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THE EFFECTS OF BUCILLAMINE ON GLUTATHIONE AND GLUTATHIONE-RELATED ENZYMES IN THE MOUSE

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Abstract—The effect of bucillamine (BA) on glutathione (GSH) and GSH-related enzymes was investigated in C57 mouse. Administration of high doses of BA (150–400 mg/kg) produced a dose-dependent depletion (20–44%) of hepatic GSH, which was similar in magnitude to that produced by equimolar doses of other sulphydryl drugs studied previously. GSH depletion after acute BA administration correlated well with the elevation of serum glutamic-pyruvic transaminase (SGPT) (6–9-fold increase above control). The increase in SGPT after chronic administration (7 days), although significantly higher than the controls, was however much less than after acute administration. The hepatic GSH concentrations of mice given 7 days of BA were similar to the controls, again correlating well with SGPT activity. Administration of BA (150–400 mg/kg) caused also a significant dose-dependent increase in the oxidized glutathione (GSSG) in blood by 2–7-fold, as well as a dose-dependent increase in blood glutathione *S*-transferase (GST) activity (2–13-fold). In an *in vitro* experiment, hepatic GST activity was activated by various concentrations of BA (1 μM–1 mM). There was little or no effect on GSSG reductase and on glutathione peroxidase (GSH-Px) after acute administration of BA. Chronic administration of BA had no effect on hepatic GSSG reductase and GSH-Px, but GSSG reductase activity in blood was increased significantly by 4-fold. It is possible that BA may affect the redox status through auto-oxidation and oxidation with endogenous thiols such as glutathione, affecting GSH concentrations and the GSH/GSSG ratio in tissues and, thus, having both metabolic and toxicological consequences. Whether or not the induction of GST activity *in vivo* in blood and *in vitro* in liver enzyme preparations shared the same underlying mechanism(s) requires further investigation.

Bucillamine (BA*) [*N*-(2-mercapto-2-methylpropionyl)-L-cysteine] is a newly developed drug used in the treatment of rheumatoid arthritis [1, 2]. The exact mechanism of action of BA is not known, but many studies to date suggest that it is an immunomodulator; increasing the ratio of suppresser/helper T cell populations, augmenting the activity of natural killer cells and plasma interferon activity [3] and increasing the phagocytic activity of macrophages [4]. Another feature of BA is its similarity in chemical structure to D-penicillamine (PA), another substituted cysteinyl compound and slow-acting anti-rheumatic drug. Unlike PA, BA contains two sulphydryl groups (Fig. 1). The similarity in their chemical structures implies that they may have similar actions and exert similar anti-rheumatic effects, a view that requires further confirmation.

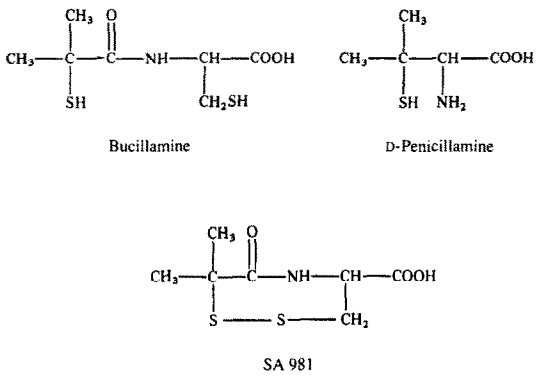

Fig. 1. Chemical structures of D-penicillamine, bucillamine and the intramolecular disulphide of bucillamine (SA981).

Sulphydryl (SH) groups are highly reactive moieties with important functions in many biological processes. The integrity of the SH groups of intracellular and plasma membrane proteins and soluble thiols are essential to a large number of biological functions [5–8]. In addition, low molecular weight non-protein thiols have been shown also to have a protective effect against free radical-, radiation- and chemically reactive metabolite-induced toxicity [6, 9–11], but many thiols may themselves cause glutathione (GSH) depletion and thus lead to oxidative stress and even toxicity [12, 13]. Initial metabolic studies have shown that BA may form the intramolecular disulphide as well as other mixed disulphides [14, 15]. As the oxidation of SH groups may affect the redox state and the availability of SH groups of structurally and functionally important protein and non-protein

* Abbreviations: BA, bucillamine; BSA, bovine serum albumin; CDNB, 1-chloro-2,4-dintrobenzene; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; GSSG reductase, glutathione reductase; GSH-Px, glutathione peroxidase; MOPS, morpholinopropanesulphonic acid; PA, D-penicillamine; NADPH, nicotinamide adenine dinucleotide, reduced form.

endogenous thiols, the effect of BA on tissue GSH was investigated in the present study. Other GSH-related enzymes such as glutathione *S*-transferase (GST), glutathione reductase (GSSG reductase) and glutathione peroxidase (GSH-Px), which are essential to the overall regulation of GSH and redox balance, were investigated also.

MATERIALS AND METHODS

Reagents. Bucillamine was a gift from Santen Pharmaceutical Co. (Osaka, Japan). D-Penicillamine, glyoxalase I (Grade IV, from yeast), reduced and oxidized glutathione (GSH and GSSG), glutathione *S*-transferase (GST, from equine liver), methylglyoxal, reduced nicotinamide adenine dinucleotide (Type X), *t*-butyl hydroperoxide, GSH reductase (Type III), morpholinopropane-sulphonic acid (MOPS), Folin and Cicocalteu's Phenol reagent and other general reagents were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Perchloric acid (60%) was obtained from Merck (Darmstadt, F.R.G.). All solvents were redistilled before use.

Determination of tissue glutathione (GSH). The liver aliquot (0.3–0.5 g) was homogenized with ice-cold 1 M perchloric acid (3 mL) containing 2 mM EDTA. The homogenate was then centrifuged (2000 *g*) for 15 min at 4° to obtain a clear supernatant. An aliquot (0.2 mL) of the acidic supernatant was neutralized with 0.1 mL of 2 M potassium hydroxide containing 0.3 M MOPS. The mixture was centrifuged for 30 sec at 2000 *g*. The sample was assayed for GSH immediately after neutralization by the glyoxalase I method [16].

For blood GSH, an equal volume of ice-cold 2 M perchloric acid containing 4 mM EDTA was added to the blood sample (0.5 mL) immediately after it was obtained. The acidic supernatant was obtained by centrifugation and reduced GSH determined as described previously.

Effect of bucillamine on reduced GSH in blood and liver. Male C57 mice (25–30 g), with free access to food and water, were given various doses of BA in saline (150–400 mg/kg, i.p.). Control animals received saline solution only. After 3 hr, blood was obtained by heart puncture while the animal was under ether anaesthesia. Aliquots of blood (0.5 mL) were taken for the determination of reduced glutathione (GSH) concentration and also the GSH-related enzyme activities. Aliquots (0.5 g) of liver were also taken for the determination of tissue GSH concentration and GSH-related enzymes activities (GST, GSH-Px and GSSG reductase).

Determination of glutathione S-transferase (GST), glutathione reductase (GSSG reductase) and glutathione peroxidase (GSH-Px) activities. Liver samples (0.5 g) were homogenized in 10 mL ice-cold 0.01 M phosphate 0.15 M KCl buffer at pH 7.4. The homogenate was centrifuged at 10,000 *g* for 15 min at 4°. The supernatant was used as the source of liver GST, GSSG reductase and GSH-Px. Blood samples (0.5 mL) were also homogenized (by hand) in the same buffer and the supernatant was used as the source of blood GST, GSSG reductase and GSH-Px. All samples were kept on ice at all times prior to use.

GST activity was determined by a modification of the method described previously [17], using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The incubation mixture contained 0.5 mM CDNB, 1 mM GSH and 10–20 μg protein (cytosolic + microsomal) in 0.1 M phosphate buffer (pH 6.5) with 0.1 mM EDTA. The formation of the conjugate of GSH and CDNB was monitored at 340 nm.

GSH-Px activity was measured by the coupled enzyme procedure [18] based on a NADPH-coupled reaction whereby oxidized glutathione (GSSG) produced by GSH peroxidase and hydroperoxide was reduced by exogenous glutathione reductase and NADPH. The decrease in absorbance of NADPH at 340 nm was measured over 5 min. The reaction mixture (3 mL) consisted of 0.24 mM hydroperoxide/*t*-butyl hydroperoxide, 1 mM GSH, 1.5 units of yeast GSSG reductase and 1.4 mM NADPH in 0.15 M phosphate buffer (pH 7.5) with 5 mM EDTA. One unit of GSH-Px activity is expressed as the oxidation of 1 nmol NADPH/min/mg protein.

GSSG reductase was assayed by the method of Carlberg and Mannervik [19]. The rate of oxidation of NADPH by GSSG was measured as the decrease in absorbance at 340 nm over 5 min. One unit of GSSG reductase activity is expressed as the oxidation of 1 μmol NADPH/min/mg protein.

Protein concentration in the liver fractions (cytosolic + microsomal) was measured by Lowry's method using bovine serum albumin as standard [20].

Effect of bucillamine on glutathione S-transferase (GST) activity in vitro. Livers from male C57 mice (25–30 g) given previously free access to food and water, were excised immediately after the animals were killed, and homogenized in ice-cold 0.01 M phosphate 0.15 M KCl buffer at pH 7.4. The homogenate was centrifuged at 10,000 *g* for 15 min at 4°. The supernatant was used as the source of glutathione *S*-transferase enzymes (GST).

Bucillamine (1 μM–1 mM, final concentration) was incubated with the enzyme preparation (1 mg/mL) at 37° for a series of incubation times (0–240 min). The GSH-related enzymes activities were determined, as described previously, by taking aliquots (50 μL) of a diluted solution of the incubation mixture (1 in 50) for each assay system. Protein concentrations were also determined to calculate the enzyme activity.

Statistical analysis. All results are reported as mean ± standard deviation of the mean. Differences between means were determined using Student's *t*-test.

RESULTS

Effect of bucillamine (BA) on reduced glutathione (GSH) and oxidized glutathione (GSSG) and serum glutamic-pyruvic transaminase (SGPT) activity

As shown in Fig. 2, BA caused a dose-dependent (150–400 mg/kg) depletion of hepatic GSH (20–44%) and GSSG (18–61%). When calculated on an

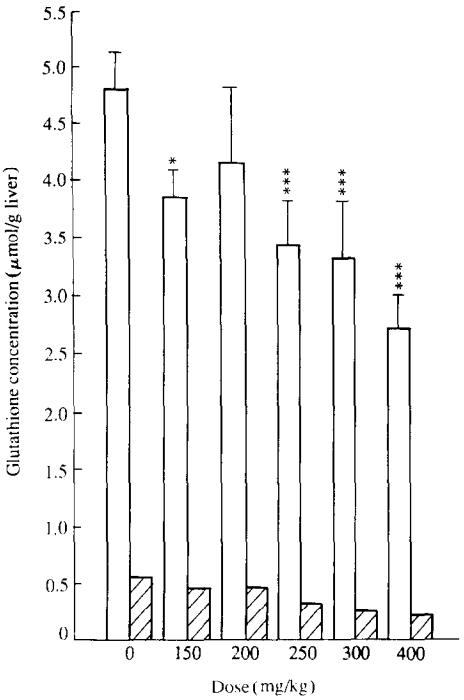

Fig. 2. Effect of bucillamine (150–400 mg/kg, i.p.) on hepatic reduced glutathione GSH (□) and oxidized glutathione GSSG (▨) 3 hr after administration.

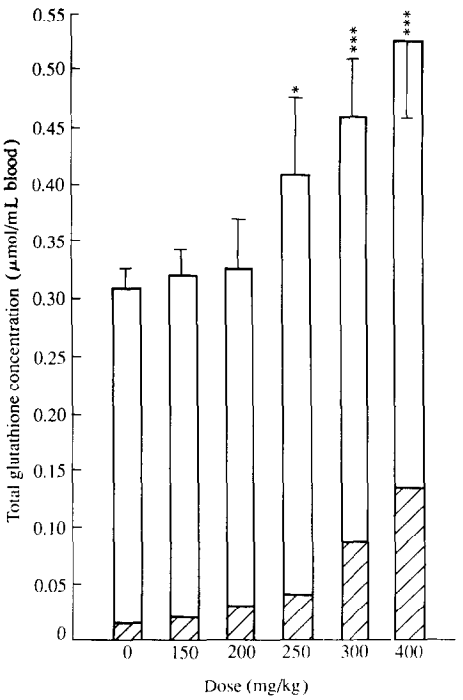

Fig. 3. Effect of bucillamine (150–400 mg/kg, i.p.) on blood reduced glutathione GSH (□) and oxidized glutathione GSSG (▨) 3 hr after administration.

equimolar basis, the depletion of hepatic GSH by BA was similar in magnitude to that caused by other sulphydryl drugs studied previously [21].

BA had little effect on reduced GSH in blood (Fig. 3). However, the amount of total glutathione (GSH + GSSG) increased dose-dependently with BA from 250 mg/kg upwards. This increase in total glutathione (GSH + GSSG) was due mainly to an increase in GSSG level, which may be a result of more GSSG being translocated from the liver to blood.

There was a dose-dependent increase (6–9-fold above control) of serum glutamic-pyruvic transaminase activity in mice, 3 hr after BA administration (Table 1). The increase in SGPT correlated with the depletion of hepatic GSH.

When BA was administered for 7 days, the SGPT activity was 68.4 ± 7.4 units/L, 3 hr after the final dose of BA was given, compared to 28.6 ± 5.9 units/L in the controls. This was much lower than when BA was administered acutely (Table 1). The hepatic GSH concentrations in mice after chronic BA administration were similar to the controls. The lack of significant GSH depletion in the liver correlated with the lesser degree of liver toxicity, as indicated by a smaller increase in SGPT activity.

Table 1. Effect of bucillamine on serum glutamic-pyruvic transminase (SGPT) activity in mice 3 hr after the final i.p. administration

Treatment	SGPT activity (units/L)	% Depletion of GSH
Control	28.6 ± 5.9	0
BA, single dose		
150 mg/kg	179.0 ± 48.1*	20.0%
200 mg/kg	192.3 ± 37.3*	29.1%
250 mg/kg	262.6 ± 49.8*	31.4%
300 mg/kg	218.0 ± 32.5*	43.7%
BA, 7 days		
200 mg/kg	68.4 ± 7.4*	—

Results are mean ± SD of eight animals.
* $P < 0.001$ compared to controls using Student's t-test.

Effect of bucillamine (BA) on glutathione S-transferase (GST), glutathione reductase (GSSG reductase) and glutathione peroxidase (GSH-Px) activities

The GST activity in blood was increased substantially by BA (Table 2). The increase was dose-dependent, ranging from 2-fold (200 mg/kg BA) to 13-fold (400 mg/kg BA) when compared to control levels. GST activity in the liver was not, however, affected by BA. This may be related to the high capacity of the liver and the large quantity of GST present in the liver. GSSG reductase and GSH-Px activities in both whole blood and liver were not affected by the doses of BA used, except at 400 mg/kg of BA, when blood GSH-Px activity appeared to be higher than the controls (Table 2, not significant).

Table 2. Effect of various doses of bucillamine on glutathione reductase (GSSG reductase; unit/mg protein), glutathione peroxidase (GSH-Px; unit/mg protein) and glutathione *S*-transferase (GST; μmol/min/mg protein) activities in whole blood of mouse 3 hr after i.p. administration

Dose (mg/kg)	GSSG reductase activity (unit/mg protein)	GSH-Px activity (unit/mg protein)	GST activity (μmol/min/mg protein)
Control	1.78 ± 0.27	6.73 ± 0.67	2.50 ± 0.27
150	2.54 ± 0.91	6.68 ± 2.05	2.25 ± 1.36
200	2.31 ± 0.64	6.88 ± 0.79	5.27 ± 2.14*
250	2.58 ± 0.69	5.57 ± 2.27	22.69 ± 5.83†
300	2.00 ± 0.47	7.37 ± 2.25	22.69 ± 5.01†
400	2.06 ± 1.03	10.94 ± 4.48	33.50 ± 15.32†

Results are mean ± SD of six animals.
* $P < 0.05$, † $P < 0.001$ compared to controls using Student's *t*-test.
Unit of GSSG reductase activity: μmol/NADPH oxidized/min/mg blood.
Unit of GSH-Px activity: nmol NADPH oxidized/min/mg protein in whole blood.

Table 3. Effect of chronic administration of bucillamine (200 mg/kg; i.p. for 5 days) on glutathione-related enzyme activities in the liver and blood of mouse 3 hr after the final dose

	GSSG reductase activity (unit/mg protein)	GSH-Px activity (unit/mg protein)	GST activity (μmol/min/mg protein)
Liver			
Control	0.05	102 ± 5	6.20 ± 0.13
BA-treated	0.06 ± 0.01	102 ± 9	4.34 ± 1.31*
Blood			
Control	0.92 ± 0.32	7.54 ± 1.57	4.00 ± 2.41
BA-treated	3.57 ± 2.85*	6.11 ± 1.69	4.02 ± 2.83

Results are mean ± SD of six animals.
* $P < 0.005$ compared to controls using Student's *t*-test.
Unit of GSSG reductase activity: μmol/NADPH oxidized/min/mg protein.
Unit of GSH-Px activity: nmol NADPH oxidized/min/mg protein.

Effect of chronic administration of bucillamine (BA) on glutathione S-transferase (GST), glutathione reductase (GSSG reductase) and glutathione peroxidase (GSH-Px) activities

As shown in Table 3, GST activity in the liver was decreased by 30% after administration of BA (200 mg/kg) for 5 days, while blood GST activity was not affected. GSSG reductase activity in whole blood was increased by 4-fold to 3.57 ± 0.85 μmol/min/mg protein compared to 0.92 ± 0.32 μmol/min/mg protein in the control. However, GSSG reductase activity in the liver was not affected. There was no difference in GSH-Px activity between BA and control, both in the liver and in whole blood.

Effect of bucillamine on glutathione S-transferase (GST) activity in vitro

Bucillamine caused a dose-dependent (1 μM–1 mM, final concentration) increase in GST activity *in vitro* (Fig. 4). The increase was most significant in the first 60 min for all concentrations of BA used. There was, however, little difference between the increases in GST activity caused by high concentrations of BA (0.1 and 1 mM).

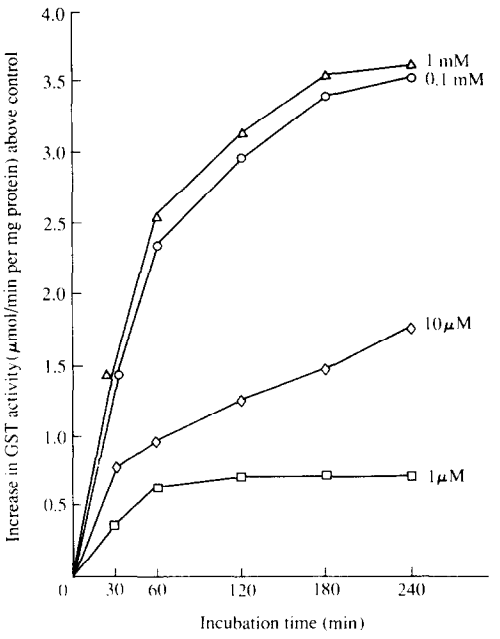

Fig. 4. Effect of bucillamine (1 μM–1 mM, final concentration) on glutathione *S*-transferase (GST) activity *in vitro*. The results are mean of 3 individual experiments, SE < 10%.

DISCUSSION

Glutathione (GSH) plays an important role, in

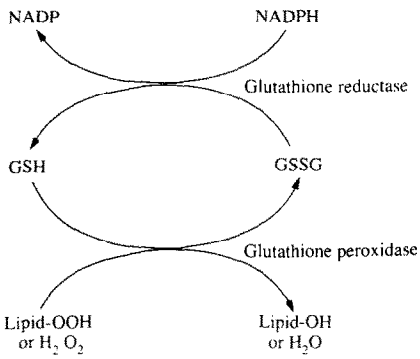

Fig. 5. Oxidation and reduction of glutathione.

many biological processes, in the intracellular protection against xenobiotics, reactive oxygen species and free radicals [22]. Conjugation of GSH with many xenobiotics or their reactive metabolites occurs either spontaneously or enzymically via the action of glutathione *S*-transferase (GST). This serves as a protective mechanism to facilitate disposition of reactive compounds [23]. The relationship between reduced and oxidized glutathione (GSH and GSSG) and other GSH-dependent enzymes is shown in Fig. 5. GSH is a substrate for GSH-Px which catalyses the reduction of hydrogen peroxide and other lipid peroxides, thus protecting cell membranes from peroxidation [7]. GSSG produced in this reaction is reduced subsequently to GSH by NADPH via the action of GSSG reductase. Therefore, it is evident that the GSH redox cycle plays a vital role in preventing oxidative stress and damage in many tissues [8, 24].

An interesting feature of bucillamine (BA) is its similarity in chemical structure to D-penicillamine (PA), but with an additional sulphydryl group. Metabolic studies have shown that BA may form disulphides, mixed disulphides with endogenous thiols and may also be oxidized to the intramolecular disulphide [14, 15]. It was, therefore, of interest to investigate whether BA would affect GSH status as did other sulphydryl drugs, such as captopril and D-penicillamine [21]. Intraperitoneal administration of BA produced a dose-dependent depletion of hepatic glutathione in mouse (Fig. 2), with both reduced glutathione (GSH) and oxidized glutathione (GSSG) being depleted. There are several possible mechanisms by which BA may interact with glutathione. Firstly, BA may be oxidized by a hepatic thiotransferase to form BA–GSH mixed disulphide directly. Secondly, hepatic GSH may be utilized in spontaneous thiol–disulphide reactions involving the BA-mixed disulphides or the intramolecular disulphide of BA. Thirdly, BA may affect the GSH-dependent enzymes such as GSSG reductase and GSH-Px, which leads to changes in the intraorgan GSH status or interorgan translocation of glutathione. This may have been the case in this study when the blood glutathione status in the same animals was considered. The concentrations of reduced GSH did not change significantly while total glutathione (GSH + GSSG) increased dose-dependently 3 hr after BA administration (Fig. 3). This increase can be attributed solely to the increase in the GSSG level in blood, which may be the result of an increase in interorgan translocation of glutathione in the form of GSSG from the liver to the blood; and/or an increase in the oxidation of GSH to GSSG, secondary to changes in the GSH-dependent enzymes activities. However, there was little or no effect on GSSG reductase and GSH-Px in these animals after an acute administration of BA. Accordingly, chronic administration of BA had no effect on hepatic GSSG reductase and GSH-Px, while hepatic GSH was depleted (Table 3). More interestingly, GSSG reductase activity in blood was increased, which may be necessary under the circumstances to reduce the high levels of GSSG translocated from extravascular sites back to GSH, in order to maintain the overall sulphydryl integrity.

Another interesting observation of this study was the different effect of BA on GST after acute and chronic administration. While blood GST was increased significantly by increasing doses of BA (Table 1) after acute administration, hepatic GST (cytosolic + microsomal) activity was decreased after chronic administration. The effect of BA on blood GST is unlikely to be an artifact since BA did not interfere with the colorimetric assay of GST. Similar activation of GST activity has been reported previously with active oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radicals [25, 26]. Taking into consideration the ability of BA to oxidize endogenous thiols or undergo auto-oxidation to form the intramolecular disulphide, it is likely that the activation of blood GST was a result of hydrogen peroxide and superoxide radicals being generated at high concentrations of BA [27] or during auto-oxidation of thiols [28]. Although hepatic GST activity did not appear to be affected in this study this signifies only the large capacity for GST activity *in vivo*, for when BA was incubated with GST *in vitro*, similar activation of hepatic GST occurred also (Fig. 4). However, the exact mechanisms and significance of this activation of GST remain to be investigated. Further studies are currently under way to investigate the possible mechanisms.

In this study, high doses of BA depleted hepatic GSH and elevated SGPT activity. Depletion of GSH is reported to enhance lipid peroxidation [29, 30] but it is as yet unknown whether depletion of GSH *per se* would lead to cell damage, although certain drug-induced toxicities were usually preceded by GSH depletion. There are some reports in the literature of liver dysfunction following BA treatment for rheumatoid arthritis [1], but the doses of BA used in this study were far in excess of therapeutic doses (4.3 mg/kg). It is uncertain whether BA at therapeutic doses would produce such a significant depletion of hepatic GSH. However, patients receiving long-term BA, whose thiols levels may be affected through disease or multiple drug treatment which could affect the GSH status or related enzyme systems, should have liver function tests performed regularly.

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REFERENCES

1. Abe C, Clinical evaluation of immunomodulators. *Int J Immunotherapy* **1**: 7–10, 1985.
2. Kashiwazaki S and Shiokawa Y, Bucillamine: a new immunomodulator. *Int J Immunother* **3**: 1–6, 1987.
3. Kosaka S, Itoh K and Kumagai K, An *in vitro* study of SA-96 (DE-019), an immunomodulator, and its application in the treatment of rheumatoid arthritis. *Tohoku J Med* **96**: 13–21, 1983.
4. Hayashi M, Abe C, Yamauchi H, Iso T and Shiokawa Y, Effects of SA96 on self-defence system in Balb/c mice. *Int J Immunother* **1**: 219–225, 1985.
5. Arias IM and Jakoby WB, *Glutathione: Metabolism and Function*. Raven Press, New York, 1976.
6. Pandeya SN, Role of sulphydryl compounds in biological systems. *J Sci Industrial Res* **38**: 568–593, 1979.
7. Reed DJ and Beatty PW, Biosynthesis and regulation of glutathione: toxicological implications. In: *Reviews in Biochemical Toxicology 2* (Eds. Hodgson E, Bird JR and Philpot RN), pp. 213–241. Elsevier/North Holland, New York, 1980.
8. Reed DJ, Regulation of reductive process by glutathione. *Biochem Pharmacol* **35**: 7–13, 1986.
9. Mitchell JB, Biaglow JE and Russo A, Role of glutathione and other endogenous thiols in radiation protection. *Pharmacol Ther* **39**: 269–274, 1988.
10. Haenen GRMM, Vermeulen NPE, Timmerman H and Bast A, Effect of thiols on lipid peroxidation in rat liver microsomes. *Chem Biol Interact* **71**: 201–212, 1989.
11. Yeung JHK, Effect of sulphydryl drugs on paracetamol-induced hepatotoxicity in mice. *Drug Metab Drug Int* **6**: 295–301, 1988.
12. Helliwell TR, Yeung JHK and Park BK, Hepatic necrosis and glutathione depletion in captopril-treated mice. *Br J Exp Path* **66**: 67–78, 1985.
13. Munday R, Toxicity of thiols and disulphides: involvement of free-radical species. *Free Radical Biol Med* **7**: 659–673, 1989.
14. Takashina H, Horiuchi M, Miyagi S, Uemura O and Tadashi I, Study on metabolism of dithiol compound II. Determination of *N*-(2-mercapto-2-methylpropanoyl)-L-cysteine (SA96) and its metabolites in blood and urine of rats and dogs. *Yakugaku Zasshi* **105**: 671–676, 1985.
15. Morikawa N, Matsumoto J, Kito H and Iso T, Metabolic fate of *N*-(2-mercapto-2-methylpropionyl)-L-cysteine (SA96) II. Absorption, distribution, metabolism and excretion during repeated administrations to male rats. *Iyakuhin Kenkyu* **16**: 808–817, 1985.
16. Akerboom TPM and Sies H, Assay of glutathione, glutathione disulphide and glutathione mixed disulphides in biological samples. *Methods Enzymol* **77**: 373–382, 1981.
17. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases: the first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
18. Paglia DE and Valentine WN, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* **70**: 158–169, 1967.
19. Carlberg I and Mannervik B, Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J Biol Chem* **250**: 5475–5480, 1975.
20. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
21. Yeung JHK, Breckenridge AM and Park BK, Drug–protein conjugates VI. Role of glutathione in the metabolism of captopril and captopril plasma protein conjugates. *Biochem Pharmacol* **32**: 3619–3625, 1983.
22. Meister A, New aspects of glutathione biochemistry and transport-selective alteration of glutathione metabolism. *Nutr Rev* **42**: 397–410, 1984.
23. Boyland E and Chasseaud LF, The role of glutathione and glutathione *S*-transferases in mercapturic acid biosynthesis. *Adv Enzymol* **32**: 172–219, 1969.
24. Inoue M, Nobukuni Y, Ando Y, Hirota M, Hirata E and Morino Y, Interorgan metabolism of glutathione as the defence mechanism against oxidative stress. In: *Nephrotoxicity of Antibiotics and Immunosuppressants* (Eds. Tanabe T, Hook JB and Endou H), pp. 51–60. Elsevier, Amsterdam, 1986.
25. Aniya Y and Anders MW, Activation of rat liver microsomal glutathione *S*-transferase by reduced oxygen species. *J Biol Chem* **264**: 1998–2002, 1989.
26. Mureta T, Hatayama I, Satoh K, Tsuchida S and Sato K, Activation of rat glutathione transferases in Class Mu by active oxygen species. *Biochem Biophys Res Commun* **171**: 845–851, 1990.
27. Mita S and Matsunaga K, Differences in the effects of the antirheumatic drugs, bucillamine and D-penicillamine, on mitogen-induced proliferation of mouse spleen cells. *Agents Actions* **30**: 363–368, 1990.
28. Misra HP, Generation of superoxide free radical during the autooxidation of thiols. *J Biol Chem* **249**: 2151–2155, 1973.
29. Younes M and Siegers CP, Lipid peroxidation as a consequence of glutathione depletion in rat and mouse liver. *Res Commun Chem Pathol Pharmacol* **27**: 119–128, 1980.
30. Levine WG, Glutathione, lipid peroxidation and regulation of cytochrome P-450 activity. *Life Sci* **31**: 779–784, 1982.