Pharmaceutical Research, Vol. 4, No. 4, 1987

Report

Pharmacokinetics of Gold Sodium Thiomalate in Rabbits

Srikumaran Melethil[1] and Darryle Schoepp[1,2]

Received November 6, 1986; accepted April 7, 1987

Male, New Zealand white rabbits (3.5–4.3 kg) received a single 2-mg/kg dose of gold sodium thiomalate (Myochrysine) via intramuscular ($N = 4$) and intravenous ($N = 3$) routes. Blood samples were drawn from the marginal ear vein for a period of 5–10 days. The concentration of gold in whole blood was determined using graphite furnace atomic absorption spectrophotometry. The blood concentration–time profiles obtained following both routes of administration were best described by a two-compartment open model with first-order absorption for the intramuscular route. Gold was absorbed rapidly with a mean (harmonic) absorption half-life of 9.0 min, with a peak concentration of 6.0 ± 1.0 μg/ml ($N = 4$). Blood concentrations declined in a biphasic manner; the mean α half-lives were 0.738 and 1.78 hr for the iv and im routes, respectively. The corresponding terminal (β) half-lives were 54.1 and 63.0 hr. The estimated volume of the central compartment (70 to 93 ml/kg) agreed closely with the rabbit blood volume. The mean (±SD) extent of the dose absorbed following intramuscular injection was 68.9 ± 12.4%.

KEY WORDS: pharmacokinetics; gold; rabbits; intramuscular; intravenous; bioavailability.

INTRODUCTION

Gold compounds have been widely used as antirheumatic agents during the last 60 years. However, very little is known about the pharmacokinetics of gold in humans and animals. Gerber and co-workers (1) reported that following intravenous (iv) administration of gold (^{195}Au) thiomalate, plasma concentrations of gold in six arthritic patients declined in a monoexponential fashion, with a mean half-life of 5.2 days. Unfortunately, the first sample was not drawn until 24 hr postadministration. The results of Harth (2) suggested that the pharmacokinetics of gold may be better described by a two-compartment model. The only detailed investigation on the pharmacokinetics of iv and intramuscular (im) Myochrysine in humans involved a recent study by Waller and co-workers with two subjects (3). Following iv (5 mg) and im (10 mg) administration, plasma gold concentration–time curves were described by a triexponential equation, with mean terminal half-lives of 13.3 and 12.2 days in the two subjects (3). Unfortunately, the sampling protocol was not adequate for the determination of the absorption rate constant. Walz and co-workers (4,5) reported a biphasic decline of gold in rats following im gold sodium thiomalate (6 mg/kg), with mean α and β half-lives of 17.9 and 72 hr, respectively. Other pharmacokinetic parameters were not reported. In contrast, Mason and McQueen (6) reported that a three-compartment model best described the disposition kinetics of im gold (2 mg/kg) in rats; the half-lives for the three phases of disposition and absorption were 15.0, 52.3, 573, and 0.189 hr, respectively. In view of the limited amount of information available in this area, experiments were conducted to compare the pharmacokinetics of gold in rabbits following iv and im administration of gold sodium thiomalate.

EXPERIMENTAL

Male, New Zealand white rabbits weighing approximately 4 kg (3.5–4.3 kg) received a single 2-mg/kg dose of gold sodium thiomalate (Myochrysine, Merck, Sharp and Dohme) via iv ($N = 3$) and im ($N = 4$) routes. The gold content of Myochrysine was determined to be 48%. One-milliliter blood samples were drawn at periodic intervals from the marginal ear veins of the rabbit for a period of 8–10 days, except in two cases where sampling was carried out only for 5–6 days. Samples were drawn approximately every 15 min for the first 2 hr and every 30 min for the next 2 hr. Hourly samples were drawn during the 4- to 8-hr interval, followed by a 12-hr sample; then samples were drawn every 12 hr until the end of the first week. After that, sampling was on a 24-hr basis until the end of the study. All samples were stored refrigerated in heparinized plastic tubes until assayed. The time lag between collection and assay was about 3–4 days.

The gold concentration in blood was measured by the method previously reported by Melethil and co-workers after minor modifications for sample volume (7). A 20-μl aliquot of suitably diluted (20–40 times) blood samples or standard solutions of gold prepared in rabbit blood (0–12 μg/ml) was introduced into the graphite furnace using an Eppendorf pipette and the resulting atomization peak at 242.8 nm was recorded. Calibration curves prepared using peak heights

[1] Schools of Pharmacy and Medicine, University of Missouri—Kansas City, M3-206A, 2411 Holmes, Kansas City, Missouri 64108-2792.

[2] Present address: CNS Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285.

0724-8741/87/0800-0332$05.00/0

obtained from standard solutions were used to determine the concentration of gold in the blood samples. The lowest detectable concentration (before sample dilution) with the method was 0.5 μg/ml.

The blood concentration–time data were fitted to a suitable model using nonlinear regression analysis (8) and standard pharmacokinetic methods (9). The criteria (F test) discussed by Boxenbaum and co-workers (10) and Akaike's Information Criteria (11) were used in deciding on the most appropriate model describing the concentration–time data. In addition, the residuals (observed concentration–calculated concentration) were examined using the run test to check for bias in curve fitting (12). Model-independent analysis was conducted using statistical moment theory (13). Areas under the blood concentration–time curves were calculated using the trapezoidal rule and extrapolated to infinity $(AUC)_0^\infty$. Statistical comparisons were done using the t test for independent samples (14).

RESULTS AND DISCUSSION

Gold concentrations in blood approached limits of detection within 8–10 days after injection via both routes of administration. Representative blood concentration–time curves for gold following iv and im administration of gold sodium thiomalate are shown in Figs. 1 and 2. The profiles (including those not shown) are biphasic and best described by a two-compartment open model, with first-order absorption for the intramuscular route. The parameters estimated via nonlinear regression analysis were α, β, k_{21}, V_c, and k_a [symbols as defined earlier (9)]. For the seven sets of data (three iv and four im), r^2 for regression ranged from 0.978 to 0.996, with r^2 being greater than 0.99 in four cases. Except for α and k_{21}, there was a high degree of confidence in the parameter estimates as was indicated by the low coefficient of variation (less than 10%) and narrow 95% confidence interval. This was not the case for α and k_{21}. The coefficients for these two parameters ranged from 15.8 to 32.2% in five cases, 36.6 and 42% in one iv case (rabbit 6), and 92.6 and 95.1% in one im case (rabbit 2). In three of the seven cases, the 95% confidence interval included the value of zero. Westlake has previously pointed out that wide confidence intervals are to be expected as a general rule, unless the data strictly conform to the model chosen (15). The lack of confidence in estimating α and k_{21} in this study may be due to the fact that their values are close to each other. Wagner has reported that as the value of α approaches that of k_{21}, the two-compartment model collapses to the one-compartment type (16). In this connection it is interesting to note that the correlation matrix for regression analysis obtained in this study for both the iv and the im data showed a high correlation (>0.99 in six animals and 0.97 in one) between α and k_{21}. In principle, when two parameters show such a high degree of correlation, only one of them is needed for curve fitting. However, log concentration vs time profiles appeared biphasic and the F test described by Boxenbaum and co-workers (10) and Akaike's Information Criteria (11) confirmed a two-compartment model (i.e., the need for α and k_{21} to characterize the data). The strong correlation between α and k_{21} for this compound may be due to the high affinity of gold for albumin, which makes distribution between blood and various tissues difficult. Previous *in vitro* binding studies from our laboratory have shown that in the concentration range of 2–10 μg/ml (similar to those encountered in the present study), gold was essentially 100% bound to bovine serum albumin (7). Data from other investigators have shown that rabbit and human albumin also bind gold to such a high degree (17,18).

Values for model parameters are shown in Table I. The

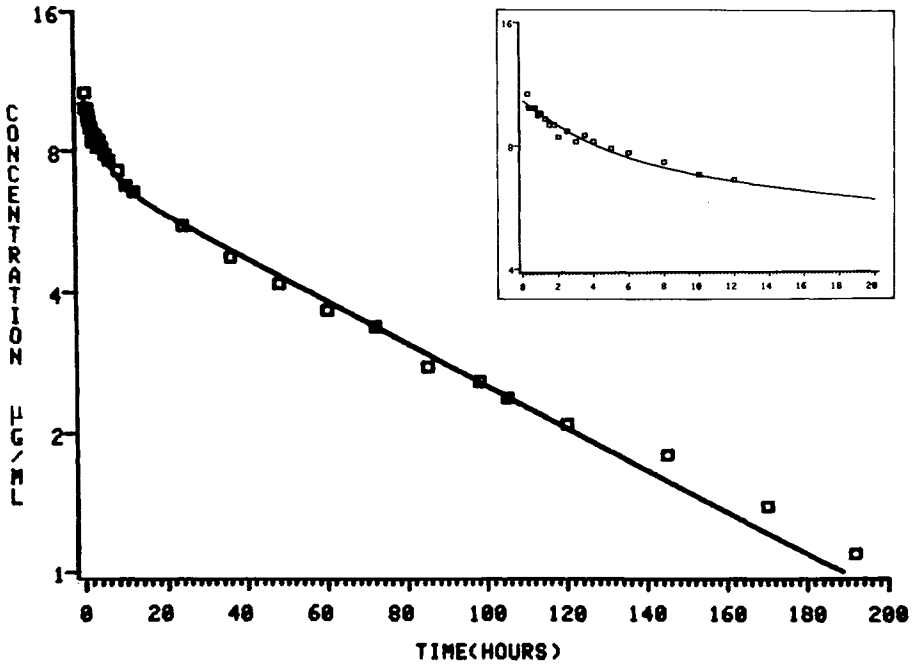

Fig. 1. Blood concentrations of gold as a function of time after iv bolus injection of 2 mg/kg of gold sodium thiomalate in rabbit 7. The line represents the least-squares fit according to a two-compartment model. The inset shows the data for the first 20 hr on an expanded scale.

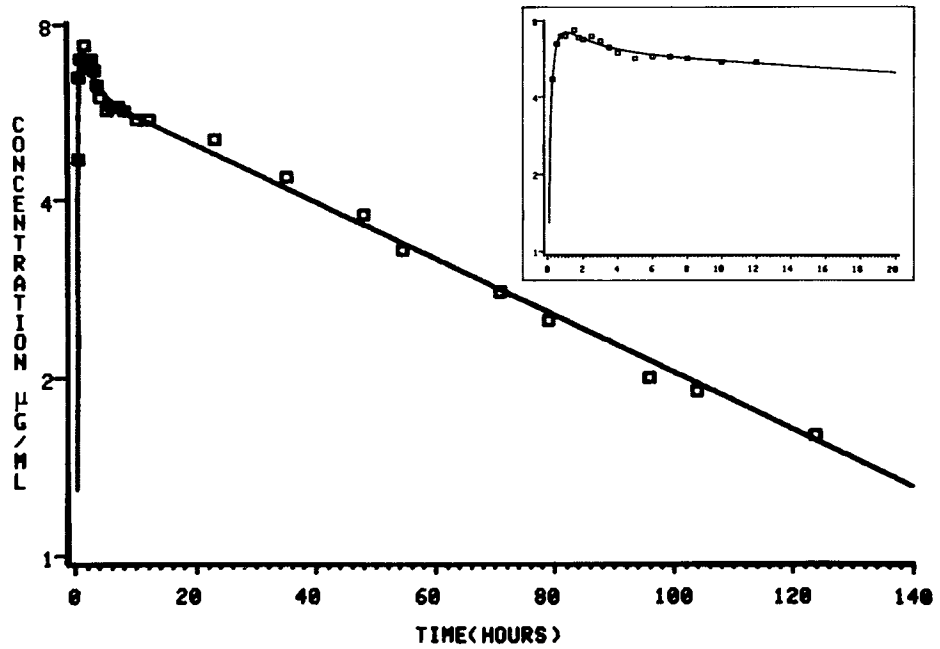

Fig. 2. Blood concentrations of gold as a function of time after im injection of 2 mg/kg of gold sodium thiomalate in rabbit 1. The line represents the least-squares fit according to a two-compartment model with first-order absorption. The inset shows the data for the first 20 hr on an expanded scale.

parameters estimated by regression analysis (α, β, k_{21}, V_c, and k_a) were used to calculate k_{10}, k_{12}, and V_B (volume of distribution in the β phase). For the im and iv routes, α values (mean ± SD) were 0.389 ± 0.225 and 0.939 ± 0.910 hr^{-1}, respectively. These differences were not significantly different ($P > 0.05$), as can be readily observed. If rabbit 6, which appears to be an outlier is excluded, the mean for the iv route becomes similar (0.424 hr^{-1}) to that for the im route. Since absorption was very rapid, it is reasonable to expect that it does not interfere significantly with the distributive

Table I. Pharmacokinetic Parameters for Gold in the Rabbit

Parameter	Rabbit No. 1	2	3	4	5	6	7	Mean ± SD im	Mean ± SD iv
Weight (kg)	3.92	3.50	3.75	4.08	4.39	4.27	4.32	3.81 ± 0.248	4.33 ± 0.0602
Route	im	im	im	im	iv	iv	iv	—	—
Dose (mg)[a]	3.76	3.36	3.60	3.92	4.21	4.10	4.15	3.66 ± 0.239	4.15 ± 0.0551
α (hr^{-1})	0.536	0.126	0.283	0.611	0.600	1.97	0.248	0.389 ± 0.225	0.939 ± 0.910
$t_{1/2}$, α (hr)	1.29	5.50	2.45	1.13	1.16	0.352	2.79	1.78[b]	0.738[b]
β (hr^{-1})	0.0111	0.0109	0.0113	0.0108	0.0119	0.0161	0.0105	0.0110 ± 0.222E	0.0128 ± 2.91E[c]
$t_{1/2}$, β (hr)	62.4	63.6	61.3	64.2	58.2	43.0	66.0	63.0[b]	54.1[b]
k_{12} (hr^{-1})	0.125	0.0126	0.737	0.135	0.267	0.441	0.0666	0.252 ± 0.328	0.258 ± 0.187
k_{21} (hr^{-1})	0.408	0.112	0.205	0.473	0.322	1.53	0.178	0.300 ± 0.469	0.677 ± 0.743
k_{10} (hr^{-1})	0.0146	0.0123	0.0156	0.0140	0.0221	0.0208	0.0147	0.0141 ± 1.38E	0.0192 ± 3.95E
V_c (ml/kg)	—	—	—	—	84.1	69.6	93.2	—	82.3 ± 11.9
V_B (ml/kg)	—	—	—	—	152.2	84.1	127.8	—	121.4 ± 34.5
Cl (ml/hr)[d]	—	—	—	—	7.92	5.78	5.79	—	6.50 ± 1.23
k_a (hr^{-1})	3.44	3.94	3.95	7.14	—	—	—	4.62 ± 1.70	—
$(AUC)_0^\infty$ [($\mu g \times hr$)/ml]	566.1	440.3	399.0	395.1	531.8	709.2	716.4	450.1 ± 79.98*	652.5 ± 104.6
FD/V_c (μg/ml)	8.19	5.45	6.23	5.18	11.4	13.8	10.3	6.26 ± 1.36*	11.8 ± 1.79
MRT (hr)	88.2	90.2	85.5	94.6	83.1	69.7	93.9	89.6 ± 3.84	82.2 ± 12.1
V_{SS} (ml/kg)					658.2	402.9	543.7	—	538.8 ± 127.9

[a] Expressed as gold.
[b] Harmonic mean.
[c] $E = 10^{-3}$.
[d] Dose/$(AUC)_0^\infty$.
* Significantly different from iv ($P < 0.05$).

phase. The mean values of k_{21} (whose values approximate values for α) for the im and iv routes were 0.300 ± 0.469 and 0.677 ± 0.743 hr^{-1}, respectively (see Table I). The $t_{1/2}(\beta)$ values exhibited considerably less variability, with values for six of seven animals ranging from 58.2 to 64.2 hr and one animal (rabbit 6) having a value of 43 hr. For the im and iv routes, the β values were not significantly different ($P > 0.05$); the $t_{1/2}(\beta)$ values (harmonic means) were 63.0 and 55.1 hr, respectively. The values obtained for V_c (see Table I) were approximately 25% higher than the blood volume in rabbits, which range from 55.6 to 57.3 ml/kg (19). In addition, V_c was about 70% of the total volume of distribution (V_B). Therefore, volume terms for distribution of gold may be expected to be similar in magnitude to the blood volume. Results from our previous *in vitro* studies have shown that gold is essentially 100% bound to bovine serum albumin (7). In view of the similarity in binding of gold to bovine and rabbit albumin, the observed relationship between blood volume and volume of distribution is expected. Waller and co-workers (3) reported similar findings in humans.

In the postdistribution phase, the fraction of drug in the central compartment is equal to the ratio α/k_{10}. The mean values for this ratio following im and iv routes were 0.78 and 0.67, respectively. Since gold is extensively bound to plasma proteins, a large fraction of the total body content of gold is expected to be in the central compartment.

As can be seen (Fig. 2), absorption following im administration was very rapid; t_{max} was 1.3 ± 0.58 hr. The mean im absorption rate constant of 4.62 hr^{-1} was in good agreement with the value of 3.67 hr^{-1}, reported by Mason and McQueen, in rats (6). Mean residence time (MRT) values for the drug following iv and im administration, estimated using statistical moments (13), were approximately of equal magnitude (see Table I). It has been suggested that the mean absorption time (MAT) can be estimated from the difference between the MRT (im) and the MRT (iv). In this study, absorption is about 400–500 times faster than elimination [$t_{1/2}(\beta)$, ~50–60 hr). The iv and im profiles were quite similar (see Figs. 1 and 2). Therefore, our results show that this approach may not be possible for drugs like gold thiomalate, whose absorption is severalfold faster than its elimination. For example, rabbit 7 has a MRT (iv) of 93.9 hr, while three of the rabbits (numbers 1–3) have MRT (im) values ranging from 85.5 to 90.2 hr. However, since this study was not done in a crossover design, we recognize that interrabbit variation may be a factor that may make MAT estimations difficult in this study. The ratio of MRT/$t_{1/2}$ in this study (six of seven rabbits) ranged from 1.39 to 1.47. For the iv bolus route, $t_{1/2}$ = 0.693 MRT. Therefore, the expected value of the ratio is 1.44. The reason that the MRT (im) values were close to those for the iv route is due to the extremely rapid absorption in relation to elimination.

No data are currently available on the extent of absorption of an intramuscularly administered dose in animals. The β values for the im (0.0110 ± 0.000222 hr^{-1}; $N = 4$) and iv (0.0128 ± 0.00291 hr^{-1}; $N = 3$) were not significantly different. Therefore, the areas under the concentration–time curve extrapolated to infinity (AUC) for the two routes were compared without correction for β (20). Area values were significantly lower for the im route ($P < 0.05$). By comparing individual area values with that of the mean area value for the iv route, the percentages of the dose absorbed (F) were 86.8, 67.5, 61.1, and 60.1% for rabbits 1, 2, 3, and 4, respectively. Estimates of FD/V_c were also significantly higher for the iv route compared to the im route (see Table I). An F value of 52.7% is obtained from the ratio of these two values and is in reasonable agreement with the F value obtained from (AUC) ratios (mean, 68.9%). A recent article has cited several other examples in humans where im doses have been incompletely absorbed (21). A likely explanation may be the precipitation of the drug at the injection site, followed by removal by phagocytosis.

There has been only one definitive study on the pharmacokinetics of gold thiomalate in humans. Using two subjects, Waller and co-workers (3) reported that (~0.15 mg/kg) following iv administration of gold thiomalate (5 mg or ~0.15 mg/kg), plasma gold concentrations ranged between 0.03 and 1 μg/ml and declined in a triphasic manner, with a terminal half-life of 10.5 and 16.1 days. In these two subjects, a 10-mg dose given im resulted in concentration profiles and terminal half-lives (12.2 days in both subjects) similar to those obtained following the 5-mg iv dose. Apart from the interspecies and concentration differences, the observed biphasic decline in rabbits (as opposed to the triphasic decline of plasma gold in humans) may also be due to the fact that whole blood concentrations were used in the present study.

Bi- or triphasic decline of plasma (blood) gold suggests the existence of regions in the body that do not equilibrate rapidly with plasma gold ("deep compartments"). Therefore, studies aimed at evaluating the blood concentration–response relationship should be conducted only after such equilibration has been achieved. Based on the terminal half-lives reported by Waller *et al.* (3), patients have to be on gold for at least 60 days before such equilibration can be expected. In a thorough review on the subject of monitoring plasma gold concentrations in rheumatoid arthritis, Lorber (22) has pointed out that the existing controversy regarding the concentration–effect relationship stems from the facts that, first, many such investigations have been conducted during early therapy and, second, gold dosing is empirical. A greater understanding of gold pharmacokinetics in humans, particularly regarding its distribution behavior, should be valuable in the optimization of gold dosage in rheumatoid arthritis.

The volumes of the central compartment (V_c) and distribution (V_B) for humans, normalized for body weight, were about 50 and 134% of the rabbit values. The low whole-blood clearance of gold in rabbits is consistent with its extensive binding to plasma proteins. The plasma clearance of gold in humans, normalized for body weight, was about fourfold lower than blood clearances observed in rabbits. In spite of these differences, the fraction of the dose absorbed (F) was quite similar in both species. In the human study, F values in the two subjects were 0.64 and 1.13 (mean, 0.89); the corresponding value in rabbits was 0.69. While studies with larger numbers of subjects are needed, these preliminary studies suggest that the rabbit may be a satisfactory model for bioavailability studies for im gold thiomalate in humans.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Richard N. Reopelle for providing the rabbits and Dr. Carl Metzler for helpful dis-

cussions on data analysis. A part of this study was conducted while the authors were at the College of Pharmacy, North Dakota State University, Fargo, North Dakota 58105.

REFERENCES

1. R. C. Gerber, H. E. Paulus, R. Bluestone, and M. Lederer. *Arth. Rheum.* 15:625–629 (1972).
2. M. Harth. *Clin. Phrmacol. Ther.* 15:354–360 (1974).
3. E. S. Waller, J. W. Massarella, J. E. Crout, and G. J. Yakatan. *Res. Comm. Chem. Pathol. Pharmacol.* 37:33–47 (1982).
4. D. T. Walz, M. J. DiMartino, L. W. Chakrin, B. M. Sutton, and A. Misher. *J. Pharmacol. Exp. Ther.* 197:142–152 (1976).
5. D. T. Walz, D. E. Griswold, M. J. DiMartino, and E. E. Bumbier. *J. Rheumatol.* 7:82–824 (1980).
6. R. W. Mason and E. W. McQueen. *Proc. Univ. Otago Med. School* 55:11–13 (1977).
7. S. Melethil, A. Poklis, and A. J. Sagar. *J. Pharm. Sci.* 69:585–587 (1980).
8. C. M. Metzler, G. L. Elfring, and A. J. McEwen. *Biometrics* 30:562–563 (1974).
9. M. Gibaldi and D. Perrier. *Pharmacokinetics,* Marcel Dekker, New York, 1975, pp. 48–55, 80–83.
10. H. G. Boxenbaum, S. Riegelman, and R. M. Elashoff. *J. Pharmacokin. Biopharm.* 2:123–148 (1974).
11. K. Yamaoka, T. Nakagawa, and T. Uno. *J. Pharmacokin. Biopharm.* 6:165–175 (1978).
12. N. R. Draper and H. Smith. *Applied Regression Analysis,* John Wiley & Sons, New York, 1975, pp. 95–99.
13. K. Yamaoka, T. Nakagawa, and T. Uno. *J. Pharmacokin. Biopharm.* 6:547–558 (1978).
14. G. W. Snedecor and W. G. Cochran. *Statistical Methods,* Iowa State University, Ames, 1978, pp. 100–106.
15. W. J. Westlake. *J. Pharm. Sci.* 60:882–885 (1971).
16. J. G. Wagner. *Fundamentals of Clinical Pharmacokinetics,* Drug Intelligence Publications, Ill., 1975, pp. 154–155.
17. E. G. McQueen and P. W. Dykes. *Ann. Rheum. Dis.* 28:437–442 (1969).
18. E. Jellum, J. Aaseth, and E. Munthe. *Proc. Roy. Soc. Med.* 70 (Suppl. 3):136–139 (1977).
19. S. H. Weisbroth, R. E. Flatt, and A. L. Kraus. *The Biology of the Laboratory Rabbit,* Academic Press, New York, 1974, p. 57.
20. J. G. Wagner. *J. Pharm. Sci.* 36:652–653 (1967).
21. C. B. Tuttle. *Am. J. Hosp. Pharm.* 34:965–968 (1977).
22. A. Lorber. *Clin. Pharmacokin.* 2:127–146 (1977).