Biochemical Pharmacology, Vol. 23, pp. 163–165. Pergamon Press, 1974. Printed in Great Britain.

SHORT COMMUNICATIONS

The effect of gold salts on the purine:pyrophosphate phosphoribosyltransferase* enzymes of human blood cells

(*Received* 16 *March* 1973; *accepted* 25 *June* 1973)

UNPREDICTABLE bone marrow depression and agranulocytosis are side effects of chrysotherapy. There is evidence that some of these cases are due to individual immune reactions,[1] but others remain unexplained and may have a biochemical basis. Patients with a clinically silent biochemical defect might be expected to show abnormal sensitivity to gold if the combined effect of the metal and the biochemical abnormality interfered with an essential biosynthetic mechanism such as nucleic acid production.

In spite of a recent report of [^{14}C]glycine incorporation into nucleic acid purines in mixed leukocytes,[2] there is considerable evidence that bone marrow cells do not synthesize purine ribonucleotides *de novo*.[3–5] However, they do contain AMP and IMP:pyrophosphate phosphoribosyltransferases (EC 2.4.2.7 and EC 2.4.2.8 respectively) as well as the enzymes which catalyse the purine ribonucleotide interconversion reactions.[6] The former enzymes catalyse the formation of purine 5′-phosphates from free purine bases and phosphoribosylpyrophosphate.

This communication reports the results of studies on the effects of some gold compounds on the purine phosphoribosyltransferases which are present in peripheral granulocytes and erythrocytes, these cells being formed in the bone marrow. The action of the gold compounds on partially purified HxGu PRTase from erythrocytes has also been studied.

METHODS

Heparinized blood samples were taken from healthy volunteers. The blood was allowed to sediment in the presence of 1·5% (w/v) dextran at ambient temperature before removing the leucocyte-enriched supernatant to prepare a pure granulocyte suspension as described previously.[7] The remaining erythrocytes were washed twice with an equal volume of 0·85% (w/v) sodium chloride solution. The cells were disrupted using ultrasonic vibrations at 8 μm peak amplitude, 10 sec for granulocytes and 5 sec for erythrocytes though in some cases the erythrocytes were lysed by hypotonic shock.

Partially purified HxGu PRTase (approx. 180-fold purification) was prepared using the method of Krenitsky *et al.*[8] scaled down to using 50 ml of erythrocytes and omitting the final chromatography on a Sephadex column.

The microradiochemical assays of the enzymes have been described elsewhere,[9,10] however, the tris-HCl buffer was used at pH 8·0 instead of 7·5. The concentrations of cell protein or enzyme used in the assays and the periods of incubation at 37° were as follows: granulocytes, 1·0–1·25 mg/ml of incubation medium for 10 min; erythrocytes, 1·5–1·75 mg/ml of incubation medium for 5 min; purified enzyme, 0·015 mg/ml of incubation medium for 2 min. After terminating the enzyme reaction by addition of 5 μmole of EDTA, the ^{14}C-nucleotides were separated from the ^{14}C-substrates by high voltage paper electrophoresis using sodium lactate solution (0·02 M, pH 3·6, 160 V/cm). The location and subsequent scintillation counting of the ^{14}C-nucleotides has been described previously.[10]

We investigated the effect of sodium aurothiomalate (Myocrisin), aurothioglucose (Solganal), sodium chloroaurate, 2-mercaptoethanol and sodium thiomalate on the enzyme activity *in vitro*.

The protein content of the cell preparations was measured by the method of Lowry *et al.*[11]

RESULTS

The activity of HxGu PRTase in both granulocyte and erythrocyte preparations was profoundly affected by sodium aurothiomalate. Figure 1 shows that the effect is biphasic and whereas drug con-

* EC 2.4.2.7 AMP:pyrophosphate phosphoribosyltransferase. EC 2.4.2.8 IMP:pyrophosphate phosphoribosyltransferase. (The purine substrate specificity of this enzyme includes guanine as well as hypoxanthine, it is therefore sometimes referred to as hypoxanthine-guanine phosphoribosyltransferase or HxGu PRTase).

centrations of 0·01–0·1 mM are inhibitory, higher concentrations are not. The purified enzyme, provided it was prepared from fresh unfrozen erythrocytes, gave a similar shaped curve (Fig. 1).

The results were the same when either [^{14}C]hypoxanthine or [^{14}C]guanine was used as the purine substrate. Addition of 5 mM 2-mercaptoethanol protected the enzyme against this inhibition by sodium aurothiomalate.

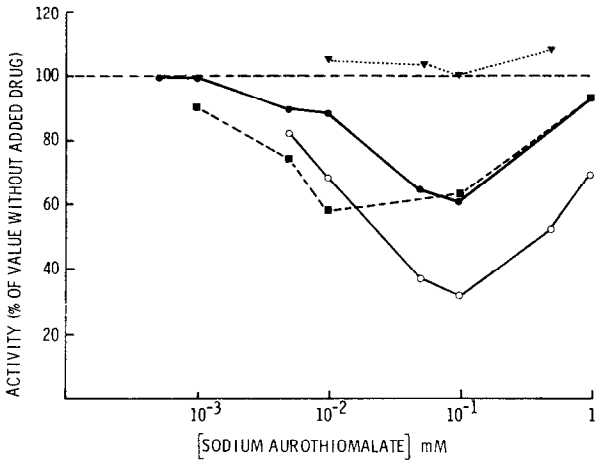

FIG. 1. The effect of *in vitro* sodium aurothiomalate on the HxGu PRTase activity of: disrupted granulocytes (○), haemolysates (●), purified enzyme from haemolysate (■) and disrupted granulocytes with 5 mM 2-mercaptoethanol (▼). The hypoxanthine concentration was 0·6 mM, PRPP was 1·0 mM and Mg^{2+} 5·0 mM.

Sodium chloroaurate (pH 8·0) did not give this biphasic dose–response curve. Higher concentrations of this gold salt gave increasing inhibition with both granulocyte and erythrocyte preparations (Fig. 2). The other gold-containing drug, aurothioglucose, behaved similarly to sodium aurothiomalate although the inhibitory effects at 0·1 mM were less marked. Sodium thiomalate (0·01–10·0 mM) alone had no effect on enzyme activity. However, an equimolar mixture of sodium thiomalate and sodium chloroaurate (both at pH 8·0) produced a biphasic response similar to that obtained with sodium aurothiomalate (Fig. 2).

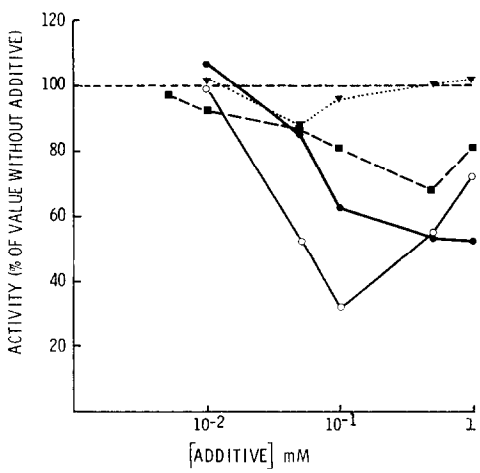

FIG. 2. The activity of granulocyte HxGu PRTase in the presence of various gold salts and other additives: aurothioglucose (■), sodium chloroaurate (●), sodium thiomalate (▼) and an equimolar mixture of both sodium chloroaurate and sodium thiomalate (○). The substrate concentrations were: hypoxanthine = 0·6 mM, PRPP = 1·0 mM and Mg^{2+} = 5·0 mM.

When the cell preparations from either granulocytes or erythrocytes were frozen and stored at −20° for 1–2 months there was no loss of enzyme activity but the response to sodium aurothiomalate was completely abolished. This lack of response to the drug was also observed when the enzyme was purified from frozen, stored erythrocytes. Heating the disrupted granulocytes or haemolysates to 75° for 10 min (as in the purification procedure) made no difference to the biphasic response curve produced by sodium aurothiomalate. The haemolysates were also treated with 0·8 M urea, a reagent which is known to dissociate the subunits of aspartate transcarbamylase,[12] but apart from inhibiting the enzyme by about 30% in the absence of drug there was no change in the shape of the sodium aurothiomalate dose–response curve.

AMP:pyrophosphate phosphoribosyltransferase was neither inhibited nor stimulated by sodium aurothiomalate in the same range of concentrations as were used in the experiments with HxGu PRTase.

DISCUSSION

The results described in this communication indicate that gold salts at certain concentrations are inhibitors of HxGu PRTase presumably because they combine with the enzyme sulphydryl groups. However, the results showing that sodium aurothiomalate is no longer inhibitory after freezing the enzyme suggest that these sulphydryl groups may be associated with the tertiary or sub-unit structure rather than with the catalytic sites. The work of Chilson *et al.*[13] has shown that freezing modifies the activity and sub-unit structure of mammalian lactic dehydrogenase and other enzymes. Freezing may cause non-specific disulphide bridging and aggregation.[14] It is of interest that heating the enzyme did not alter its susceptibility to inhibition by sodium aurothiomalate, suggesting that the change produced by freezing is a highly specific one.

The biphasic dose response curves of the enzyme to sodium aurothiomalate and less markedly to aurothioglucose as opposed to sodium chloroaurate indicate that this phenomenon is due to the presence of both gold and a thiol sulphur atom in the drug molecule. Previous work[10] has shown that HxGu PRTase has properties which are compatible with its being subject to allosteric regulation. We suggest that the thiomalate group within the drug molecule influences the site to which gold binds on the enzyme. The observation that sodium aurothiomalate is inhibitory towards HxGu PRTase at the concentrations which are usually found in the plasma during therapy (0·015–0·090 mM) is of clinical interest.

Acknowledgements—We are grateful for the technical assistance of R. Grimes (sandwich student) and D. Fyfe. We would like to thank May & Baker Ltd. for the gift of sodium aurothiomalate, and the Schering Corporation, N.J. for the aurothioglucose.

Division of Inherited Metabolic Diseases,
Medical Research Council Clinical Research Centre,
Northwick Park,
Watford Road,
Harrow, Middlesex HA1 3UJ, *England*

WENDY J. WESTWICK
JENNIFER ALLSOP
RICHARD W. E. WATTS

REFERENCES

1. E. J. DENMAN and A. M. DENMAN, *Ann. rheum. Dis.* **27,** 582 (1968).
2. H. S. DIAMOND, M. FRIEDLAND, D. HALBERSTAM and D. KAPLAN, *Arch. rheum. Dis.* **28,** 275 (1969).
3. L. G. LAJTHA and J. R. VANE, *Nature (Lond.)* **182,** 191 (1958).
4. R. M. S. SMELLIE, R. Y. THOMSON and J. N. DAVIDSON, *Biochim. biophys. Acta* **29,** 59 (1958).
5. R. Y. THOMSON, G. RICCERI and M. PERETTA, *Biochim. biophys. Acta* **45,** 87 (1960).
6. R. ABRAMS and M. BENTLEY, *Arch. biochem. Biophys.* **56,** 184 (1955).
7. W. J. WESTWICK, J. ALLSOP and R. W. E. WATTS, *Biochem. Pharmac.* **21,** 1955 (1972).
8. T. A. KRENITSKY, R. PAPAIOANNOU and G. B. ELION, *J. biol. Chem.* **244,** 1263 (1969).
9. B. M. DEAN, R. W. E. WATTS and W. J. WESTWICK, *FEBS Lett.* **1,** 179 (1968).
10. J. A. CRAFT, B. M. DEAN, R. W. E. WATTS and W. J. WESTWICK, *Eur. J. Biochem.* **15,** 367 (1970).
11. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
12. J. C. GERHARDT and A. B. PARDEE, *J. biol. Chem.* **237,** 891 (1962).
13. O. P. CHILSON, L. A. COSTELLO and N. O. KAPLAN, *Fedn Proc.* **24,** S-55 (1965).
14. J. LEVITT, *J. theor. Biol.* **3,** 355 (1962).