Bioavailability of dietary glutathione: effect on plasma concentration

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HAGEN, TORY M., GRAZYNA T. WIERZBICKA, A. H. SILLAU, BARBARA B. BOWMAN, AND DEAN P. JONES. Bioavailability of dietary glutathione: effect on plasma concentration. Am. J. Physiol. 259 (Gastrointest. Liver Physiol. 22): G524-G529, 1990.—Plasma glutathione (GSH) concentration in rats increased from ~15 to 30 μM after administration of GSH either as a liquid bolus (30 μmol) or mixed (2.5-50 mg/g) in AIN-76 semisynthetic diet. GSH concentration was maximal at 90–120 min after GSH administration and remained high for over 3 h. Administration of the amino acid precursors of GSH had little or no effect on plasma GSH values, indicating that GSH catabolism and resynthesis do not account for the increased GSH concentration seen. Inhibition of GSH synthesis and degradation by L-buthionine-[S,R]-sulfoximine and acivicin showed that the increased plasma GSH came mostly from absorption of intact GSH instead of from its metabolism. Plasma protein bound GSH also increased after GSH administration, with a time course similar to that observed for free plasma GSH. Thus dietary GSH can be absorbed intact and results in a substantial increase in blood plasma GSH. This indicates that oral supplementation may be useful to enhance tissue availability of GSH.

GLUTATHIONE (GSH) is the most abundant low-molecular-weight thiol found in mammalian cells and functions in the metabolism of xenobiotics and carcinogens (2), in maintenance of the cellular thiol-to-disulfide ratio (29), and in supply of a nontoxic reservoir of cysteine (10). GSH appears to be synthesized in all mammalian cells and is normally maintained at millimolar concentrations (14). However, the synthetic capacity is insufficient to maintain GSH concentrations when tissues are exposed to certain drugs or their metabolites (e.g., acetaminophen), redox cycling compounds (e.g., menadione), peroxides (e.g., tert-butyl hydroperoxide), X rays, or ultra violet radiation. Depletion of GSH impairs the ability of cells to protect against these agents and results in injury or death. Thus administration of supplemental GSH might allow the detoxication mechanisms to continue protecting cells from injury.

Some epithelial cells have the capacity to take up exogenous GSH. Uptake by isolated cells of rat lung (type II) (7), renal proximal tubule (6), and small intestinal epithelium (15) is Na⁺ dependent and inhibited by structural analogues of GSH. Detailed studies with vesicles from enterocytes and renal cortex (15, 17) showed that GSH transport occurred in the basolateral membranes and had characteristics of an electrogenic Na⁺-coupled symport system. Uptake of GSH by this system protected cells from injury by redox cycling agents or tert-butyl hydroperoxide (6, 7, 15). This indicates that methods to increase plasma GSH may be useful to prevent or treat certain types of toxicological and pathological processes.

Earlier studies with vascularly perfused small intestine of rat showed that intact GSH was transported from the lumen into the vascular perfusate (8). The process was Na⁺ dependent and occurred even in the presence of acivicin (a specific inhibitor of γ-glutamyltransferase) and buthionine sulfoximine (an inhibitor of GSH synthesis). Thus, although breakdown and resynthesis of GSH occur in the intestine, uptake of intact GSH also occurs. In an initial in vivo experiment, administration of a bolus of GSH in solution increased the plasma GSH concentrations (8).

The purpose of the present study was to examine the bioavailability of orally administered GSH by analyzing its effect on plasma GSH. We administered GSH either as a bolus by gavage or in the diet as a solid mixed with the semisynthetic powdered diet, AIN-76. GSH and GSH-protein mixed disulfides were measured in the blood plasma obtained from venous catheters or by cardiac puncture. The former was used in combination with the in vivo GSH-depletion model of Scott and Curthoys (23) to determine whether the intestinal absorption of intact GSH, rather than its degradation and resynthesis, contributes to increases in plasma GSH in vivo. The results demonstrate that administration of GSH, either in a liquid bolus or in solid food, can increase plasma GSH. Thus at least a portion of ingested GSH is directly available for utilization by cells with a capacity to take up GSH.

MATERIALS AND METHODS

**Materials.** GSHII, glutathione disulfide (GSSG), 1,10-phenanthroline, sulfosalicylic acid, heparin (sodium salt), 1-fluoro-2,4-dinitrobenzene, dithiothreitol, γ-glutamyltransferase, and iodoacetic acid were purchased from Sigma Chemical (St. Louis, MO). AIN-76 semisynthetic diet was prepared by ICN Biochemicals (Cleveland, OH). L-Buthionine-[S,R]-sulfoximine (BSO) was purchased from Bachem (Torrence, CA). Acivicin (L-
[αS,βS]-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) was a gift from Dr. J. P. McGovern, Upjohn (Kalamazoo, MI). [35S]GSH (sp act 770 mCi/mmol) was purchased from Du Pont-New England Nuclear (Boston, MA). All surgical equipment and materials were purchased from the Emory University Veterinarian’s office. All other chemicals were reagent grade and obtained from local sources. Deionized or distilled water was used throughout for analytic purposes.

Animal housing and care. Male Sprague Dawley rats (outbred albino, SASCO, Oregon, WI) weighing between 175 and 200 g, were placed on a reversed light-dark cycle for 2 wk before the experiment and fed rat chow and water ad libitum. In studies examining GSH absorption from solid food, the pelleted chow was replaced by AIN-76 diet 7 days before the experiment. Rats were allowed to eat this diet for 1 h immediately after dark to accustom them to eat quickly and synchronize their eating for studies of the time course of absorption.

Jugular vein catheterization. The left external jugular vein was catheterized by the method of Juarbe and Sillau (13). Briefly, rats were anesthetized with a mixture of xylazine (0.03 mg/kg) and ketamine hydrochloride (0.1 mg/kg), the head and neck area were shaved, and the exposed areas were swabbed with povidone-iodine (Betadine). An incision was made in the neck, and the exterior jugular vein was exposed. Silicone tubing (0.02 in. ID × 0.037 in. OD, American Scientific Products, McGaw Park, IL) was filled with a normal saline solution containing heparin, inserted into the vein, and secured with ties. The catheter was exteriorized through a small incision on the head. The neck incision was closed with sutures, and 1,000 IU of penicillin G was administered intramuscularly. A housing with a removable cap was sutured to the head to protect the catheter. Rats had 1 day for recovery before experiments were begun.

Analysis of GSH. GSH was measured by the high-performance liquid chromatographic (HPLC) method of Reed et al. (22). Blood plasma was derivatized with 40 mM iodoacetic acid and 1.5% (vol/vol in absolute ethanol) 1-fluoro-2,4-dinitrobenzene to yield S-carboxymethyl DNP derivatives. The derivatives were separated on a 10-μm Ultrasil-amine column (Beckman Instruments, Norcross, GA) using 80% methanol (vol/vol) in H2O for solvent A and 5 M sodium acetate (pH 4.5) containing 64% (vol/vol) methanol for solvent B. The column was equilibrated at 1 ml/min with 90% solvent A before injection of samples and maintained at 90% solvent A for 10 min after sample injection. A linear gradient to 95% solvent B was then run over 50 min. Derivatives were detected at 365 nm and quantified relative to standards by integration (Hewlett-Packard model 3390A integrator).

Assessment of plasma GSH after gavage. Each rat was given 0.3 μl of 0.9% (wt/vol) NaCl containing no additions, GSH, or amino acids by stomach gavage between 8 and 10 A.M. on successive days. At times indicated, blood (0.15 ml) was drawn through the venous catheter into a heparinized syringe. Deproteinized plasma was prepared and derivatized as previously described (16, 22). Because plasma GSH concentration can be altered by release of GSH from lysed erythrocytes or by oxidation of GSH, analysis needed to be rapid and without damage to erythrocytes during their separation from the plasma. Using techniques as previously described (16), we obtained blood from catheters and heart puncture without hemolysis (<0.2%). The extent of oxidation during sampling and derivatization by this method is ~20% (16). In some experiments, the protein precipitate was solubilized by addition of 0.1 M NaOH, and GSH bound to protein via disulfide linkages was released by addition of 5 mM dithiothreitol. Protein was again precipitated by addition of 10% (wt/vol) trichloroacetic acid, and the supernatant was derivatized for HPLC analysis.

GSH absorption after treatment with acivicin and BSO. To assess the significance of gastrointestinal absorption of GSH, plasma concentrations were depleted by the method of Scott and Curthoys (23). Rats were treated with BSO (300 mg/kg) to inhibit synthesis and then treated with acivicin (10 mg/kg) to inhibit degradation. Circulating GSH was then removed by infusion of γ-glutamyltransferase (0.4 mg/h) and followed by infusion of acivicin to inhibit this enzyme. Scott and Curthoys reported that acivicin treatment inhibited >97% of renal γ-glutamyltransferase, and BSO treatment depleted renal GSH levels by 65% (23). Infusion of γ-glutamyltransferase after acivicin infusion depleted plasma GSH concentrations by >95% (23). Therefore this method allows the study of GSH absorption with minimal interference by normal GSH metabolites.

Plasma GSH concentrations after feeding AIN-76 diet. On the day of the experiment rats were fed AIN-76 powdered diet supplemented with 2.5, 5, 10, 20, 30, or 50 mg GSH/g AIN-76 or the equivalent of 10 mg GSH/g in the form of cystine, glutamate, and glycine. Paired controls were given only AIN-76. The experiment was begun when food was placed in the cages; all food was removed after 1 h. Beginning at 30 min and continuing for 3 h, a rat and its control were anesthetized by a ketamine-xylazine solution and blood was taken (1.0 ml) via cardiac puncture.

RESULTS

Absorption of GSH after administration in a liquid. We previously found that plasma GSH increased nearly threefold in rats 90 min after gavage with 90 μmol GSH in saline (8). The current study was designed to provide a more complete description of the time course of changes in plasma GSH, to measure the concentration dependence of transport in vivo, and to determine whether a similar increase in plasma GSH occurs when GSH is included as a supplement in the diet. For the experiments where GSH was administered by gavage, we implanted a catheter in the jugular vein of rats for blood sampling. With this method, blood sampling was less stressful to the animal, multiple samples could be obtained from an individual rat, and the same rat could subsequently be used as its own control. Results show that endogenous GSH values averaged 12 μM (Fig. 1). A statistically significant increase (P < 0.05) in plasma GSH was detectable 90 min after gavage, with GSH concentrations reaching ~40 μM. Plasma values declined slightly at 2 h...
but were still elevated 3 h after gavage. Thus oral administration of GSH causes a significant increase in plasma GSH concentration that is maintained for over 2 h.

To address whether this increase could be due to synthesis of GSH from amino acids released by hydrolysis of GSH, we administered an equivalent amount of the amino acid constituents of GSH. Measurement of plasma GSH over the same time course showed that no increase occurred (Fig. 1). Thus synthesis from its amino acid precursors cannot account for the elevation in plasma GSH that is observed after administration of GSH.

To further address whether the increase was due to absorption of intact GSH, we inhibited breakdown and resynthesis of GSH in rats by administration of acivicin (10 mg/kg) and buthionine sulfoximine (300 mg/kg), respectively (23). To deplete residual plasma GSH, γ-glutamyltransferase (0.4 mg/h) was injected intravenously and then inactivated by subsequent administration of acivicin (23). Thus the contribution from tissue synthesis and release of GSH to the plasma was greatly reduced. The acivicin-BSO-γ-glutamyltransferase treatment decreased the plasma GSH concentration to <10% of normal (Fig. 2; from 12 to 1.1 μM), in agreement with the results from Scott and Curthoys (23). The time course of plasma GSH (Fig. 2) after administration by gavage showed accumulation of GSH similar to that observed without treatment. Plasma GSH values increased to 31 μM at 90 min after gavage with an initial rate of GSH appearance calculated to be 0.5 nmol·min⁻¹·ml plasma⁻¹. The maximum concentration was comparable to that without the acivicin-BSO treatment even though the treatment results in substantial inhibition of GSH hydrolysis and synthesis. Thus GSH hydrolysis and resynthesis appear to contribute little to the initial rise or maximal value of plasma GSH concentration.

Although the plasma GSH-to-GSSG ratio is high and GSSG contributes little to the total plasma GSH pool, a substantial amount of GSH is present as a mixed disulfide with protein (16). To determine whether oral GSH altered the amount of GSH bound to plasma proteins, we also analyzed protein-bound GSH in the plasma of rats treated with acivicin BSO and given GSH by gavage. Values of protein-bound GSH before gavage were 22 nmol/mg protein and increased after 90 min to 41 ± 13 nmol/mg protein (Fig. 3). The time course roughly paralleled the changes in free GSH, and GSH returned to nearly control values by 3 h after administration (Fig. 3). Thus GSH absorption causes a significant increase in protein-bound GSH as well as in free plasma GSH.

To determine the proteins to which GSH bound, we investigated the binding of [35S]GSH to plasma proteins. Proteins in the blood plasma were obtained by collecting nondeproteinated plasma from acivicin-BSO-treated rats that had been given a [35S]GSH solution. Samples were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (nonthiol-reducing conditions) followed by autoradiography. A densitometric scan of the autoradiogram showed that only one protein bound GSH (data not shown). Comparison with known serum proteins as markers revealed that the protein species that bound GSH comigrated with rat serum albumin. Thus serum albumin, which is known to contain a thiol and form GSH-protein mixed disulfide (11), appears to account for the binding of GSH by proteins in the plasma.

**Absorption of GSH from a solid diet.** Although these data show that gastrointestinal absorption of GSH oc-
curs from a liquid bolus, dietary intake of GSH probably would come mostly from solid foods. Since the rate of movement of a solid meal through the gastrointestinal tract is slower than that of a liquid (19), a greater proportion of GSH may be broken down or not absorbed. We tested GSH absorption from solid foods by mixing different concentrations of GSH with the AIN-76 diet. Rats were allowed to eat either GSH-supplemented or nonsupplemented AIN-76 diet for up to 1 h. Variations occurred in the amount of food ingested for each rat, but the typical amount of food consumed was ~15 g. At various times after food was given, blood was obtained by heart puncture from a rat fed GSH-supplemented diet and from a control fed diet without GSH. Administration of 5 mg GSH/g diet resulted in an increase in plasma GSH from a basal level of 16 μM to 41 at 90 min (Fig. 4). Thus the amount and time course of appearance in plasma is consistent with that observed in studies employing a liquid bolus of GSH. The rate of clearance of GSH from the plasma was relatively slow, with GSH concentrations remaining elevated relative to control values 3 h after food intake.

As discussed above for GSH given in a liquid, the lumen of the intestine contains γ-glutamyltransferase, which breaks down GSH. Therefore the increase in plasma GSH from GSH in the solid diet could be due to breakdown of GSH, absorption of its constituent amino acids, and subsequent synthesis and efflux of GSH from the liver. To address this possibility, we administered an equivalent amount of the amino acid constituents of GSHI mixed in the AIN-76 diet. Results showed that the amino acids did not increase plasma GSH concentrations, indicating that synthesis of GSH does not account for the elevation seen when 5 mg GSH was added per gram of diet (Fig. 4).

To determine the dependence of plasma GSH concentra-

FIG. 3. Measurement of mixed disulfides of GSH with plasma proteins in acivicin-BSO-treated rats after gavage with GSH. Plasma proteins were precipitated from plasma of rats treated with acivicin-BSO-γ-glutamyltransferase (23), and protein-bound GSH was assessed by HPLC after solubilization, addition of 5 mM dithiothreitol to release GSH, and derivatization. Averages of 2 experiments are given with ranges of values indicated by error bars.

FIG. 4. Effect of 5 mg GSH/g diet on plasma GSH values after administration in an AIN-76 powdered diet. Rats were allowed to eat GSH- or amino acid-supplemented AIN-76 diet for up to 1 h. Blood (1 ml) from a rat and its paired control were taken at times indicated and plasma GSH concentrations were determined. Results show an increase in plasma concentration that is similar to that in Fig. 1. Animals receiving AIN-76 without either GSH or amino acids show no increase above zero time (data not shown). Open squares, plasma GSH values after consumption of 5 mg GSH/g in AIN-76 diet; filled squares, plasma GSH values after consumption of an equimolar concentration of Gln, Cys, and Gly. Comparable time courses were obtained from rats given 2.5 or 10 mg GSH/g diet, but maximal value was not as great. Data for 120- and 180-min samples for GSH-treated rats were from single animals with 5 mg GSH/g; similar results were obtained in experiments with 2.5 or 10 mg GSH/g. * Statistically different from control preparations (P < 0.05).

DISCUSSION

Little information is available as to the fate of GSH following various routes of administration. Wendel and co-workers reported that plasma half-life of GSH was 1.9 min in mice (27) and humans (26) after intravenous administration of millimolar concentrations. Ammon et al. (1) reported a similar rapid elimination of GSH after an intravenous dose in rats and concluded that this disappearance was due to oxidation of the GSH. Until the present study, no comparable investigations using oral administration in rats have been performed. Our results show that GSH in either solid or solution results in prolonged increases in plasma GSH. Maximal values of GSH occurred 90–120 min after administration and declined slowly. Comparison of plasma GSH concentrations in controls and acivicin-BSO-treated animals show
that hepatic GSH synthesis and efflux are likely to contribute to the sustained elevated in plasma GSH only after 2 h. Thus, in addition to hepatic synthesis and release, oxidation-reduction reactions, and extrahepatic uptake and degradation, GSH absorption can significantly affect the plasma GSH pool.

The concentration dependence of GSH absorption into the blood plasma indicates that GSH absorption is a regulated process. Intestinal absorption can be affected by a variety of factors, and absorption of some nutrients is strictly controlled by hormones. Hepatic GSH efflux is stimulated by α1-adrenergic agonists (24), and recent studies in our laboratory have shown that certain hormones can stimulate GSH absorption two- to eightfold over basal levels in intestinal segments (Hagen and Jones, unpublished observations). Thus the extent of GSH absorption may depend on the amounts and particular hormones present during and following food intake.

Variation in the GSH-to-GSSG ratio can also affect metabolism and transport processes (29), and this may be important in the concentration dependence of the increase in plasma GSH. Hebbel et al. (9) showed that inhibition of erythrocyte Ca2+ adenosinetriphosphatase occurred after intracellular oxidation of GSH. Conversely, study of protein-disulfide exchange with the ouabain-resistant K+ -Cl− transporter in erythrocytes showed stimulation of K+ transport after intracellular GSH depletion (18). Inhibition and stimulation in these two systems could be partially reversed by allowing GSH synthesis to replate erythrocyte GSH concentrations (9, 18). In light of this type of control, regulation of GSH uptake may be important. The decreased intestinal uptake at higher GSH concentrations may reflect this regulation and indicate that a mechanism may exist to protect against a marked increase in thiol-to-disulfide ratio.

Little is known about the average daily intake of GSH, the amounts of GSH in various food sources, or the importance of dietary GSH in human health or pathologies. Certain meats and vegetables have GSH contents in the millimolar range (4, 12, 28). If an intake of 500 g of food containing 1 mmol GSH/kg is assumed, an individual would consume ∼150 mg GSH/day. This amount (∼10 µmol/kg) is less than the amount administered by gavage to rats and substantially less than the amount found to be optimal for increasing plasma GSH when GSH was mixed with dry diet (5 mg/g; rats consumed ∼15 g food/day or 75 mg/day, ∼1,200 µmol/kg). Our initial studies of GSH absorption in humans given 15 mg/kg (50 µmol/kg) as a bolus in solution showed that this amount gives a two- to fivefold increase in plasma GSH (T. M. Hagen, R. Weber, H. L. Bonkovsky, and D. P. Jones, unpublished observations). Thus it appears that dietary intake of GSH by humans is low relative to the amounts likely to give a substantial increase in plasma GSH.

GSH transport into epithelial cells of lung, kidney, and intestine allows GSH to be maintained better than by synthesis alone and improves the function of GSH-dependent detoxication systems. Thus increasing plasma GSH concentrations by oral administration can increase the availability of GSH for transport into these tissues. This provides the basis for the therapeutic or prophylactic use of GSH against a wide variety of pathophysiological conditions.

Conditions where GSH supplementation may be useful include the disease states that adversely affect plasma GSH concentrations, such as hepatic dysfunction or cirrhosis (3), or those that affect the epithelial cells which can utilize exogenous GSH for protection (5, 7, 20, 25). Because renal proximal tubule cells can take up GSH, oral administration may aid in prevention of radical-induced injury after ischemia (21). However, clinical trials will be needed to determine the effectiveness of therapeutic oral GSH administration in humans.

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