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The systemic availability of oral glutathione

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Summary. When the plasma glutathione concentration is low, such as in patients with HIV infection, alcoholics, and patients with cirrhosis, increasing the availability of circulating glutathione by oral administration might be of therapeutic benefit.

To assess the feasibility of supplementing oral glutathione we have determined the systemic availability of glutathione in 7 healthy volunteers.

The basal concentrations of glutathione, cysteine, and glutamate in plasma were 6.2, 8.3, and 54 $\mu mol \cdot l^{-1}$ respectively. During the 270 min after the administration of glutathione in a dose of 0.15 $mmol \cdot kg^{-1}$ the concentrations of glutathione, cysteine, and glutamate in plasma did not increase significantly, suggesting that the systemic availability of glutathione is negligible in man.

Because of hydrolysis of glutathione by intestinal and hepatic γ-glutamyltransferase, dietary glutathione is not a major determinant of circulating glutathione, and it is not possible to increase circulating glutathione to a clinically beneficial extent by the oral administration of a single dose of 3 g of glutathione.

Key words: Glutathione; systemic availability, cysteine, glutamate

Glutathione plays an important role in the detoxification of reactive oxygen intermediates generated intracellularly and at sites of inflammation, in the detoxification of toxic electrophilic metabolites of xenobiotics, in cellular immune function, and as a source of cysteine for various organs [Bilzer and Lauterburg 1991; Gmunder et al. 1990: Higashi et al. 1977]. Since several disease states associated with glutathione deficiency have been identified there is growing interest in the therapeutic use of sulphydryls.

The plasma concentration of glutathione has recently been reported to increase in the rat following oral administration of glutathione [Hagen et al. 1990]. If oral glutathione were systemically available in humans, pharmacological doses of oral glutathione might be used to protect against the toxicity of xenobiotics and to normalize plasma glutathione in disease states with low glutathione, such as patients with HIV infection [Buhl et al. 1989], alcoholics [Lauterburg and Velez 1988], and patients with hepatic cirrhosis [Chawla et al. 1984; Burgunder and Lauterburg 1987].

In this study we have assessed the systemic availability of oral glutathione in healthy volunteers.

Subjects and methods

Protocol

Seven healthy volunteers, four women and three men, aged 22 to 47 y, participated in the study, which was approved by the local ethics committee. After an overnight fast an indwelling catheter was placed in an antecubital vein and two blood samples were obtained 30 min apart to establish baseline values for plasma sulphydryls. The subjects then took 0.15 $mmol \cdot kg^{-1}$ of glutathione orally (Boehringer Robin, Milan, Italy) dissolved in 200 ml of water. Further blood samples were obtained at 30 min intervals up to 270 min. The subjects did not eat or drink for the duration of the study. Previous studies have shown that the plasma concentration of endogenous glutathione does not change significantly over this period of time [Burgunder et al. 1989; Porta et al. 1991].

Analytical methods

Heparinized blood (5 ml) was immediately mixed with 0.4 ml of L-serine/Na-borate (final concentration 20 $mmol \cdot l^{-1}$) in order to prevent catabolism of glutathione by γ-glutamyltransferase. After centrifugation at 3500 × g for 2 min the plasma was derivatized with monobromobimane, and unbound glutathione and cysteine in plasma were quantified by HPLC [Newton et al. 1981; Aebi et al. 1991]. Total glutathione and total cysteine (i.e. the unbound sulphydryl, its disulphide, and protein and non-protein mixed disulphides) were assayed by the same method after reduction of plasma samples with dithiothreitol [Aebi et al. 1991].

Glutamate in plasma was measured enzymatically [Beutler and Michal 1974].

Statistics

The results were analysed by one-way and repeat-measures analysis of variance followed by Scheffé's test for multiple contrasts. The data are given as mean (SD).

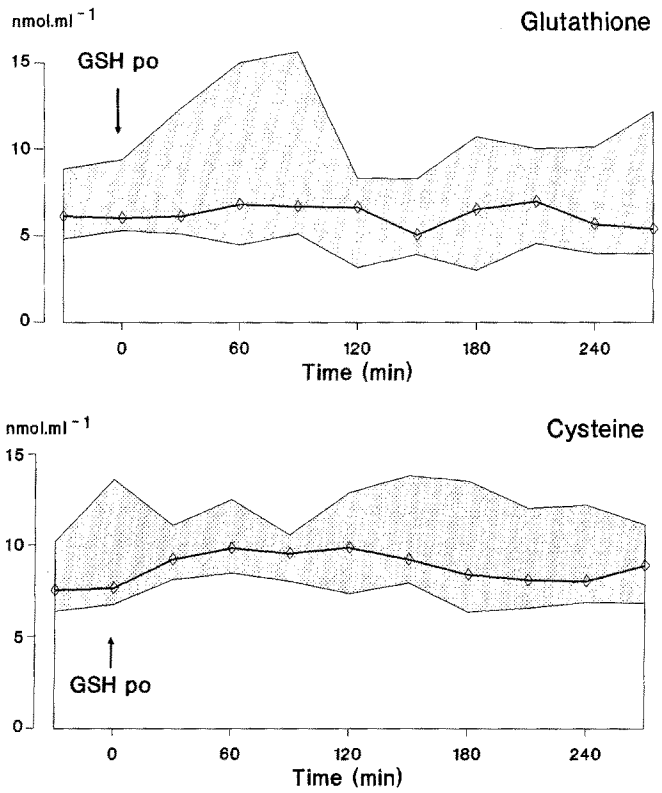

Fig. 1. Plasma concentrations of unbound glutathione (top) and unbound cysteine (bottom) in seven healthy volunteers after the oral administration of 0.15 mmol · kg^{-1} glutathione. Median and range

Results

No volunteer experienced any adverse effects from oral glutathione. The basal concentrations of glutathione and cysteine were 6.2 (1.2) and 8.3 (1.9) μmol · l^{-1} respectively. The time-courses of the two thiols in plasma after oral glutathione are shown in Fig. 1. Plasma glutathione increased transiently in two women within the first 2 h after ingestion. One man had a slight (i.e. less than two-fold) rise in plasma glutathione for 4 h. In the other four subjects there was no increase in plasma glutathione, and at no time during the study was the mean plasma concentration of glutathione significantly different from baseline. Plasma cysteine tended to increase after glutathione, but its mean concentration was at no time significantly different from the basal value.

The basal concentrations of total glutathione, total cysteine, and glutamate were 11.5 (4.3), 266 (65), and 54 (14) μmol · l^{-1} respectively. The concentrations and the ratio of total to unbound glutathione did not increase significantly during the study (Fig. 2).

Discussion

These data show that a single oral dose of 0.15 mmol · kg^{-1} of glutathione does not significantly increase plasma glutathione concentrations in man. We have previously estimated the input of glutathione into the circulation of healthy subjects at 23.5 μmol · h^{-1} · kg^{-1} [Burgunder and Lauterburg 1987]. In spite of this substantial influx the plasma concentration of glutathione is in the low micromolar range because of its short half-life of around 2.5 min [Wendel and Cikryt 1980; Burgunder and Lauterburg 1987]. In order to double the plasma concentration of glutathione the input of exogenous glutathione into the circulation would have to equal the endogenous release into the circulation and thus amount to approximately 25 μmol · h^{-1} · kg^{-1}. Thus, 0.075 mmol · kg^{-1} glutathione would be required to maintain the increased plasma concentration for 3 h, i.e. the systemic availability of glutathione would have to be around 50%.

Intact glutathione may be absorbed to this extent in the rat [Hagen et al. 1990], but our data suggest that the systemic availability of oral glutathione is negligible in man. In man and guinea-pigs the activity of hepatic γ-glutamyltransferase is an order of magnitude higher than in rats. Consequently, the hepatic extraction of glutathione in the portal venous blood of guinea-pigs exceeds 85% [Speisky et al. 1990] and a similar value may be expected in man. Since glutathione is also metabolized by intestinal γ-glutamyltransferase, it is not surprising that there was no sustained increase in plasma glutathione in our subjects.

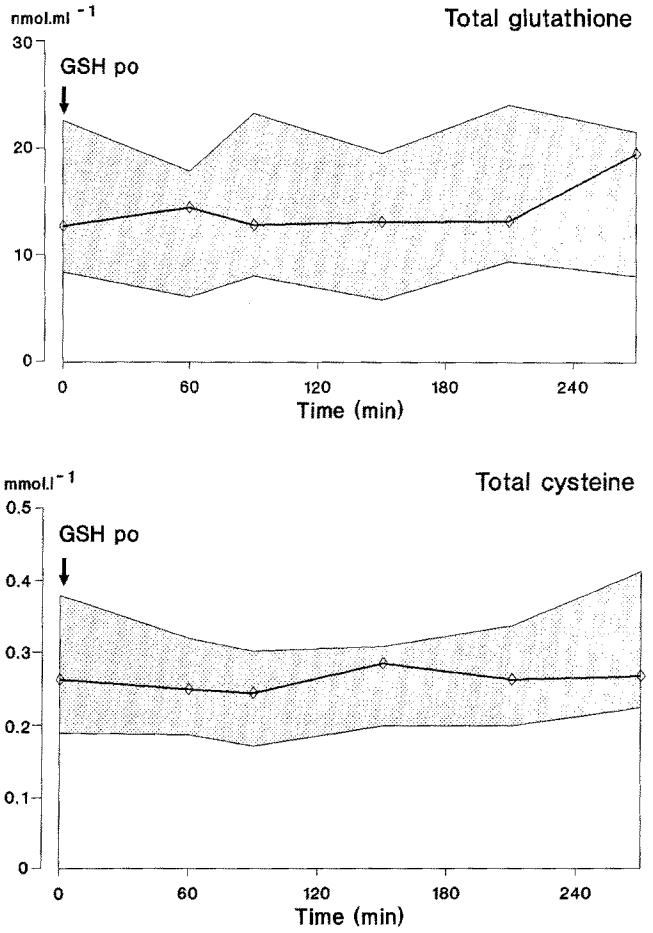

Fig. 2. Plasma concentrations of (top) total glutathione and (bottom) total cysteine (i.e. free sulphydryl, its disulfide, and protein and non-protein mixed disulfides) in seven healthy volunteers after the oral administration of 0.15 mmol · kg^{-1} glutathione. Median and range

Although only minimal amounts of intact glutathione reach the systemic circulation, one would expect the concentrations of its constituent amino acids to increase. However, we did not observe significant increases in plasma cysteine or glutamate after oral glutathione. Since the extent of first-pass metabolism and the rate of turnover of these amino acids are not known, it is difficult to predict how much of these amino acids would have to be absorbed in order to result in measurable increases in their concentrations in peripheral blood.

Low circulating glutathione and low tissue glutathione concentrations have been documented in alcoholics, and in patients with hepatic cirrhosis, HIV infection, and lung disorders [Buhl et al. 1989; Burgunder and Lauterburg 1987; Chawla et al. 1984; Lauterburg and Velez 1988; Cantin et al. 1989]. Whether the glutathione deficiency in these states contributes to morbidity and whether correction of the deficit might alter their natural history can only be determined by increasing the availability of glutathione by pharmacological interventions. The present data indicate that this goal can not be achieved with oral glutathione in doses of up to 3 g, and other avenues to replete glutathione should be explored.

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