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Hypouricaemic effect after oral administration in chickens of polyethylene glycol-modified uricase entrapped in liposomes

Y. NISHIDA, N. KAMATANI, T. MIYAMOTO, *Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan 113*

Liposomal-entrapped methoxypolyethyleneglycol (PEG) modified uricase was used to study the plasma urate lowering effect after its oral administration to chickens. Plasma uric acid concentrations fell gradually and were accompanied by a rise in plasma uricolytic activity. Serum from chickens given PEG-modified uricase liposomes did not react with native uricase in immunodiffusion plates. These results suggested the clinical usefulness of liposome-entrapped PEG modified uricase in the treatment of hyperuricaemia.

Liposomes have been studied as a carrier system to facilitate the oral administration of therapeutic agents such as hormones and enzymes (Fendler & Romero 1977; Finkelstein et al 1978). However, the entrapment of proteins within liposomes may not render them non-immunogenic. Repeated administration of enzymes may produce antibody and cause immune reactions. Abuchowski et al (1977) reported a method for preparing long-living, non-immunogenic enzymes, suitable for human treatment, by covalently attaching methoxypolyethyleneglycol (PEG) to the enzyme. These modified enzymes retained enzymic activity and had neither immunoreactivity nor immunogenicity.

In the present experiment, uricase modified by PEG was entrapped in liposomes and studied for its hypouricaemic activity by oral administration to chickens. The clinical usefulness of this preparation in the treatment of hyperuricaemia is discussed.

Materials and methods

Uricase from *C. utilis* was obtained from Seikagaku Kogyo Co. PEG, mol. wt 5000, was purchased from Union Carbide. Coupling of enzymes to activated PEG was by the method of Abuchowski (Abuchowski et al 1977; Davis et al 1981). Cyanuric chloride (4·6 g) was dissolved in 300 ml of anhydrous benzene containing 10 g of anhydrous sodium carbonate. PEG was added to the mixture and stirred for 3 days at room temperature (20 °C). The solution was filtered and 300 ml of light petroleum (bp 40–60 °C) added and the precipitate dried. 500 mg of uricase was dissolved in 10 ml of 0·1 M phosphate buffer pH 7·5. Activated PEG in a five-fold molar excess was added to this solution.

The liposomes were prepared essentially as described by Poste & Papahadjopoulos (1976). Lecithin, cholesterol and dicetyl-phosphate (molar ratio 7 : 2 : 1) were dissolved in 6 ml chloroform. After rotary evaporation, a uniformly thin lipid layer was formed in round bottom flask. 10 mg of PEG modified uricase (1 mg = 5·0 units) was dissolved in 6 ml, 0·15 M phosphate buffer pH 7·4. This solution was added to the round bottomed flask containing the lipid film. After dispersion of the lipid film by vortexing under a N_2 atmosphere, the mixture was sonicated for 10 min in a Ultrasonic H180G. The liposomal PEG modified uricase preparation was centrifuged at 10 000*g* for 30 min and the precipitate discarded. The free enzyme was removed from the liposomal PEG modified uricase preparation by centrifugation at 100 000*g* for 90 min and the supernatant was discarded. The liposomal pellet was resuspended in phosphate buffer pH 7·4, so that a homogeneous suspension of inverted micelles was formed.

Liposomes containing PEG modified uricase in a total volume of 6 ml were administered orally to three chickens. 1·0 ml blood samples were collected in chilled heparinized tubes at indicated times and centrifuged immediately. Plasma uric acid concentrations were determined immediately by the colorimetric method (Caraway & Marable 1960) using the Wako kit. Uricolytic activity was assayed using uric acid as substrate. 50 µl of plasma was added to 3·0 ml, 0·066 M glycine buffer pH 9·3 containing 1 mM uric acid and the decrease in absorbance was measured spectrophotometrically at 293 nm as uric acid converted to allantion.

Antibody to uricase was obtained from rabbit injected with uricase in Freunds' adjuvant. Injections were repeated three times every week. The serum was obtained two weeks after the last injection and stored at −30 °C.

The gel diffusion plate was prepared with 0·6% agarose in barbital buffer pH 8·6. Serum obtained from chickens administered PEG modified uricase liposomes 3 times weekly was set up in immunodiffusion plates to test for precipitating activity with uricase. Plates were incubated overnight at 4 °C and stained with Coomassie blue.

Results

The effects of oral administration of liposomal PEG modified uricase on blood uric acid concentrations in chickens are shown in Fig 1. Following oral administration, plasma uric acid fell gradually to about 50% of control value at 120 min.

* Correspondence.

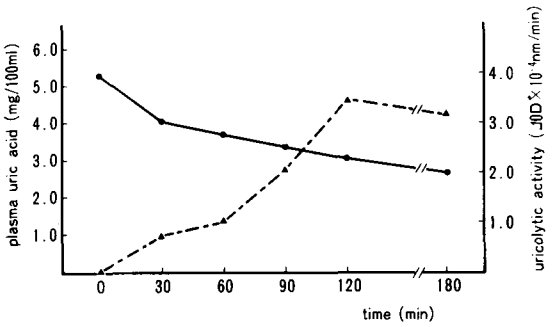

FIG. 1. Effect of oral administration of PEG modified uricase entrapped into liposomes on plasma uric acid (●——●) and plasma uricolytic activity (▲---▲) in chickens. The values show the mean of three chickens.

Plasma uricolytic activity after oral administration of the liposomes is also shown in Fig. 1. Uricolytic activity of plasma obtained before administration was not detected, but this rose gradually and reached a maximum at 120 min.

PEG modified uricase showed no precipitation reaction with uricase antibody on immunodiffusion plates. Also, serum obtained from chickens given PEG modified uricase liposomes did not react with native uricase or PEG modified uricase.

Discussion

Liposomes have been shown to have protective effects against proteolytic digestive enzymes (Davis et al 1981). The presence of phospholipids inhibits proteolytic enzyme activity. However it is controversial whether liposomes are transported intact across the intestinal mucosa. Patel & Ryman (1976) and Dapergolas & Gregoriadis (1976) showed that intragastric administration of insulin liposomes led to hypoglycaemic activity in rats. Fendler (1977) and Finkelstein & Weismann (1978) reported also that substances entrapped in liposomes can be absorbed into the circulation and still remain intact in the liposomes following oral administration. In contrast, Deshmukh et al (1981) showed that liposomes did not carry drugs through the intestinal wall.

In the present experiment, oral administration to chickens of PEG modified uricase entrapped in liposomes produced a 50% fall in blood uric acid accompanied by a rise in plasma uricolytic activity. The possibilities remain that PEG modified uricase is absorbed in its free untrapped form to a small degree or liposomal-entrapped PEG modified uricase can be absorbed from the gastrointestinal tract. The absorption of liposomes may be influenced by their lipid composition.

On the other hand, the immunopotentiating properties of liposomes were recognized by Shek & Sabiston (1981). Liposomal-associated protein antigen was reported to be a potent stimulator of immunological memory (Allison & Gregoriadis 1974) and to reduce the risk of a systemic immunological reaction, uricase was modified by PEG. It has been shown that activated PEG masks antigenic determinants of the enzyme molecule without loss of enzymatic activity (Abuchowski et al 1977). Nishimura et al (1981) and Chen et al (1981) showed that uricase modified with activated PEG has neither immunoreactivity or immunogenicity, but enzymatic activity remains. In the present experiment, the serum of the chickens administered PEG modified uricase entrapped into liposomes showed no precipitating antibodies to uricase. The hypouricaemic effect of PEG modified uricase in mice or man has been shown by Davis et al (1981), and Abuchowski et al (1981). However, the uricase may undergo digestive degradation during gastrointestinal passage after oral administration.

Therefore, PEG modified uricase entrapped into liposomes may have useful properties in oral enzyme therapy for gout.

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