were injected intravenously through the tail vein and then killed at 1, 5, 24 or 72 hours after the sodium aurothiomalate treatment. For controls, mice were injected intravenously with 0.9% saline (37°C), anaesthetised with ether and bled through the jugular vein. Kidney, liver, pouch lining tissue, and exudate fluid were removed for the determination of gold.

EXUDATE LEUCOCYTE COUNT
At various times after the injection of sodium aurothiomalate or saline into the mice, control and test mice were killed and 1 ml of cold 0-9% saline was injected into the pouch. The exudate was collected and cooled in an ice bath. The exudate volume was noted. All the leucocytes in...
Effect of sodium aurothiomalate on inflammation

CHEMOTAXIS AND PHAGOCYTOSIS OF EXUDATE LEUCOCYTES
Chemotaxis of the exudate leucocytes was assessed by the modified Boyden chamber technique. The chamber consisted of two compartments and a Millipore filter of pore diameter 3 μm was placed between the two compartments. Exudate leucocytes were suspended in Hanks’s balanced salt solution with 1% heat inactivated (56°C) fetal calf serum. The viability of the exudate cells was determined by the trypan blue exclusion technique. A 100 μl aliquot of the cell suspension (5 × 10^6 cells/ml) was taken and added to the upper compartment. The synthetic peptide formylmethionyl-leucyl-phenylalanine was prepared with the same Hanks’s balanced salt solution at a concentration of 10^−8 mol/l and was used as chemoattractant and placed in the lower compartment.

The chambers were then incubated for 90 minutes at 37°C. At the end of the incubation, the filters were removed and rinsed in Hanks’s balanced salt solution and fixed in ethanol, followed by staining with Masson haemalum.

Cell migration in the filter papers was assessed microscopically using the leading front technique. Each experiment was repeated five times.

The phagocytic activity of the exudate leucocytes was assessed with zymosan particles, which were clearly identifiable microscopically in the phagocytic cells. Zymosan (6 mg; Sigma) in 1 ml of sterile saline was injected into the air pouch of the control and test mice. Five hours later the mice were killed and the air pouches lavaged. The exudates were washed with Hanks’s balanced salt solution and centrifuged. The cell pellets were then mixed with 1% egg albumen solution and the cell suspension was smeared on glass slides and stained with Masson haemalum. The number of zymosan particles phagocytised by 200 neutrophils was randomly counted from four slides for each mouse.

DETERMINATION OF GOLD IN TISSUES
The dry ashing method recommended by Grahame et al was used for the determination of gold in various tissues. Tissue and fluid samples of about 0.5 g were weighed in tared porcelain crucibles. The samples were dried overnight at 60°C. The dried samples were then charred over a hot plate for about two hours, after which they were ashed in a furnace at 480°C for at least eight hours. The residues were dissolved in 5 ml 20% hydrochloric acid with moderate heating. The solutions were filtered into 10 ml calibrated flasks using an ashless Whatman 42 filter paper. The crucibles were rinsed with 1% hydrochloric acid with moderate heating at least twice and then filtered. The solutions were analysed for gold with a Hitachi Z-7000 polarised zeeman atomic absorption spectrometer with a graphite furnace.

STATISTICAL ANALYSIS
All the data were summarised and tabulated as mean (standard error). The significance of the result was analysed by Student’s t test. A value of p<0.05 was considered to be significant.

Results
To determine whether different methods of giving sodium aurothiomalate to mice affected the efficacy of the gold in suppressing the acute inflammation, the following experiment was carried out. Three days after the injection of carrageenan into the 6 day old air pouch, sodium aurothiomalate was injected into the mice by three different routes: (a) intravenously through the tail vein (group II); (b) intramuscularly into the muscle of the right thigh (group III), and (c) directly into the air pouch (group IV). A second injection of the same dose was given 10 days after the induction of inflammation. Group I was used as a control group which received no sodium aurothiomalate. Mice from all four groups were killed four days after the second injection of the gold compound. Figure 1 shows that the leucocyte count in the exudate of all the test groups (II, III, IV) was significantly lower (p<0.05) than that of the control group (I). Figure 1 also shows that mice in group II which received an intravenous injection of sodium aurothiomalate showed a significant decrease in gold retention. Figure 2 shows that the total exudate leucocytes in the inflammatory air pouches of mice treated with different routes of administration of sodium aurothiomalate (gold sodium aurothiomalate) (GSTM)). n=8; *p<0.05 = significantly lower when compared with other test groups (III and IV), a* p<0.05 significantly lower when compared with Group IV, Group I, control; Group II, intravenous injection of GSTM; group III, intramuscular injection of GSTM; and Group IV, direct injection of GSTM into the air pouch.
significantly lower (p<0.05) number of leucocytes than those of the other two test groups (III and IV). The amount of gold in the pouch lining tissue of group II mice was similar to that of group III mice, but significantly lower (p<0.05) than that of group IV mice, which received sodium aurothiomalate injected directly into the inflammatory cavity.

Gold was determined in plasma at one and five minute intervals immediately after a single injection of sodium aurothiomalate. Figure 2 shows that the mice injected both intravenously and intramuscularly with sodium aurothiomalate had significantly higher (p<0.05) gold concentrations than the group in which sodium aurothiomalate was injected directly into the air pouch.

Figure 3 shows the amounts of gold in liver, kidney, plasma, exudate fluid and pouch lining tissue at various times after a single intravenous injection of sodium aurothiomalate. One hour after injection, the highest concentration of gold was in the plasma, followed by the kidney, liver and pouch lining tissues. Five hours after injection, the amount of gold in kidney tissue was almost three times that present after one hour. At 24 hours after injection the amount of gold in the pouch lining tissue reached its peak value, whereas the concentrations of gold in other organs were decreasing. At 72 hours after injection, the concentration of gold in the tissues of most of the organs was reduced to negligible levels, except for the kidney which retained 0.435 µg/g fresh weight. Figure 4 shows that there was no significant difference in the exudate volume between the control and test mice at any time. The total number of exudate leucocytes in the test animals at 24 and 72 hours after injection showed significantly lower (p<0.05) values than those of the control mice.

An experiment was conducted to determine whether gold has a paradoxical, locally pro-inflammatory action by injecting various

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### Figure 2
Amount of gold in plasma after a single injection of sodium aurothiomalate (GSTM); n=8.

### Figure 3
Concentration of gold in liver, kidney, plasma, exudate fluid, and pouch lining tissue at various times after an intravenous injection of a single dose of sodium aurothiomalate; n=8.

### Figure 4
The inflammatory reactivity of exudate leucocytes and exudate volume from the experiment of fig 3. *p<0.05 = significantly lower compared with control mice at the same time.
amounts of sodium aurothiomalate directly into the inflammatory air pouch. Figure 5 shows that the total number of exudate leucocytes in all the test groups was lower compared with the control values. No significant difference was found among all the test groups.

The phagocytic capacity of exudate neutrophils was determined using zymosan as the indicator. Figure 6 shows that the amount of zymosan phagocyted by exudate neutrophils in all the test groups was significantly lower (p<0.05) than that of the control group and the group injected with saline. The chemotaxis of the exudate neutrophils was also studied. Figure 7 shows that a significant reduction (p<0.05) in neutrophil chemotaxis was found in all the groups treated with sodium aurothiomalate. There was a significant difference (p<0.05) between the groups injected intravenously and intramuscularly with sodium aurothiomalate and the group injected directly into the air pouch.

Discussion
These findings show that there is no direct correlation between the concentration of gold in the inflamed pouch lining tissue (facsimile synovium) and the suppression of the acute inflammation in the cavity. However, all the test groups, regardless of the different routes of administration of sodium aurothiomalate showed a significant inhibitory action on the carrageenan induced inflammation. Figure 1 raises two interesting questions: (a) why is there a significant difference in the suppression of active inflammation in the test groups between the groups injected intravenously and intramuscularly with sodium aurothiomalate when the inflamed pouch lining tissue of these groups did not show any significant difference in the amount of gold?; (b) although a direct injection of sodium aurothiomalate into the inflammatory cavity (group IV) gave the highest concentration of gold in the pouch lining tissue, why was the suppression of the number of leucocytes in the inflammatory exudate the least in this group?

To explain these findings the structural characteristics of the 6 day old air pouch must be considered. Edwards et al. showed that the tissue lining of the 6 day old air pouch has many similar features to joint synovium. In our previous work, we showed that the lining tissue of the 6 day old air pouch developed into a very effective barrier for retaining, removing
and depositing substances injected into the air cavity. Figure 2 shows that the amount of gold in plasma after a single dose of sodium aurothiomalate injected into the air pouch was the lowest, followed by an injection of sodium aurothiomalate intravenously and intramuscularly. It is therefore reasonable to assume that the rate of escape of the injected gold from the cavity into the blood might not be as rapid as that injected into the muscle (group III) and into the blood circulation (group II). This might explain why the pouch lining tissue in the group of mice receiving a direct injection of sodium aurothiomalate into the cavity showed the highest level of gold retention. As gold has been shown by in vitro studies to inhibit phagocytosis, lysosomal enzyme release and the leukotriene production of leucocytes,16-18 it would be expected that a direct presentation of sodium aurothiomalate to the leucocytes at the inflammatory site would show the best inhibitory effect of the acute inflammation compared with the other two routes of administration. However, this is not so.

Figure 5 shows that there was no significant difference in leucocyte suppression when increasing doses of sodium aurothiomalate were injected into the inflammatory pouch. As gold concentrations in the inflamed pouch lining tissue do not reflect the efficacy of the action of gold on the suppression of the acute inflammation in the cavity, we speculate that the significant decrease of exudate leucocytes after sodium aurothiomalate treatment is most likely due to the suppressing effect of the gold on the functional activities of the circulating leucocytes. Therefore, it appears that the concentrations of gold present in the circulating blood is an important factor which determines the degree of inflammatory suppression. This inhibitory effect is slowly taking place in the circulating leucocytes.

Figure 4 shows that the significant suppression of acute inflammation was encountered 24 hours after the intravenous injection of sodium aurothiomalate. It is interesting to note that the action of gold showed less effectiveness in reducing the volume of exudate fluid compared with the exudate leucocytes. This suggests that the blood vessels of the inflammatory site are the least affected by gold. Using tissue oedema as a measure of the efficacy of gold in the treatment of arthritis, such as the measurement of the inflamed paws in animals19 might therefore possibly lead to wrong conclusions being drawn.

As the inflammatory effect of carrageenan has been clearly shown to cause a massive influx of predominantly neutrophilic leucocytes from the blood circulation into the cavity,13 14 it is reasonable to assume that the significant decrease of exudate leucocytes at 24 and 72 hours after injection may be attributable to the action of gold in inhibiting the emigration of the circulating neutrophils from the blood towards the carrageenan in the cavity. This assumption is well supported by the finding (fig 7) that gold inhibits the chemotactic response of the neutrophils to different degrees via different routes of sodium aurothiomalate injection. A similar observation was made in the action of gold on neutrophils towards various types of irritants.16-19 This study also shows that gold could suppress the phagocytic capability of the neutrophils. It cannot be ruled out that gold also alters other responses of the circulating neutrophils, as shown by in vitro studies.20-22

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