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Erythrocyte Membrane Gold Levels After Treatment with Auranofin and Sodium Aurothiomalate

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Abstract Triethylphosphine gold-2,3,4,6-tetra-*o*-acetyl-L-thio-D-glucopyranoside (auranofin and sodium aurothiomalate; Myocrisin®) are two chemically different gold compounds used to treat rheumatoid arthritis. This study highlights the interaction, in vivo, of these drugs with erythrocyte membrane in patients with rheumatoid arthritis. Fifty-eight patients with definite or classical rheumatoid arthritis were included in this study and randomly allocated to three groups as 18 patients in the Myocrisin® group, 20 patients in the auranofin group, and 20 patients in the placebo group. The drugs appeared to react, in vivo, in different ways. With Myocrisin®, the level of gold in erythrocyte membrane was, initially, very high and decayed exponentially afterwards, whereas auranofin produced a constant high level up to 36 weeks. The erythrocyte membrane gold level in nonsmokers was higher than that in smokers in the auranofin group, and it decreased with an increase in the number of cigarettes smoked (r=0.836 P<0.01); no such correlation was observed in the Myocrisin® group. In a changeover study, auranofin appeared to change the nature of erythrocyte membrane after reacting with it and rendering it incapable of picking up any gold from Myocrisin®. In the case of auranofin, the hemolysate membrane gold level was found to correlate with clinical improvement.

Keywords Auranofin · Myocrisin® · NSAIDs · Rheumatoid arthritis · Gold drugs

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Introduction

The use of gold drugs in the treatment of rheumatoid arthritis has been well established [1–3], but their mechanism of action is still unknown. Similarly, very little is known about the in vivo chemistry of gold. A variety of gold compounds having different clinical efficacy are in use. All the gold compounds with the exception of gold-2,3,4,6-tetra-*o*-acetyl-L-thio-D-glucopyranoside (auranofin) are administered parenterally. Auranofin is an oral gold compound with less toxic effects as compared with the parenterals. Although these drugs have been successfully used for quite a long time, there exists no specific parameter, such as the plasma gold and hemolysate gold levels or the hemolysate-to-plasma gold ratio [4], which could correlate with their clinical efficacy or toxicity. In the present study, an attempt has been made to find such a correlation between the erythrocyte membrane gold level or hemolysate-to-plasma gold ratio and clinical improvement after treatment with sodium aurothiomalate (Myocrisin®) and auranofin. Myocrisin® is a Au(I)-thiolato compound, whereas auranofin is a phospine-Au(I)-thiolato complex. The structures of these drugs are shown in Fig. 1.

Subjects and Methods

Fifty-eight patients with definite or classical rheumatoid arthritis, according to the criteria of the American Rheumatology Association, were included in this study and randomly allocated to three groups as 18 patients in the Myocrisin® group (eight male and ten female; median age 50, range 35–76), 20 patients in the auranofin group (seven male and 13 female; median age 53, range 30–72), and 20 patients in the placebo group (nine male and 11 female, median age 55 years, range 36–69). All had active synovitis unresponsive to nonsteroidal anti-inflammatory drugs. They had not previously been treated with gold in any form and had not received penicillamine, levamisole, immunosuppressive drugs, or corticosteroids in the 3 months preceding this study.

The patients in the Myocrisin® group were given intramuscular injections of Myocrisin® (50 mg) weekly for 12 weeks following an initial test injection of 10 mg. Thereafter, the frequency of injection was varied from 1 to 4 weeks according to clinical response. The auranofin group received auranofin 3 mg b. d. for 24 weeks. The placebo group received placebo tablets identical to auranofin twice daily. In this trial, ten patients, after completing the one treatment, received the second treatment as well. The sequence of therapy was: Myocrisin®-to-auranofin (four patients) and auranofin-to-Myocrisin® (six patients).

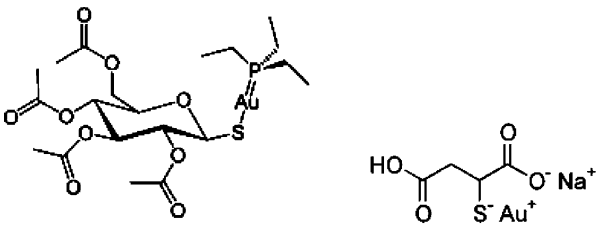


Fig. 1 Structure of drugs under investigation

Table 1 Gold Levels (μg mL^{-1}) of Patients Withdrawing from the Trial

Treatment	Week	Reason for withdrawal (number of patients)	Plasma gold	Hemolysate gold	Membrane gold
Myocrisin®	13	Rash (1)	3.1	0.43	ND[a]
	3	Nitritoid reaction (1)	1.95	1.38	ND[a]
	22	Proteinuria (1)	1.1	0.23	0.05
Auranofin	6	Diarrhea (1)	0.33	0.19	0.04
	7	Leucopenia (1)	0.32	0.20	0.05
	10	Hematuria (1)	0.20	0.17	0.13
Placebo	2	Diarrhea (1)	–	–	–
	6	Hematuria (1)	–	–	–
	15	Lack of effect (4)	–	–	–

[a] *ND* Not detectable

The participants were educated about the type of study, safety of medicine, and possible undesirable effects, and consent was obtained. All the procedures followed were in accordance with the current revision of the Helsinki Declaration, and all the subjects used in this study gave informed consent. The study was approved by the Ethics Committee of the University of Sargodha Medical College. The study was carried out at the affiliated hospital of the University of Sargodha under responsibility of a rheumatologist at the hospital.

Blood was taken by venesection prior to the next gold or placebo dose at 0, 3, 6, 12, 24, and 36 weeks. The blood fractions were obtained by centrifuging 5 mL heparinized blood sample at 3,000×*g* for 10 min. The plasma was removed by suction, and cells were washed twice with isotonic saline solution and lysed with an equal volume of distilled water for 2 h at 4 °C. Hemolysate was removed by suction after recentrifuging the sample. Whole-blood, plasma, and hemolysate gold levels were determined by graphite-furnace atomic absorption spectrometry as reported in literature [5,6]. The limit of quantification and accuracy of the method were better than 1 ng mL^{-1} and 93%, respectively. Erythrocyte membrane gold level was determined by subtraction of plasma and lysate gold from whole-blood gold. The clinical assessment of disease activity was performed on a weekly basis by the hospital staff at the university hospital. Pain score, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were selected as useful parameters for this study. Statistical comparison was

Table 2 Clinical Response of Patients Between Week 0 and Weeks 12 and 24 of the Trial (Wilcoxon's Matched-pairs Signed-rank Test was Used)

Compound	Week	Fall in		
		Pain score (visual analogue scale, 1–10)	ESR (mm h^{-1}, Westergren)	CRP (mg L^{-1})
Auranofin	12	8.5±1.5 to 1.7±0.5 ($p<0.05$)	NS[a]	NS[a]
	24	NS[a]	43±15 to 32±5 ($p<0.01$)	36±8 to <1 ($p<0.05$)
Myocrisin®	12	8.0±1.3 to 2.0±0.4 ($p<0.05$)	38±11 to 29±6 ($p<0.05$)	28±7 to <1 ($p<0.01$)
	24	8.0±1.3 to 1.8±0.5 ($p<0.01$)	38±11 to 25±4 ($p<0.01$)	40±12 to <1 ($p<0.05$)
Placebo	12	NS[a]	NS[a]	NS[a]
	24	8.7±1.3 to 6.5±1.1 ($p<0.01$)	NS[a]	NS[a]

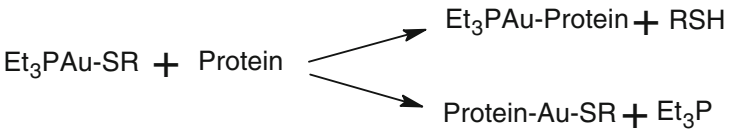
[a] *NS* Not significant

performed by Wilcoxon's matched-pairs signed-rank test or linear regression as appropriate. Correlations were tested for the level of significance using a Student's *t* test. Clinical improvement was indicated by the fall of pain score, ESR, and CRP values.

Results and Discussion

Of the 58 patients, 46 completed the 9-month study. The largest dropout occurred in the placebo group, due to lack of effect. Only a few patients discontinued active therapy. There was no obvious correlation between gold levels and dropout due to rash or other toxic reaction with either Myocrisin® or auranofin. However, the number of dropouts was rather small (Table 1). Both the drugs produced a clinical improvement as indicated by falls in ESR and CRP between 0 and 24 weeks (Table 2).

About 95% of the patients in the auranofin group continued to show appreciable concentration of erythrocyte membrane gold through the period of 36 weeks, whereas very few patients (~2%) possessed this form of gold in the Myocrisin® group. Figure 2 gives the median values along with their interquartile ranges of gold concentration at different intervals of time. With Myocrisin®, the erythrocyte membrane gold level was, initially, very high and decayed exponentially afterwards, whereas auranofin produced a constant high level up to 36 weeks. This result confirms that the two drugs react in vivo in different ways such that the membrane possesses enhanced affinity for auranofin gold. This may be attributed to following exchange reactions with the membrane proteins.



The patients who showed an improvement in disease conditions after 24 weeks of therapy both with Myocrisin® and auranofin also exhibited an initial high level with

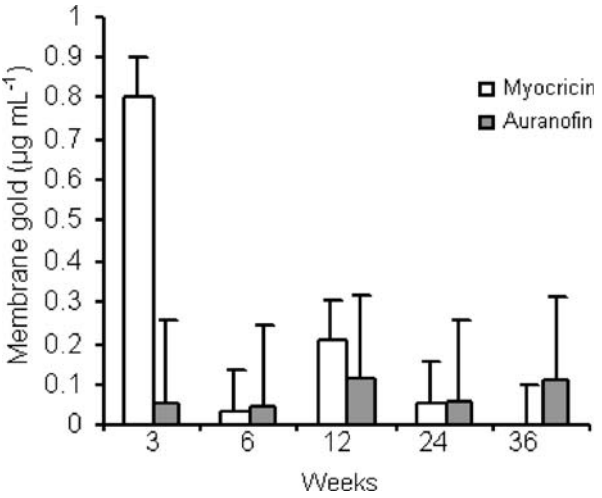


Fig. 2 Erythrocyte membrane gold levels. The heights of the bars are medians, and *error bars* represent interquartile range

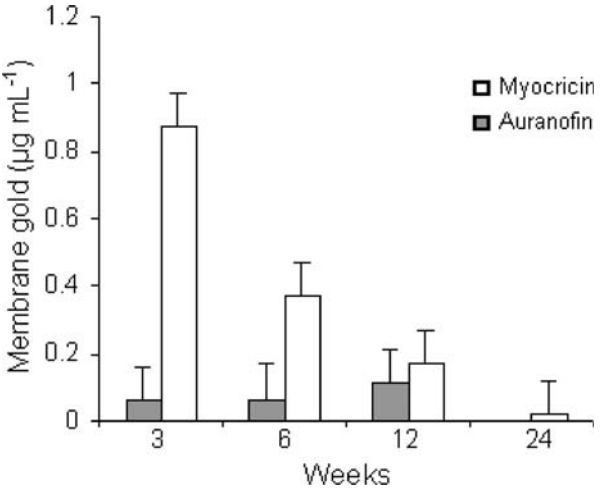


Fig. 3 Erythrocyte membrane gold levels in the improved patients. The heights of the bars are medians, and *error bars* represent interquartile range

Myocrisin®, which decayed exponentially ($r=-0.833$, $P<0.0005$) with time, and a continuous high level ($r=0.957$, $P<0.05$) was observed with auranofin up to 12 weeks (Fig. 3). In nonimprovers, there was no such correlation in the Myocrisin® group, whereas a similar trend was noted in the auranofin group (Fig. 4). About 97% of the patients on "dual" treatment carried the membrane gold in the auranofin group, whereas only 8% had appreciable concentration following Myocrisin®. This is suggestive of the fact that auranofin is changing the nature of the membrane after reacting with it and rendering it incapable of picking up any gold from Myocrisin®. This was confirmed by the case of four patients where the treatment was changed from Myocrisin® to auranofin and the membrane still picked up gold from auranofin.

A significant correlation ($r=-0.858$, $P<0.05$) between hemolysate and the membrane gold levels was found after 3 and 24 weeks of treatment with auranofin. The lysate gold level decreased with an increase in the membrane gold concentration (Fig. 5). There was no

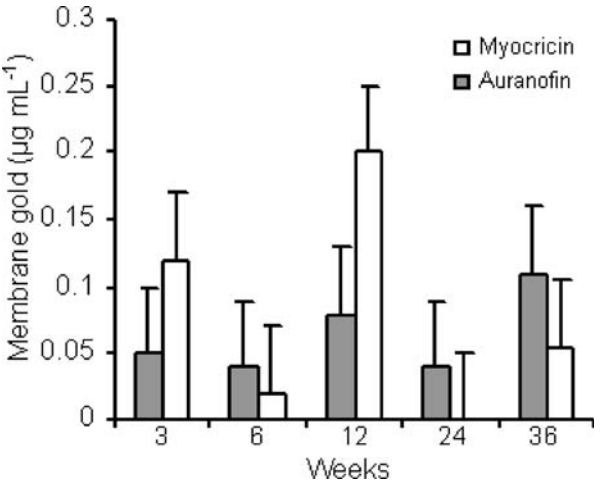


Fig. 4 Erythrocyte membrane gold levels in the unimproved patients. The heights of the bars are medians, and *error bars* represent interquartile range

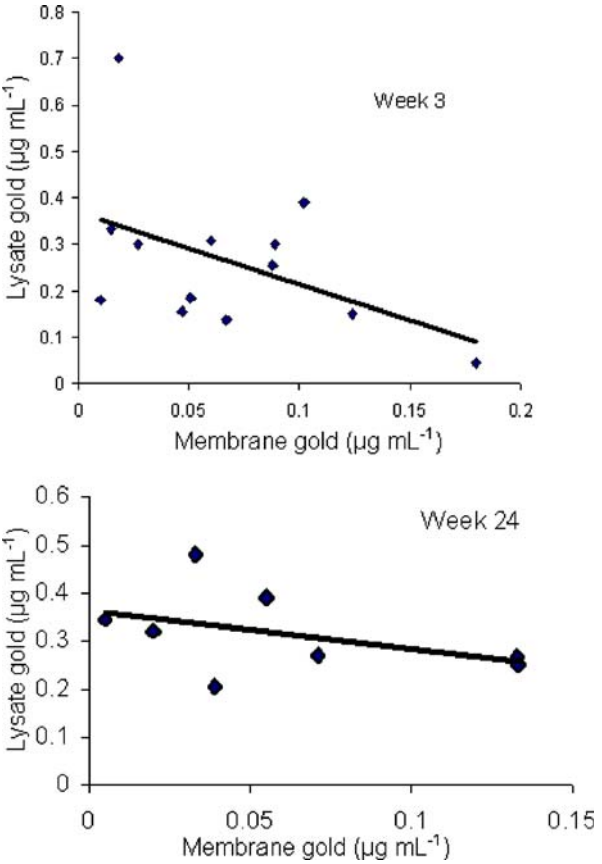


Fig. 5 A relationship between erythrocyte membrane gold and lysate gold levels after 3 and 24 weeks of auranofin therapy

such correlation in the Myocrisin® group, and the correlation between the membrane gold and the plasma gold levels was not significant ($r=-0.212$, $P<0.05$) in case of auranofin as well (Fig. 6).

The membrane gold level was higher in nonsmokers than that in smokers in the auranofin group (Fig. 7), and it decreased with an increase in the number of cigarettes smoked (Fig. 8). The correlation was significant ($r=0.836$, $P<0.01$) statistically. In Myocrisin®, no such correlation was observed. The decrease in the membrane gold level with more smoking suggests that the membrane gold is being leached out by the higher level of cyanide in such patients [7]. Thus, auranofin and Myocrisin® appear to produce chemically distinct metabolites. The auranofin gold seems to produce more mobile complexes in vivo and is easily taken up by the membrane, possibly through a ligand exchange process. Based on these observations, it can be concluded that clinical improvement, as indicated by a significant fall of pain score, ESR, and CRP values, is correlated with the gold level in erythrocyte membrane in case of auranofin.

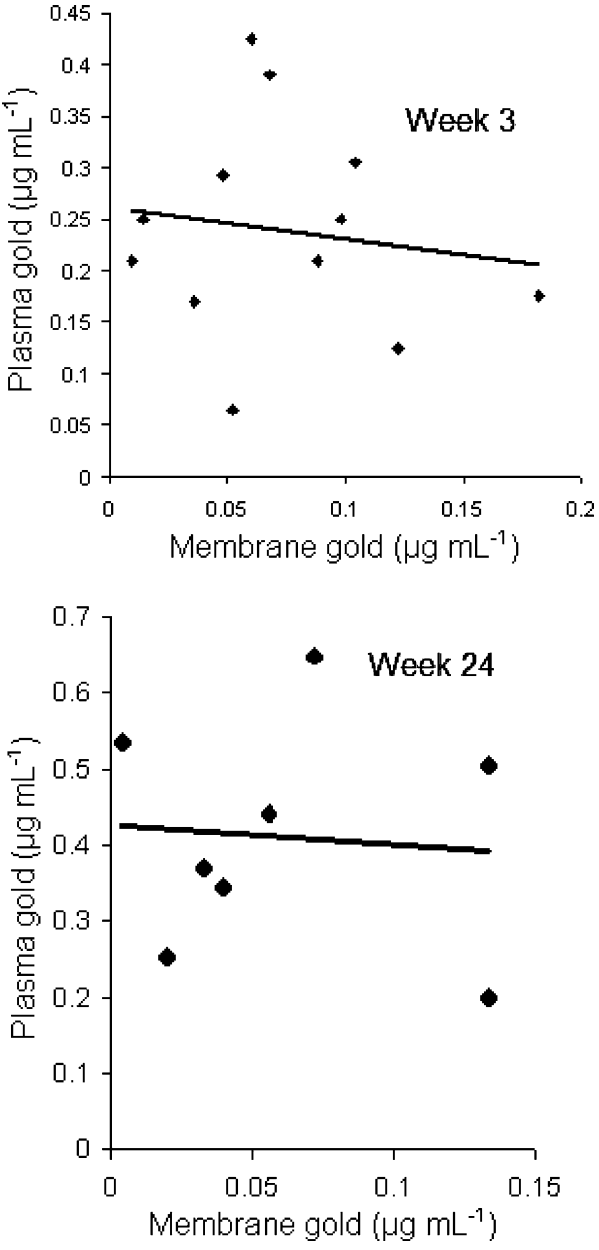


Fig. 6 A relationship between erythrocyte membrane gold and plasma gold levels after 3 and 24 weeks of auranofin therapy

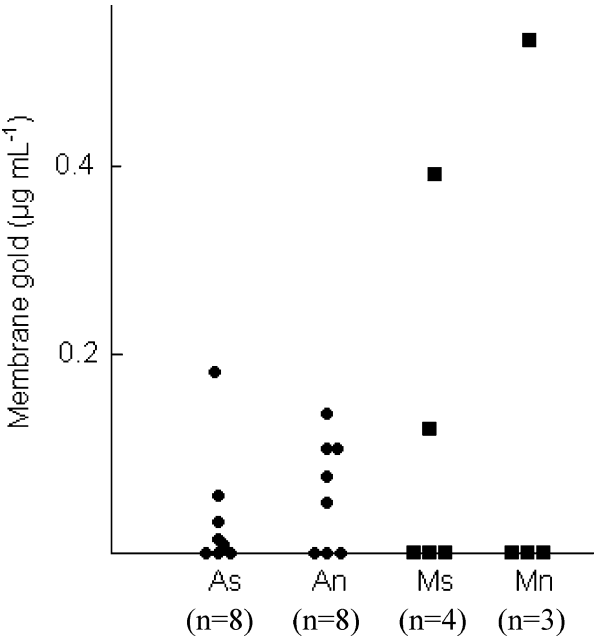


Fig. 7 A comparison of the membrane gold for auranofin (smokers and nonsmokers) with that for Myocrisin® (smokers and nonsmokers). *As* Auranofin (smokers), *An* auranofin (nonsmokers), *Ms* Myocrisin® (smokers), *Mn* Myocrisin® (nonsmokers)

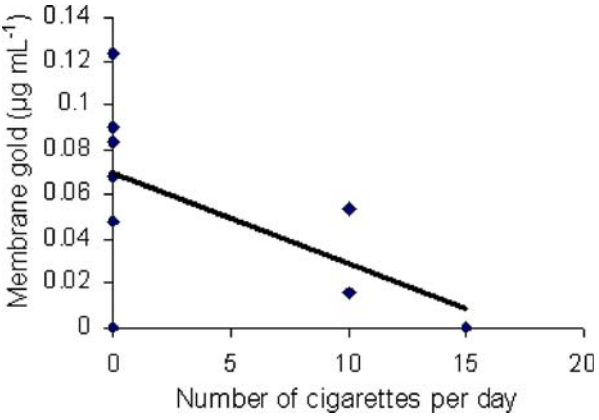


Fig. 8 A relationship between the number of cigarettes smoked and membrane gold levels after 3 weeks of auranofin therapy

Reference

1. Empire Rheumatism Council (1961) Gold therapy in rheumatoid arthritis. Ann Rheum Dis 20:315–334
2. Sigler JW, Bluhm GB, Duncan H, Sharp JT, Ensign DC, McCrum WR (1972) A double-blind study of the effects of gold salts in the treatment of rheumatoid arthritis. Arthritis Rheum 15:125–126
3. Tiekink ER (2003) Phosphinegold(I) thiolates—pharmacological use and potential. Bioinorg Chem Appl 1(1):53–67
4. Lwis D, Capell HA, McNeil CJ, Iqbal MS, Brown DH, Smith WE (1983) Gold levels produced by treatment with auranofin and sodium aurothiomalate. Ann Rheum Dis 42:566–570
5. Kamel H, Brown DH, Ottaway JM, Smith WE (1976) Determination of gold in blood fractions by atomic absorption spectrometry using carbon rod and carbon furnace atomisation. Analyst 101:790–797
6. Egila J, Littlejohn D, Smith WE, Sturrock RD (1992) Gold concentrations in blood fractions of patients with rheumatoid arthritis treated with Myocrisin. J Pharm Biomed Anal 10(9):639–644
7. Tsuge K, Kataoka M, Seto Y (2000) Cyanide and thiocyanate levels in blood and saliva of healthy adult volunteers. J Health Sci 46(5):343–350