

Therapeutic trials in muscular dystrophy

1 Gold in murine dystrophy¹

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SUMMARY A trial of sodium aurothiomalate as an antiproteinase drug in the treatment of murine muscular dystrophy is reported. A blind controlled comparison of high (25 $\mu\text{g}/10$ g body weight) and low dose gold (5 $\mu\text{g}/10$ g body weight) with saline-injected control animals was made, all injections being given three times weekly. The body weights and functional ability of the mice were assessed at weekly intervals. No significant difference between the groups was observed. A trial of very high dose chrysotherapy (500 μg of gold/10 g body weight) also showed no therapeutic benefit.

The aetiology of the human and animal hereditary muscular dystrophies is still unclear, and despite many previous therapeutic attempts no widely accepted remedy is available for any of the muscular dystrophies. There is a marked increase of several proteinases in human and animal dystrophic muscle (Weinstock *et al.*, 1958; Tappel *et al.*, 1962; Pennington, 1963; Park and Pennington, 1965; Kar and Pearson, 1972), while most other non-lysosomal enzymes are decreased in the muscle. It seems likely that these abnormally increased levels of proteinases may cause the rapid breakdown of muscle proteins, whether they are derived from the muscle fibres themselves or from macrophages infiltrating the degenerating muscle. Thus, if it were possible to inhibit these proteinases the rate of skeletal muscle degeneration might be slowed down. A recent report suggests that a number of microbial antiproteinases improve the growth of dystrophic chicken muscle in tissue culture (McGowan *et al.*, 1976). As part of a series of studies searching for agents of therapeutic value in the muscular dystrophies, such as ethylene derivatives and penicillamine (Enomoto and Bradley, 1977a; Bradley, Gardner-Medwin, and Enomoto, in preparation), we investigated the effect of a number of antipro-

teinases on murine muscular dystrophy *in vivo* (Enomoto and Bradley, 1977b), and here report trials of chrysotherapy.

Sodium aurothiomalate has been widely used for the treatment of rheumatoid arthritis (Lockie *et al.*, 1958; Smith *et al.*, 1968). It is postulated that lysosomes from macrophages and other cells within the rheumatoid joint release hydrolytic enzymes which degrade the joint cartilage, and that these enzymes are inhibited by gold salts (Lockie *et al.*, 1958; Persellin and Ziff, 1966; Janoff, 1969). Parenterally administered gold salts enter skeletal muscles (Bertrand *et al.*, 1948; Jeffrey *et al.*, 1958), particularly where the muscle is damaged (Yarom *et al.*, 1976). The gold might thus inhibit the abnormally increased lysosomal enzymes, slowing the rate of muscle degeneration in muscular dystrophy.

Methods

Thirty-three mice with hereditary muscular dystrophy (Bar Harbor 129 ReJ dy/dy) weighing over 10 g at 40 days of age were used. These less severely affected mice were chosen because dystrophic mice weighing under 10 g at 40 days have a short survival period (see Results), which is less useful for a therapeutic trial.

Body weights and functional capacity were assessed weekly. The maximum slope up which the mouse could climb was measured using a fine wire mesh screen. The motility assessment score was obtained by one of us (AE) scoring the follow-

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ing factors on a four point scale (normal 0; slightly abnormal 1; moderately abnormal 2; severely abnormal 3): general condition, maximum speed of running, ability to hang from a horizontal bar by the forelimbs, and hind limb posture. The sum of these four functions was termed the *assessment score*.

The mice were divided into three groups, each consisting of five males and six females. The dystrophic mice were collected into triplets with body weight and functional grouping as similar as possible, and then allotted randomly to group A, B, or C. This procedure was adopted since the prognosis depends on the early functional status (see Results), and it was, therefore, important to ensure that the groups were initially identical.

Sterile sodium aurothiomalate solutions were made in concentrations of 0.1 and 0.02 mg of gold/ml of 0.9% sodium chloride solution, and administered three times per week by intraperitoneal injection at a dose of 0.25 ml/10 g body weight. The trial was conducted in a blind controlled fashion, the assessor (AE) being unaware of the coding of the solutions. Mice of group A received the high dose (25 μ g of gold per 10 g body weight), the mice of group C received the low dose (5 μ g of gold per 10 g body weight). The control group B received 0.9% sodium chloride solution alone. When the mice died, several muscles were removed and examined histologically. Treatment was continued for the whole life of the animals.

In a second experiment, we studied the effect of very high doses of sodium aurothiomalate (500 μ g gold/10 g body weight) on 10 dystrophic mice. These were injected subcutaneously three times per week. The matched control group of 10 dystrophic mice received saline solution. Two additional functional tests were included in the assessment—the fastest time for the mouse to run one metre, and the minimum width on a gradually narrowing bar which the mouse could walk before falling off (average of at least five trials). In the former test, the mouse was induced to run by sprinkling it with 70% alcohol.

Results

SURVIVAL OF CONTROL DYSTROPHIC MICE

A pilot study of the survival of 30 untreated dystrophic mice demonstrated that the prognosis depended on the initial body weight, 94% of those greater than 10 g at 40 days surviving to 90 days of age, compared to 8% of those less than 10 g.

BLIND CONTROLLED STUDY

Survival period and maximum body weight The

Table shows the mean lifespan, maximum body weight, and age at the maximum body weight of the three treatment groups. There was no statistically significant difference between the groups. The proportion of animals surviving at each time during the trial was similar in each group.

Table *Life span and body weights of dystrophic mice in a controlled trial of chrysotherapy*

	Group A (high dose)	Group B (control)	Group C (low dose)
Life span (days)	276 \pm 117 (100–438)	281 \pm 176 (101–635)	225 \pm 88 (74–381)
Maximum body weight (g)	17.6 \pm 1.9 (14.7–21.5)	18.1 \pm 2.2 (14.0–22.4)	16.7 \pm 2.3 (13.8–20.5)
Age at maximum body weight (days)	129 \pm 36 (69–172)	127 \pm 29 (95–188)	102 \pm 28 (39–130)

Mean \pm SD (range in each group).

Body weight The mean body weight of surviving animals at each time point during the trial was similar.

Motility assessment scores The Figure shows that no significant difference appeared between the three groups during the experimental period.

Maximum slope The mean maximum slope which the mice could climb showed wide variations, and there was no significant difference between the group.

Histological examination of the muscle No histological difference between the three groups was detected.

VERY HIGH DOSE EXPERIMENT

No statistically significant difference was found between the experimental and control group, nor were toxic signs observed.

Discussion

Injected gold is retained for a long period in the body. Patients on maintenance chrysotherapy excreted 39, 16, 12, and 10% of the injected dose in the first, second, third, and fourth weeks after the last injection respectively (Mascarenhas *et al.*, 1972). After a single intravenous tracer dose of ^{195}Au -sodium aurothiomalate, 43% of the gold remained in the human body 60 days later (Gerber *et al.*, 1974). A similar prolonged retention is shown in animals (Jeffrey *et al.*, 1958). Chrysotherapy, therefore, leads to a slow accumulation of gold in the tissues, which is of value in the potential use of gold for the treatment of muscular dystrophy.

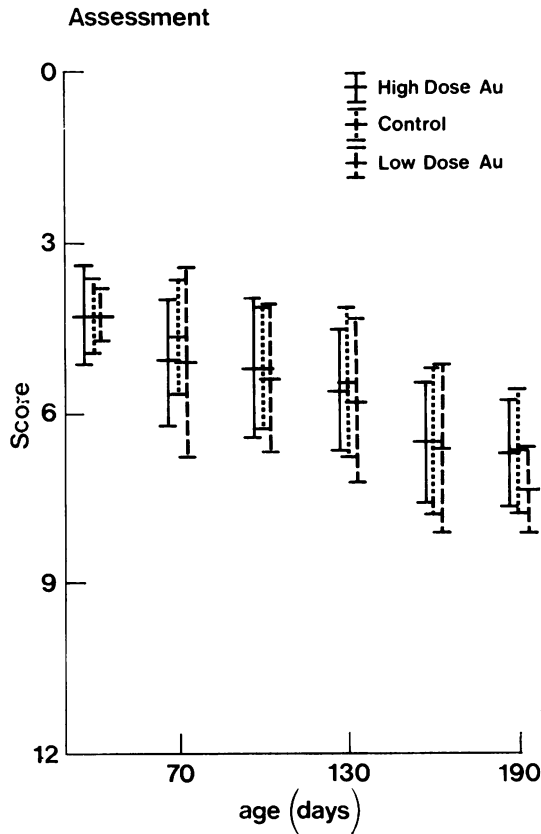


Figure Motility assessment score (see Methods) of dystrophic mice in controlled trial of chrysotherapy.

Initial study of dystrophic (dy/dy) mice showed that survival depended greatly on their state at weaning. Affected mice can be recognised from age 25–35 days, and sometimes earlier. About a third of these have died by 60 days, the mortality being confined to those less than 10 g body weight by 40 days. In view of the slow accumulation of gold in the tissues, treatment might not be expected to produce an effect for several weeks. It was, therefore, decided to select for study mice of greater than 10 g weight at 40 days, which were likely to survive beyond this period.

Injected gold enters normal muscles, though the concentration is much below that in organs containing cells of the reticuloendothelial system. Thus, in necropsy specimens from a patient with rheumatoid arthritis who received 2.5 g gold in the four years before death, the gold concentration in the muscle was 7 $\mu\text{g/g}$ wet weight, while that in the liver was 125 $\mu\text{g/g}$ wet weight (Gottlieb *et al.*, 1972). Similarly, in animals the concentra-

tion in normal muscle ranged from 5% to 25% of that in liver, and was similar to that in plasma (Bertrand *et al.*, 1948; Jeffrey *et al.*, 1958; McQueen and Dykes, 1969). However, in the synovium, damage by rheumatoid or chemical arthritis causes a fourfold increase in the accumulation of gold (Bertrand *et al.*, 1948; Lawrence, 1961). The gold is concentrated in lysosomal bodies in synovial cells and macrophages (Jessop *et al.*, 1973). Though chemical analysis of the increased accumulation in muscle produced by damage has not been performed, it has been shown that, as in the synovium, damage to muscle increases the uptake of gold into regenerating muscle fibres (Yarom *et al.*, 1976). It might thus be expected that dystrophic muscle fibres would slowly accumulate gold with every episode of necrosis and regeneration.

Though it has been demonstrated that gold inhibits macrophage lysosomal enzymes *in vitro* (Persellin and Ziff, 1966; Ennis *et al.*, 1968; Janoff, 1969), and also inhibits phagocytic activity of macrophages and polymorphs *in vivo* (Jessop *et al.*, 1973; Vernon-Roberts *et al.*, 1973), it is not certain that the concentration of gold achieved in dystrophic muscle would be sufficient to inhibit the intrinsic muscle proteinases. *In vitro*, gold solutions at a concentration of 1 mg/ml reduce the proteinase activity of human synovial fluid to 62.5%, and of liver cell lysosomes to 30% of normal (Ennis *et al.*, 1968). The lysosomal enzyme activity is reduced to about 35% when intact macrophages are incubated in gold solutions at this concentration (Persellin and Ziff, 1966). Inhibition of lysosomal enzyme activity of 50% requires approximately 0.5 mg of gold/ml (Persellin and Ziff, 1966; Ennis *et al.*, 1968). The concentration of gold in rheumatoid synovium is probably only 5% of this value, and that in normal synovium 1.4% (Gottlieb *et al.*, 1972). However, gold specifically accumulates in the lysosomes, where concentrations may approach the levels required to inhibit lysosomal enzymes. In an attempt to increase muscle lysosomal gold concentration, the dose used in dystrophic mice in the present experiment was twice (group C), 10 times (group A), and 200 times (very high dose experiment) that used in human rheumatoid arthritis. It is interesting that no increased mortality or toxic side effects were found in these dystrophic mice.

There may be several reasons for the failure of chrysotherapy to influence the course of murine muscular dystrophy. It is possible that the increased proteinases in the muscle might be an epiphenomenon of the dystrophic process, and not the cause of the muscle degeneration. The con-

centration of gold in the skeletal muscle may not be sufficient to inhibit the intrinsic muscle proteinases, or the degree of inhibition may not be sufficient completely to block muscle degeneration. The action of gold is reversed by cysteine, and thus its main effect may be upon the proteinases B and C, sparing other proteinases (for further discussion, see McGowan *et al.*, 1976).

References

- Bertrand, J. J., Waine, H., and Tobias, C. A. (1948). Distribution of gold in the animal body in relation to arthritis. *Journal of Clinical Medicine*, **33**, 1133–1138.
- Ennis, R. S., Granda, J. L., and Pasner, A. S. (1968). Effect of gold salts and other drugs on the release and activity of lysosomal hydrolases. *Arthritis and Rheumatism*, **11**, 756–764, 1968.
- Enomoto, A., and Bradley, W. G. (1977a). Therapeutic trials in muscular dystrophy. II. Ethylene and its derivatives. *Archives of Neurology (Chicago)*, **2**, 267–270.
- Enomoto, A., and Bradley, W. G. (1977b). Therapeutic trials in muscular dystrophy. III. Microbial proteinase inhibitors in murine dystrophy. *Annals of Neurology (Chicago)*, **34**, 771–773.
- Gerber, R. C., Paulsus, H. E., Jennrich, R. I., Lederer, M., Bluestone, R., Bland, W. H., and Pearson, C. M. (1974). Gold kinetics following aurothiomalate therapy: Use of a whole-body radiation counter. *Journal of Laboratory and Clinical Medicine*, **83**, 778–789.
- Gottlieb, N. L., Smith, P. M., and Smith, E. M. (1972). Tissue gold concentration in a rheumatoid arthritic receiving chrysotherapy. *Arthritis and Rheumatism*, **15**, 16–22.
- Janoff, A. (1969). Inhibition of human granulocyte elastase by gold sodium thiomalate. *Biochemical Pharmacology*, **19**, 626–628.
- Jeffrey, M. R., Freundlich, H. F., and Bailey, D. M. (1958). Distribution and excretion of radiogold in animals. *Annals of Rheumatic Diseases*, **17**, 52–60.
- Jessop, J. D., Vernon-Roberts, B., and Harris, J. (1973). Effects of gold salts and prednisolone on inflammatory cells. 1. Phagocytic activity of macrophages and polymorphs in inflammatory exudates studies by "skin window" technique in rheumatoid control patients. *Annals of Rheumatic Diseases*, **32**, 294–300.
- Kar, N. C., and Pearson, C. M. (1972). Acid, neutral and alkaline cathepsins in normal and diseased muscle. *Enzyme*, **13**, 188–196.
- Lawrence, J. S. (1961). Studies with radioactive gold. *Annals of Rheumatic Diseases*, **20**, 341–351.
- Lockie, L. M., Norcross, B. M., and Riordan, D. J. (1958). Gold in the treatment of rheumatoid arthritis. *Journal of the American Medical Association*, **167**, 1204–1207.
- Mascarenhas, R., Granda, J. L., and Freyberg, H. (1972). Gold metabolism in patients with rheumatoid arthritis treated with gold compounds—reinvestigated. *Arthritis and Rheumatism*, **15**, 391–402.
- McGowan, E. B., Shafiq, S. A., and Stracher, A. (1976). Delayed degeneration of dystrophic and normal muscle cell culture heated with pepstatin, leupeptin and antipain. *Experimental Neurology*, **50**, 649–657.
- McQueen, E. G., and Dykes, P. W. (1969). Transport of gold in the body. *Annals of Rheumatic Diseases*, **28**, 437–442.
- Park, D. C., and Pennington, R. J. (1965). Studies on cathepsin activity in muscle particles. In *Research in Muscular Dystrophy. Proceedings of the Third Symposium on Current Research in Muscular Dystrophy*, pp. 223–235. Pitman Publishing Company: London.
- Pennington, R. J. (1963). Biochemistry of dystrophic muscle. 2. Some enzyme changes in dystrophic mouse muscle. *Biochemical Journal*, **88**, 64–68.
- Persellin, R. H., and Ziff, M. (1966). The effect of gold salts on lysosomal enzymes of the peritoneal macrophage. *Arthritis and Rheumatism*, **9**, 57–65.
- Smith, R. T., Peak, W. P., Kron, K. M., and Hermann, I. F. (1968). Increasing the effectiveness of gold therapy in rheumatoid arthritis. *Journal of the American Medical Association*, **167**, 1197–1204.
- Tappel, A. L., Zalkin, H., Caldwell, K. A., Desai, I. D., and Shibko, S. (1962). Increased lysosomal enzymes in genetic muscular dystrophy. *Archives of Biochemistry and Biophysics*, **96**, 340–346.
- Vernon-Roberts, B., Jessop, J. D., and Dore, J. (1973). Effect of gold salts and prednisolone on inflammatory cells. II. Suppression of inflammation and phagocytosis in the rat. *Annals of Rheumatic Diseases*, **32**, 301–307.
- Weinstock, M., Epstein, S., and Milhorat, A. T. (1958). Enzyme studies in muscular dystrophy. 111. In hereditary muscular dystrophy in mice. *Proceedings of the Society of Experimental Biology and Medicine*, **99**, 272–276.
- Yarom, B. R., Behar, A. J., Yanko, L., Hall, T. A., and Peters, P. D. (1976). Gold tracer studies of muscle regeneration. *Journal of Neuropathology and Experimental Neurology*, **35**, 445–457.