International Journal of Pharmaceutics, 44 (1988) 265–267
Elsevier

IJP 01511

Delivery of glutathione, as a dextran conjugate, into the liver

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(Received 31 August 1987)
(Modified version received 28 December 1987)
(Accepted 29 December 1987)

Key words: Glutathione; Dextran; Liver; Dextran conjugate of glutathione; Glutathione delivery; Acetaminophen; Buthionine sulfoximine

Glutathione (GSH) is a tripeptide (L-γ-glutamyl-L-cysteinylglycine) which is the most prevalent intracellular thiol. It plays an important role in a variety of biochemical pathways (Meister and Anderson, 1983). The detoxication potential of the liver is intimately connected to the GSH status of the organ (Jaeger et al., 1973; Schnell et al., 1983). It protects cells against damage by toxic compounds, reactive oxygen compounds, and radiation (Meister and Anderson, 1983).

Based on these findings, GSH is widely used in the treatment of hepatic disorders, allergies, poisonings of xenobiotics and heavy metals, and so on. However, extracellular GSH is impermeable to the liver and yet has a very short half-life due to its rapid renal degradation into constituent amino acids (Hahn et al., 1978; Griffith and Meister, 1979a; Wendel and Jaeschke, 1982). Although an interorganal shift of the constituent amino acids from the kidney to the liver enables hepatic resynthesis of GSH, administration of free GSH is insufficient for a stable increase in liver GSH.

In this study, we examined the idea that an intraliver delivery system for GSH might be based on a dextran conjugate of GSH that is much more effectively transported into cells than is GSH itself.

GSH was covalently coupled to soluble dextran (Dextran T-40, $M_w = 43{,}900$, $M_n = 26{,}200$, Pharmacia Fine Chemicals Co., Sweden) by the cyanogen bromide activation method (Axén and Ernback, 1971). To a stirred solution of dextran (0.2 g) in water (20 ml), cyanogen bromide was added in three portions (40, 40 and 30 mg). The pH was maintained at 11.0 during this process by addition of 4 M NaOH. Six minutes after the final addition of cyanogen bromide, the pH was adjusted to 6.5 by addition of 0.1 M HCl. Then GSH (0.4 g) was added maintaining the pH at 6.5 and coupling reaction was allowed to proceed for 24 h at 4°C. The reaction mixture was washed repeatedly with 0.1 M CH_3COOH and concentrated by filtration through an ultrafilter (UP-20 mounted in UHP-43, Toyo, Japan) under high nitrogen pressure and then freeze-dried.

The product obtained was a water-soluble powder containing 9.8 ± 1.2% w/w GSH(the average of 35 batches) according to the determination of sulfhydryl group by the method of Ellman

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(1959). The dextran conjugate of glutathione (D-GSH) released GSH gradually in alkaline media whereas it was relatively stable in acidic conditions. It was found that the content of amino group of D-GSH is very high by the measurement of amino groups with TNBS (Fields, 1971, 1972). This indicates that GSH is bound to dextran mainly through the sulfhydryl group, not through the amino group. The proportion of these linkages was estimated to be 8.5 : 1. This may contribute to chemical stability against autoxidation of the thiol group. The molecular size of D-GSH was examined by gel filtration on a Sephadex G-150 column and by HPLC on a TSKgel G3000SW column. There was good agreement between the elution peak of D-GSH detected by a differential refractometer and that measured spectrophotometrically based on the absorption of GSH. D-GSH was eluted somewhat earlier than the original dextran T-40. This indicates that the molecular size of the conjugate is increased as a result of the coupling reaction.

Mice fasted for 24 h were pretreated with buthionine sulfoximine (BSO), a potent inhibitor of τ-glutamylcysteine synthetase, to prevent intracellular glutathione synthesis (Griffith and Meister, 1979b). GSH was determined with *o*-phthaldehyde essentially as described by Hissin and Hilf (1976). The GSH level of the liver decreased to one-third of the initial level 4 h after the administration of BSO (2 mmol/kg) (Fig. 1). Intravenous administration of D-GSH to mice led to marked increase in the level of GSH. However, administration of free GSH had no significant effect on the liver GSH level.

In the studies described in Fig. 1, distribution of GSH in liver subcellular fraction was examined. The liver homogenate was centrifuged at $600 \times g$ for 5 min, $1500 \times g$ for 10 min, $10{,}000 \times g$ for 20 min, and at $105{,}000 \times g$ for 60 min and fractionated into 5 fractions. A significant increase in the GSH level was observed in each subcellular fraction after the administration of D-GSH.

Protection of mice pretreated with D-GSH against acetaminophen-induced hepatotoxicity was examined. Mice were treated intraperitoneally with a lethal dose of acetaminophen (5 mmol/kg). The

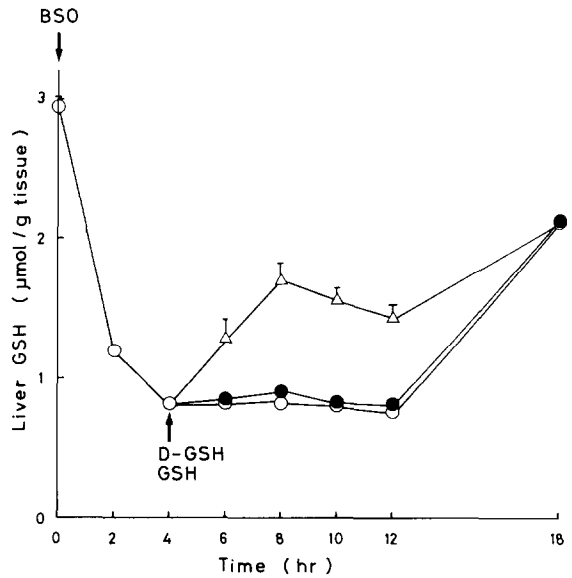

Fig. 1. Effect of D-GSH on liver GSH contents in mice treated with buthionine sulfoximine (BSO). Fasted mice were injected intraperitoneally with BSO (2 mmol/kg). Four hours later (arrow), groups of mice were given D-GSH (△, 0.39 mmol/kg in GSH equivalent), GSH (●, 0.39 mmol/kg) or saline (○, control) intravenously. Results are means ± S.E.

effect of administration of D-GSH on the survival ratio after acetaminophen administration is described in Fig. 2. Mice were treated intravenously with D-GSH (0.39 mmol/kg in GSH equivalent) or GSH (0.39 mmol/kg) both 2 h prior to and at the same time as acetaminophen administration. A 30-day survivial ratio increased progressively with coadministration of D-GSH, while little increase over the control was found when free GSH was given.

In the experiments described in Fig. 2, blood samples were collected 6 h after the administration of acetaminophen for the determination of serum alanine aminotransferase (ALT) activity. The ALT activity (normal mice, 39 ± 5 U/l) was increased after the acetaminophen administration, being 1874 ± 2463 U/l. However, the treatment with D-GSH maintained the serum ALT activity at significantly lower levels (201 ± 181 U/l).

The studies in which mice were pretreated with BSO strongly indicated that the dextran conjugate of glutathione is transported into liver cells and is intracellularly hydrolyzed to free form of GSH

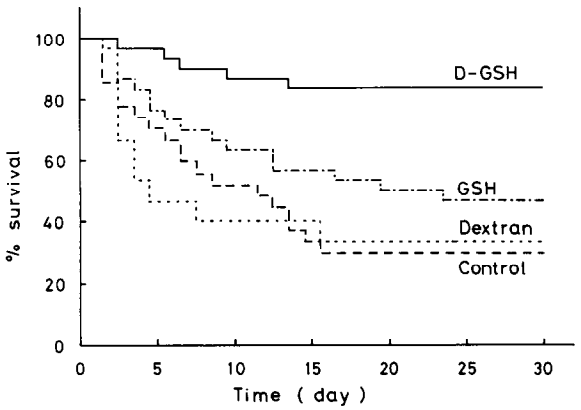

Fig. 2. Survival of mice given a lethal dose of acetaminophen; effect of administration of D-GSH. Mice were injected intraperitoneally with a 0.2 ml propylene glycol solution of acetaminophen (5 mmol/kg; control). Mice were treated by intravenous injection of a 0.2 ml saline solution of D-GSH (0.39 mmol/kg in GSH equivalent), GSH (0.39 mmol/kg) or Dextran (3.4×10^{-5} mol/kg) both 2h before and at the same time as acetaminophen administration.

because under these conditions GSH synthesis from its constituent amino acids is inhibited (Fig. 1). Furthermore, that D-GSH effectively protects mice from acetaminophen acute poisoning also suggested the intracellular GSH formation.

The present findings may provide a new approach to increase the intracellular GSH levels of tissues.

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