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Pharmacokinetics of intravenously administered glutathione in the rat

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By means of an open two compartment model with a distribution and elimination phase, the pharmacokinetic properties of intravenously administered GSH (reduced glutathione) have been investigated in the rat. After a bolus injection of four various GSH doses (50 to 300 μmol kg^{-1}), arterial plasma concentrations of GSH, GSSG (= oxidized glutathione), total thiols and soluble thiols minus GSH were elevated and then rapidly decreased non-exponentially. With increasing dose, the rate constant for drug elimination and plasma clearance increased from 0·84 to 2·44 ml min^{-1} and the half-life of the elimination phase decreased from 52·4 to 11·4 min. Both the apparent volume of distribution and the degree of penetration of GSH into the tissues were diminished with increasing dose (from 3·78 to 1·33 litres kg^{-1} and from 6·00 to 0·51 as k_{12}/k_{21}, respectively). The data indicate that GSH is rapidly eliminated. This is mainly due to rapid oxidation in plasma rather than by increased tissue extraction or volume distribution. Thus plasma GSH levels appear to be quickly regulated by which the body may maintain concentrations within narrow physiological limits.

Reduced glutathione (GSH) is a physiological tripeptide (γ-glutamyl-cysteinyl-glycine); it is present in the intracellular space of many tissues as well as in the extracellular fluid (reviewed by Ammon & Mark 1985). Previous studies have shown that exogenous GSH in concentrations similar to those present in blood plasma (0·01 mM) (Meister 1983; Meister & Anderson 1983) potentiated glucose-mediated insulin secretion from rat isolated pancreatic islets, suggesting a possible physiological role of extracellular GSH (reviewed by Ammon & Mark 1985). Furthermore, oral administration of glucose was found to increase plasma GSH in rats (Ammon et al 1985). So far there is little information on the pharmacokinetics of GSH (Wendel & Cikryt 1980) and no information about the pharmacokinetics during the late elimination phase. Therefore, the pharmacokinetics of exogenous glutathione in the rat have been investigated more precisely using a pharmacokinetic model after a single bolus of various doses of GSH had been administered.

MATERIALS AND METHODS

Animals

Wistar rats of either sex, 200–300 g, kept on a commercial diet (Altromin, Lage, W. Gemany) were housed at 24 °C and at a 12 h day/night rhythm. They were fasted for 12 h before use. All experiments were between 0800 and 1500 h.

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Chemicals

DTNB (Ellmann's reagent), oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione reductase, glyoxylase and NADPH were from Boehringer, Mannheim, West Germany. Pentobarbitone was from Serva, Heidelberg, West Germany and bovine serum albumin was from Sigma, St. Louis, USA. Methylglyoxal was from Fluka, Neu-Ulm, W. Germany. All other chemicals were from Merck, Darmstadt, West Germany in the purest form available. Reduced GSH is 99% pure (contains 1% GSSG). Freshly (immediately before the experiment) prepared isotonic solutions of GSH were used. After GSH had dissolved, the solutions contained approximately 7·3% GSSG irrespective of the concentration of the original concentration.

Method

Rats were anaesthetized by i.p. injection of pentobarbitone 50 mg kg^{-1}. Following tracheotomy for intubation, catheters were introduced into the right jugular vein for administration of GSH and into the left carotid artery for taking blood samples. After 30 min had been allowed for equilibration, animals were heparinized (5000 u kg^{-1}). GSH was administered i.v. as a bolus. Blood concentrations were followed over 30 min at times indicated in the legends of the Figures.

Preparation of blood samples

Blood samples (200 µl) taken from the cartoid artery were immediately centrifuged for 2 min at 10 000*g* and 2 °C. Plasma (80 µl) was rapidly deproteinized by mixing with 20 µl 15% (w/w) metaphosphoric acid, followed by centrifugation at 10 000*g* and 2 °C for 5 min.

Glutathione assay

One aliquot of deproteinized plasma was analysed for total glutathione (GSH & 2 GSSG) using the glutathione reductase-DTNB recycling procedure (Ellman 1959), modified by Tietze (1969). GSSG in the second aliquot was determined after the sulphydryl groups of GSH had been masked by the glyoxalase-catalysed reaction with methylglyoxal as described by Heinle (1979). GSH concentrations were calculated by subtracting GSSG from total glutathione. The third aliquot of plasma was analysed for total thiol content using Ellman's method according to Cikryt (1979). The sensitivity of the assay was 10 pmol GSH/100 µl, and the calibration curve was linear between 10 and 400 pmol/20 µl. The correlation coefficient was 0·999 and recovery was between 95·5 to 109% depending on the amount of GSH used.

Pharmacokinetic evaluations

Pharmacokinetic parameters of glutathione including total thiols were calculated by computer programs (Autoan and Nonlin). Different types of compartment models were evaluated and the best for fitted data used. The results of the study were statistically evaluated by using regression analysis for dose dependency. Two way analysis of variance (F-test) and *t*-test were used for evaluating the data.

Compartment model

The best fit for kinetics of reduced glutathione in rat plasma was the open two-compartment model with a distribution and elimination phase. For such a model, the plasma concentration Cp at any time t can be expressed by the following equation:

$$Cp = A\,e^{-\alpha t} + B\,e^{-\beta t}$$

where A is a coefficient representing the distribution phase; B and β are coefficients representing the elimination phase; α is the rate constant of distribution.

RESULTS

In Fig. 1 the effect of an intravenous bolus injection of various doses of GSH (50, 100, 200 and 300 µmol kg^{-1}) on arterial plasma concentrations of either GSH or GSSG, the sum of GSH + 2 GSSG, DTNB-reactive thiols (total thiols) and soluble thiols minus GSH is shown for 30 min. The control values obtained 5 min before the injection of GSH were 7·1 µmol litre^{-1} for GSH and 4·2 µmol litre^{-1} for GSSG. Soluble thiols and DTNB-reactive total thiols (reflecting endogenous thiols in the plasma) were only slightly different from zero (data not shown). After an injection of GSH, the plasma concentration of each of the above parameters was elevated in a dose-dependent manner followed by a rapid non-exponential decline.

In Fig. 2 the time course of GSH/GSSG ratios in the arterial plasma of rats after an intravenous injection of 50, 100, 200 or 300 µmol GSH kg^{-1} is shown. The control value was again determined 5 min before administration of GSH and found to be 1·6. Immediately after GSH injection the GSH/GSSG ratio was significantly increased. For 50 and

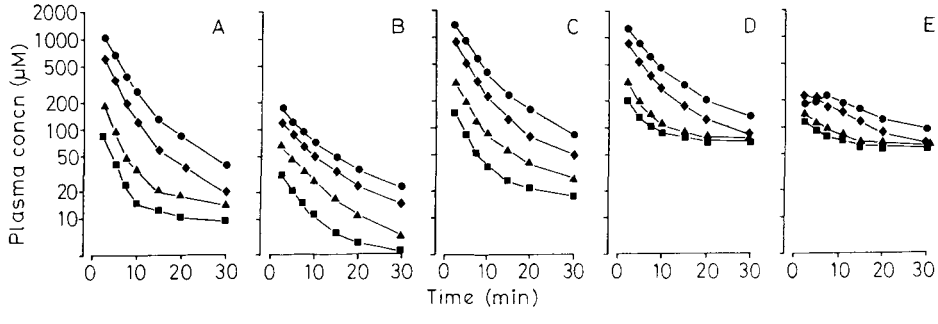

FIG. 1. Arterial plasma levels of (A) GSH, (B) GSSG, (C) sum of GSH and GSSG, of (D) DTNB-reactive thiols, and of (E) soluble thiols minus GSH after an intravenous bolus injection of various concentrations of GSH to rats. 50, 100, 200, and 300 µmol GSH kg^{-1} were injected. Control values were measured 5 min before the experiment was started and at the time (0 min) when GSH was injected. Results are given as the mean ± s.e. of 5 independent experiments. ■ 50, ▲ 100, ◆ 200, ● 300 µmol kg^{-1}.

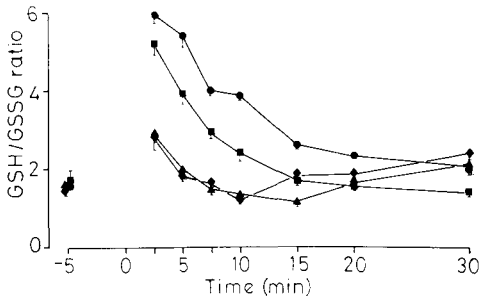

FIG. 2. GSH/GSSG-ratio in the rat arterial plasma after an intravenous injection of 50, 100, 200 and 300 μmol GSH kg^{-1} (calculated from data given in Fig. 1).

100 μmol kg^{-1} GSH, the GSH/GSSG ratios were not significantly different from each other. However, GSH doses exceeding 100 μmol kg^{-1}, i.e. 200 and 300 μmol kg^{-1} increased the ratio, at least during the first 10 min of the experiment, in a dose-dependent manner. Thereafter this increase levelled off, and the ratio was no longer different compared with all other GSH doses tested.

The pharmacokinetic parameters of plasma GSH calculated from the data derived from Fig. 1 are shown in Tables 1 and 2. While first order rate constant of distribution (α) decreased from 24·5 at 50 μmol GSH kg^{-1} to 15·1 at 300 μmol kg^{-1}, the rate constant of drug elimination (β) increased with the

Table 1. Plasma concentration of GSH: coefficients of equation at various GSH doses.

Dose of GSH (μmol kg^{-1})	50	100	200	300	*P*
A (μM)	205·2 ± 27·1	426·5 ± 28·3	1135 ± 106·1	1507 ± 158·4	<0·001
B (μM)	15·1 ± 3·0	29·9 ± 2·9	140·0 ± 39·6	296·2 ± 75·3	<0·01
α (litre h^{-1})	24·5 ± 1·1	21·8 ± 1·7	18·3 ± 1·1	15·1 ± 0·9	<0·001
β (litre h^{-1})	0·93 ± 0·21	1·48 ± 0·43	3·69 ± 0·54	3·82 ± 0·4	<0·001

Means ± s.e.; n = 5 per dose, *P* values are shown for the effect of 50 μmol kg^{-1} vs 300 μmol kg^{-1} GSH.
A = coefficient of the equation of plasma concentration representing the distribution phase.
B = coefficient of the equation of plasma concentration representing the elimination phase.
α = (apparent first-order) rate constant of distribution processes.
β = (apparent first-order) rate constant of drug elimination from the organism.

Table 2. Pharmacokinetic parameters of GSH.

Dose of GSH (μmol kg^{-1})	50	100	200	300	Linear regression
T50α (min)	1·71 ± 0·07	1·95 ± 0·14	2·30 ± 0·13	2·78 ± 0·16	r = 0·842, $P<0·001$
T50β (min)	52·4 ± 9·5	36·8 ± 7·8	12·2 ± 1·6	11·5 ± 1·4	r = −0·753, $P<0·001$
Cl_{pl} (ml min^{-1})	0·84 ± 0·13	1·32 ± 0·38	2·33 ± 0·43	2·44 ± 0·31	r = 0·676, $P<0·01$
k_{13} (litre h^{-1})	8·78 ± 0·96	11·01 ± 1·82	13·04 ± 0·53	10·35 ± 0·79	r = 0·226, n.s.
k_{12} (litre h^{-1})	14·13 ± 1·05	9·50 ± 0·82	3·67 ± 0·39	2·81 ± 0·32	r = −0·897, $P<0·001$
k_{21} (litre h^{-1})	2·52 ± 0·34	2·80 ± 0·54	5·27 ± 0·96	5·80 ± 0·98	r = 0·666, $P<0·01$
k_{12}/k_{21}	6·00 ± 0·80	4·02 ± 0·89	0·75 ± 0·09	0·51 ± 0·05	
VD (litre kg^{-1})	3·78 ± 0·62	3·49 ± 0·37	1·96 ± 0·48	1·33 ± 0·33	r = −0·73, $P<0·001$

Means ± s.e.; n = 5 per dose.
T50α half-life during distribution phase.
T50β half-life during elimination phase.
Cl_{pl} plasma clearance.
k_{12} (first-order) rate constant of transport of drug from central to peripheral compartment.
k_{13} rate constant of elimination of drug from peripheral to central compartment.
k_{21} (first-order) rate constant of transport of drug from peripheral to central compartment.
VD distribution volume.

dose of GSH injected (Table 1). Correspondingly, the half-life of the distribution phase (T50α) also increased dose-dependently (Table 2).

Table 2 shows the dependence of various pharmacokinetic parameters on the dose of GSH used. For k_{13} which reflects processes of metabolism and excretion, no significant dose-related change was detected. However, the first order rate constant of transport of GSH from blood to tissue compartment (k_{12}) decreased with increasing dose of GSH. On the other hand, the rate constant of redistribution from tissues to the central compartment (k_{21}) was significantly correlated with the dose of GSH administered. Thus the ratio k_{12}/k_{21} reflecting the degree of penetration of GSH to the tissues is diminished dependent on the GSH dose injected. Simultaneously the apparent volume of distribution (V_D) decreased with the doses of GSH. The half-life of GSH during the distribution phase, depending on the plasma concentration, was between 1·7 and 2·8 min; the half-life of the elimination phase (T50β) decreased from 52·4 min at 50 μmol GSH kg^{-1} to 11·5 min at 300 μmol GSH kg^{-1} (Table 2).

DISCUSSION

Plasma concentrations of GSH

In rats, normal concentrations of glutathione have been found to be approximately 10 μM in plasma and were present mostly as reduced glutathione (Meister & Anderson 1983; Hahn et al 1978). However, it was also observed that the concentration of GSH may differ depending on the localization of vessels from which blood has been taken: thus the highest concentrations of GSH occured in the hepatic vein and lowest ones in the renal veins (Anderson et al 1980). Moreover, feeding state and anaesthesia seem to affect plasma GSH (Anderson et al 1980). In the experiments reported here, it was reasonable to take blood from the carotid artery since, under physiological conditions, the effects of GSH on the functions of different organs (e.g. insulin secretion, reviewed by Ammon & Mark 1985) will be related primarily to the GSH concentration of arterial plasma. With anaesthetized rats the basal arterial blood concentration of GSH was found to be 7·1 μM and that of GSSG, 4·2 μM. These are similar to the findings of Hahn et al (1978) who used ^{14}C-labelled GSH.

Plasma half-life, elimination rate and distribution volume

Plasma GSH appears to be rapidly distributed when administered intravenously. Thus, the observed initial plasma half-life was found to be 1·9 min in mice (Wendel & Jaeschke 1982) and 1·7 min in man immediately after i.v. infusion of 100 mg GSH (Wendel & Cikryt 1980). Similar data were obtained in anaesthetized rats in the present study but only with respect to the distribution phase. According to the dose of GSH administered, the plasma half-life during the distribution phase varied between 1·7 and 2·8 min. Moreover, it was evident that the half-life during the distribution of GSH increased with the dose administered. This may be due to an uptake process that may become overloaded. Little GSH was taken up by tissues except the kidney which is the main organ for GSH elimination (Sekura & Meister 1974). Therefore, the distribution compartment for GSH mainly relates to extracellular space, which apparently is filled more rapidly.

Considering that the normal plasma concentration of GSH is approximately 10 μM and that immediately after distribution of GSH plasma concentrations are between 50 and 150 μM (Fig. 1), the kidneys, as the most important excretory organ for GSH, seem to possess a very high elimination capacity for GSH. Moreover, this capacity increases with the dose (50 to 300 μM) of GSH administered in the way that the plasma clearance increases 3-fold and therefore, simultaneously, the half-life of GSH during the elimination phase is diminished. The adaptation of the elimination capacity appears to bring about steady state physiological GSH levels.

GSH + 2 GSSG is distributed in about 20–25% of the body fluid since when 300 μmol GSH kg^{-1} body weight was injected, the initial plasma concentration of GSH + 2 GSSG was about 1400 μmol $litre^{-1}$. This corresponds to the volume of the extracellular space. Therefore, a rapid distribution in the extracellular space followed by slow elimination processes occur. The decrease of the apparent distribution volume (V_D) with the dose of GSH administered indicates less of it penetrates deeper compartments and/or it is increasingly metabolized (including oxidation e.g. in the blood). At first glance this is not consistent with the observation of a dose-dependent decrease in k_{12}/k_{21} which indicates less tissue penetration of GSH. However, it is conceivable that increased peripheral oxidation of GSH may be a reason for enhanced GSH disappearance. In fact, GSSG concentrations are more elevated after 5 to 30 min than could be expected from the increased dose administered (Fig. 1). This suggests that regulation of plasma GSH, i.e. its decrease, is very sensitive. Thus unphysiologically high plasma GSH concentrations may be avoided.

Oxidized glutathione very rapidly increases in a

dose-dependent manner when GSH is administered intravenously. This may be due to the fact that the solution of GSH injected contained 7·3% GSSG (spontaneous conversion from GSH to GSSG), but it may also be that rapid oxidation in the blood plasma takes place. A rapid oxidation of GSH was also found in bile (Eberle et al 1981). Since GSH is not extracted by tissues such as liver to a major degree, its oxidation (if relevant here) must take place in the plasma itself in a way similar to that of kidney, i.e. a non-enzymatic hydrogen transfer between GSH and cystinylbisglycine occurs, as described by Armstrong (1979).

Glutathione-mediated thiol-disulphide redox reactions are closely related to the GSH/GSSG redox ratio in such a way that changes of this ratio affect enzymatic glutathione oxidation reduction reactions. In tissues including liver and bile this ratio is high (99 : 1), and only a little GSSG is present (reviewed by Meister & Anderson 1983). In blood plasma this ratio was found to be 1·6 (Fig. 2). Blood tends to maintain such a ratio. Thus with 50 and 100 μmol kg^{-1} GSH, the ratio returned rapidly to control values and only after 200 and 300 μmol kg^{-1} did this ratio become significantly elevated above normal for 10 to 15 min; this suggests that only at higher plasma GSH concentrations was the capacity of the oxidation system limited.

Other plasma thiols

Following i.v. injection of GSH, the concentration of soluble DTNB-reactive thiols minus GSH increased from approximately 40 to 200 μM and thereafter showed a rapid decline. This increase is probably due to metabolism of GSH as a result of which cysteine-containing compounds are formed. Despite a rapid decrease in GSH, the plasma concentration of soluble thiols declined thereafter only slowly, and also the concentrations of soluble thiols measured in plasma after various doses of GSH differ much less from each other than in the case of plasma GSH. Moreover, it appears that the elimination rate of soluble thiols from plasma is also much less than that of GSH.

Our findings, therefore, suggest a rapid change of GSH plasma concentrations which is a prerequisite for its possible modulatory role in physiological terms.

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