THE LEVEL AND HALF-LIFE OF GLUTATHIONE IN HUMAN PLASMA

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1. Introduction

The tripeptide glutathione plays an important role within cells, where it is abundantly present in its reduced form. Very low levels are found extracellularly [1–3]. The role of extracellular glutathione is poorly understood. In [4] we suggested the kidney as an important organ in the turnover of plasma glutathione and showed that the rat liver is impermeable to GSH or GSSG. Although controversy remains regarding the physiological function, a predominant participation of the kidney in the turnover of extracellular glutathione is established [5–9]. Here, we investigated the glutathione plasma level in man taking into account the implications of a concentration gradient of >3 orders of magnitude between erythrocyte and plasma. In a single in vivo experiment we determined the apparent half-life of glutathione in human plasma to be 1.6 min.

2. Experimental

Blood (10 ml) was drawn into cooled tubes precoated with 140 IU ammonium heparinate from healthy adults and immediately centrifuged at 4°C, 3000 × *g* for 8 min. Plasma was recentrifuged at 8000 × *g* for 1 min. Protein was immediately precipitated adding 0.1 vol. ice-cold 300 g/l metaphosphoric acid containing 1 mmol/l ethylendiamine tetraacetic acid.

Sterile, pyrogen-free GSH, 100 mg (Robin, SPA, Milano) was dissolved in 1 ml sterile sodium bicarbonate solution to yield a final pH of 7.1 and dissolved to 10 ml with isotonic saline. The solution was injected within 10 s into the right vein of one of the authors (A. W., male, age 36 years, 64 kg body wt). Blood samples (10 ml) were withdrawn at the left arm vein within 20 s, transferred to an ice bath and treated as above after the experiment's end. Creatinine clearance was determined within the following 24 h interval.

Total glutathione concentrations were measured by the kinetic assay using the glutathione reductase reaction essentially as in [10]. The results are given in GSH equivalents (GSH + 2 GSSG). GSSG was measured by determining the end-point of the glutathione reductase reaction [11].

To assess the extent of hemolysis, whole blood hemoglobin was measured by a modified Drabkin procedure [12]. The very low plasma hemoglobin concentrations were determined by difference dual wavelength spectrophotometry of carbonmonoxyferrohemoglobin under the following conditions: a baseline was run at the wavelength couple 418–424.5 nm in 0.1 mol/l potassium phosphate buffer (pH 7.0); after addition of sodium dithionite (final conc. 2 mM) the cuvette was bubbled with CO for 20 s and another spectrum was run. In all samples hemolysis was <0.03%. γ-Glutamyltranspeptidase was determined according to [13].

3. Results and discussion

The mean total glutathione content in the plasma of 11 male and 9 female adults was found to be 0.34 ± 0.11 μmol/l. GSSG was ≤0.1 μmol/l in 12 subjects and 0.1–0.28 μmol/l in 8 subjects. No sex-related differences were observed. We also determined total plasma glutathione of a bilaterally nephrectomized female patient and found a level of 0.53 μmol/l. Thus the concentration gradient between intraerythrocytic and plasma glutathione amounts nearly to 4 orders of magnitude. Two possible interferences had to be ruled out: (i) a minute hemolysis, be it endogenous or

during blood sampling, may account for the amount of glutathione found; (ii) a varying activity of plasma γ-glutamyltranspeptidase may substantially degrade the glutathione present. The mean plasma hemoglobin concentration was 1.47 ± 1.02 mg/100 ml plasma and the correlation between hemoglobin and plasma glutathione concentration was $r = 0.01$. γ-Glutamyltranspeptidase activity amounted to 8.5 ± 2.6 U/l and correlated with the plasma glutathione concentration with $r = 0.001$. This means that neither of these two parameters showed any correlation with the amount of glutathione determined and might have influenced the result obtained for plasma glutathione. That the value found here is considerably lower than the data in [1–3]may be due to the controlled minimization of hemolysis [14] in our study. For laboratory animals, also much higher plasma glutathione levels were reported, e.g., 18–60 μmol/l for mice [15] and 3 μmol/l for rats [7,16].

In a single self-experiment we investigated the fate of intravenously injected glutathione in man. Fig.1

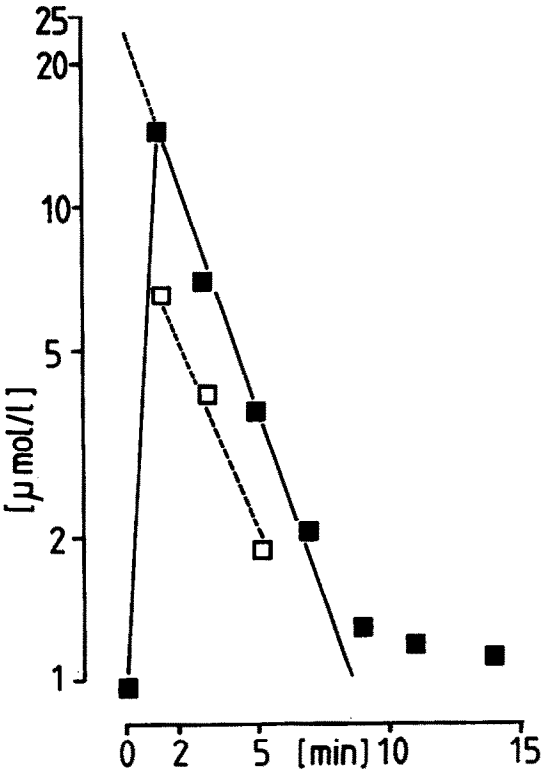

Fig.1. In vivo elimination of a single dose of 1.56 mg glutathione/kg (total 100 mg) from human plasma: (■) total glutathione; (□) oxidized glutathione.

shows that a single dose of 100 mg GSH is rapidly eliminated from plasma. We observe a distribution phase within the first 2 min and a clearance in the following 10 min. From the semilogarithmically plotted clearance phase an apparent half-life of 1.6 min is calculated. Extrapolation of this straight line to zero time shows that the tripeptide distributed completely into the extracellular space: if we assess the interstitial plus plasma volume with 21% of the subjects body weight (= 13 liters) we expected without clearance an equilibrium concentration of 25 μmol/l. It seems that a considerable proportion of the administered glutathione has been oxidized to GSSG, although we cannot exclude that this GSSG might have been released from the intracellular space. Using the borohydride reduction method [17], no mixed-disulfides could be detected. In vitro controls with the subject's whole blood or plasma indicated that neither GSH or GSSG are significantly metabolized within 20 min at 25 μmol/l.

The subject's creatinine clearance was 136 ml/min; i.e., if glutathione is cleared from the glomerular filtrate as effectively as creatinine, a half-life of 15 min is expected. Thus renal intratubular degradation alone does not account for the total plasma glutathione turnover. With a total renal plasma flow of 680 ml/min, the half-life would be 2.6 min. Even if we assume a complete 'extraction' of glutathione during one passage of the kidneys [18], still ~50% of the observed free plasma glutathione turnover must occur extrarenally. This view is substantially supported in [7] where, in the anaesthetized rat, a 50% renal glutathione turnover capacity was calculated. Whether the contribution of the extrarenal glutathione turnover is due to uptake or to degradation by other organs remains open.

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