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Is Thallium-induced Nephrotoxicity in Rats Connected with Riboflavin and/or GSH?—Reconsideration of Hypotheses on the Mechanism of Thallium Toxicity

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Key words: buthionine sulphoximine; glutathione; mechanism of action; *N*-acetylcysteine; nephrotoxicity; rat; riboflavin; thallium.

Adult female Wistar rats (Han:Wist) were injected with 2 mg of Tl_2SO_4 per 100 g body weight. Parameters of nephrotoxicity were urinary volume and protein excretion as well as blood urea nitrogen concentration. Thallium concentrations were determined in renal cortex and medulla.

There was no effect of different schedules of vitamin B_2 (riboflavin) treatment on thallium nephrotoxicity. Glutathione (GSH) concentration was not decreased by thallium in renal cortex or in medulla. The increase of GSH concentration in renal tissue by *N*-acetylcysteine pretreatment did not influence thallium nephrotoxicity. Buthionine sulphoximine diminished thallium nephrotoxicity by a significant decrease of thallium concentration in renal medulla, which was caused by enhanced urinary excretion of thallium.

From our investigations we conclude that there is no relation between thallium-induced nephrotoxicity and riboflavin and/or GSH. © 1998 John Wiley & Sons, Ltd.

INTRODUCTION

Thallium (Tl) is recognized to be one of the most toxic heavy metals. A variety of mechanisms proposed to account for the metal's toxicity are discussed in the literature. Its ability to interfere with a variety of potassium-dependent processes[1,2] is thought to play a significant role in the generation of its toxic effects.[3,4] The affinity of Tl to Na^+/K^+-ATPase is 10 times higher than that of potassium.[5] Thallium is accumulated into mitochondria and appears to act as an uncoupler of oxidative phosphorylation.[6] By *in vitro* investigations it has been shown that aerobic respiration in skin, brain and kidney tissue was inhibited markedly by the metal.[7] It has been suggested that Tl inhibits the activity of FAD enzymes such as glutathione reductase by causing a deficiency of vitamin B_2 (riboflavin) as substrate or cofactor.[3,4,8] Furthermore, Tl has an affinity for SH groups, which may contribute to Tl toxicity.[2,3,8]

The precise biochemical mechanisms underlying the clinical manifestations of thallotoxicosis are yet to be proved. The influence of Tl on the activity of Na^+/K^+-ATPase as a mechanism of toxicity supposed in the literature will be compared with our own previous data[10] in the discussion. The aim of the experiments presented in this paper was to investigate the role of riboflavin and reduced glutathione (GSH) for Tl nephrotoxicity in rats. Thallium administered at a dose of 2 mg 100 g^{-1} body wt. was proved to produce reversible functional and morphological impairment of kidney function.[10] Riboflavin depletion discussed in the literature was prevented by the administration of riboflavin. The GSH concentration in renal tissue was enhanced and decreased by *N*-acetylcysteine (ac-cys) and buthionine sulphoximine (BSO), respectively.

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MATERIALS AND METHODS

Animals and treatments

Experiments were done with 55-day-old female Wistar rats (Han:Wist) of our own outbreed. Rats were kept under standardized conditions, including a standard diet (Altromin 1316) and free access to tapwater. Animals were divided into the following groups:

Control. In previous investigations it was proved that riboflavin (denoted B2), ac-cys and BSO did not influence urinary volume or protein excretion. Therefore, to economize on rats, control values were obtained from all rats before the injection of any substance (time zero).

Tl group. A dose of 2 mg Tl_2SO_4 (Tl; Riedel-de Haen AG, Seelze, Germany) 100 g^{-1} body wt. dissolved in 5 ml of 0.9% NaCl 100 g^{-1} body wt. was injected intraperitoneally (i.p.).

Tl/B2 groups. Doses of 5 mg riboflavin-5′-phosphate-sodium salt (B2; SERVA Feinbiochemica, Heidelberg, Germany) 100 g^{-1} body wt. dissolved in

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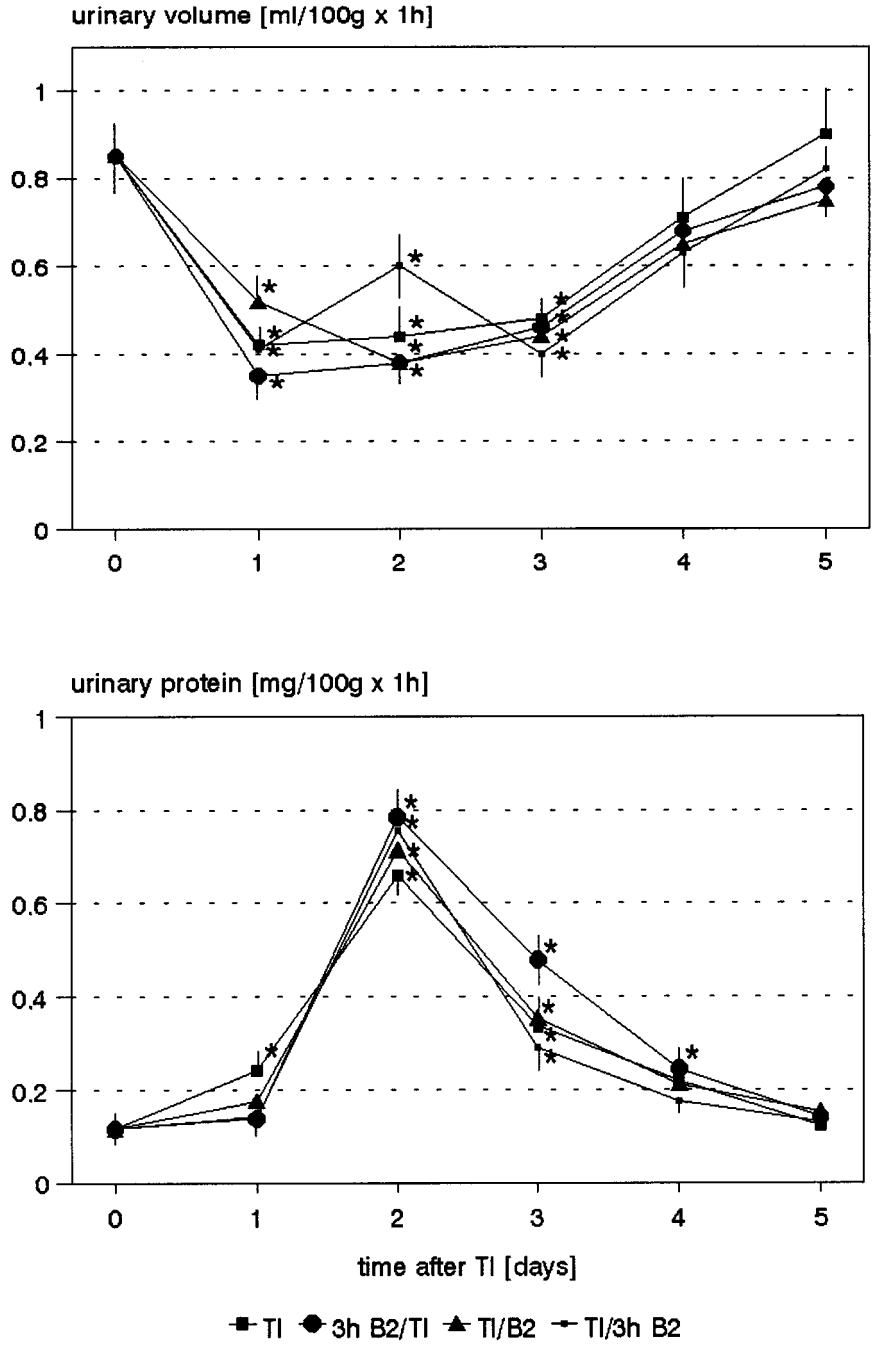

Figure 1. Influence of 5 mg B2 100 g^{-1} body wt. on Tl-induced nephrotoxic changes. Urinary volume and protein excretion were determined for 5 days after the administration of 2 mg Tl_2SO_4 100 g^{-1} body wt. Riboflavin (B2) was administered 3 h before (3 h B2/Tl), concomitantly with (Tl/B2) or 3 h after Tl (Tl/3 h B2). * Statistically significant differences to control value at time zero ($P \leq 0.05$, $n = 6$).

distilled water (1 ml 100 g^{-1} body wt. i.p.) were administered concomitantly (Tl/B2), 3 h before (3 h B2/Tl) or 3 h after 2 mg Tl 100 g^{-1} body wt. (Tl/3 h B2).

Tl/ac-cys groups. Doses of 100 mg *N*-acetylcysteine (ac-cys; Sigma-Aldrich GmbH, Germany) 100 g^{-1} body wt. were administered subcutaneously (s.c.) in 1 ml of 0.9% NaCl 100 g^{-1} body wt. 12 h before, concomitantly with and 12 and 24 h after 2 mg Tl 100 g^{-1} body wt.

Tl/BSO groups. Doses of 0.4 mmol buthionine sulphoximine (BSO; Sigma-Aldrich GmbH, Germany) 100 g^{-1} body wt. were administered s.c. dissolved in 2 ml of 0.9% NaCl 100 g^{-1} body wt. 4 h before and 24 h after 2 mg Tl 100 g^{-1} body wt.

Experimental design and sample collection

Rats were decapitated in ether anaesthesia, exsanguinated and the kidneys were removed. The effect of Tl and of the pretreatment with B2, BSO and ac-cys on the concentration of GSH and oxidized glutathione (GSSG) in renal cortex and medulla was tested. Thallium concentration was determined in renal cortex and medulla as well as in urine. Further investigations were performed separately in renal cortex and medulla.

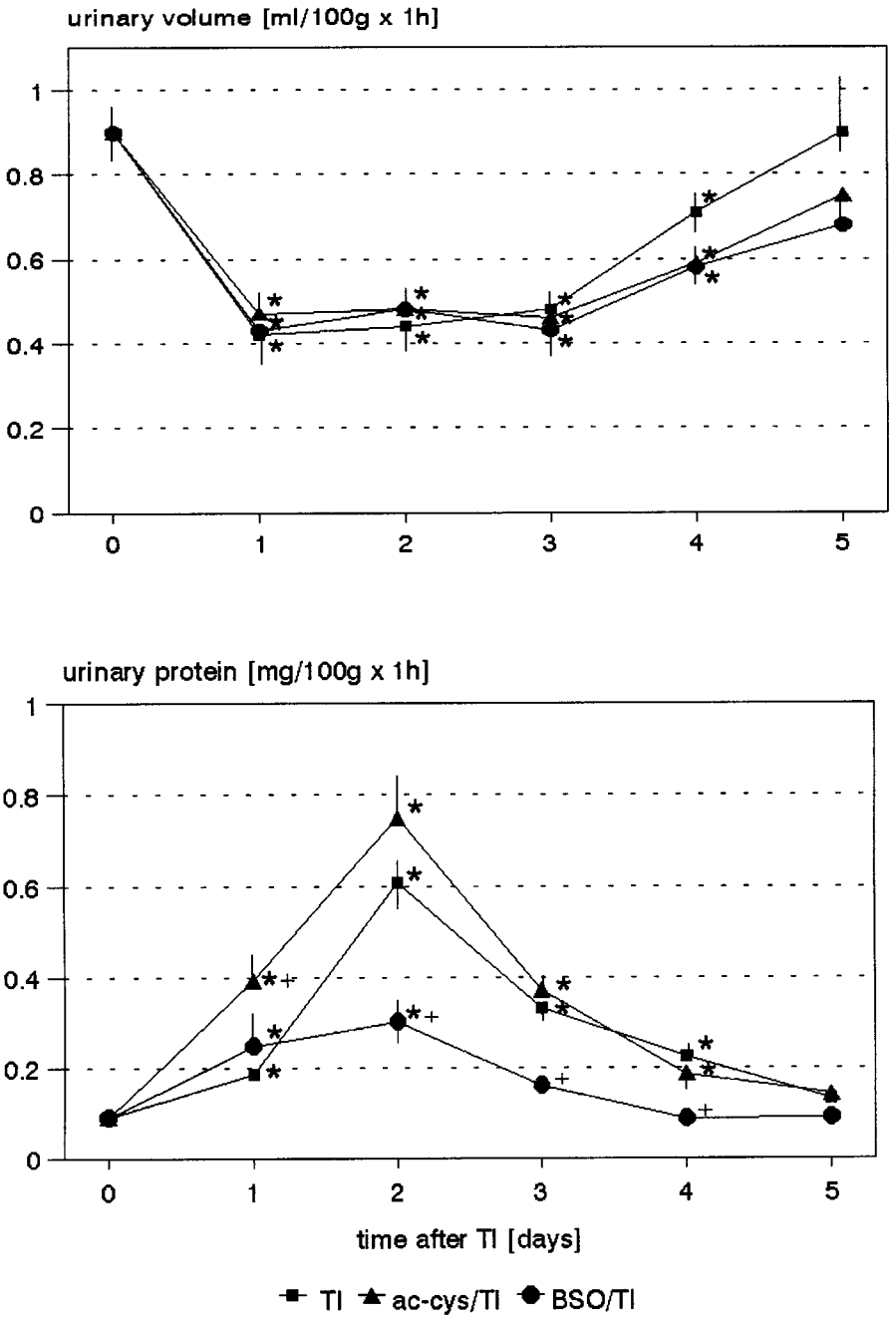

Figure 2. Influence of ac-cys and BSO on Tl-induced nephrotoxic changes. Urinary volume and protein excretion were determined for 5 days after the administration of 2 mg Tl_2SO_4 100 g^{-1} body wt. ac-cys: four doses of 100 mg 100 g^{-1} body wt. (ac-cys/Tl) BSO: two doses of 0.4 mmol 100 g^{-1} body wt. (BSO/Tl). * Statistically significant differences to control value at time zero ($P \leqslant 0.05$, $n = 6$). + Statistically significant different from rats that were given Tl only ($P \leqslant 0.05$, $n = 6$).

Diuresis experiments were done with conscious rats at different times after Tl administration without water loading. Urination was induced by slight manual pressure on the suprapubic region before and at the end of the 1-h collecting period. Volume and protein excretion were determined. Rats were sacrificed after the last diuresis experiment in ether anaesthesia to obtain blood and kidneys for the determination of blood urea nitrogen (BUN) and Tl concentration, respectively.

Analysis

Reduced glutathione was determined colorimetrically[11] and GSSG fluorimetrically,[12] separately in renal cortex and medulla.

Thallium in kidney cortex and medulla was determined by flameless atomic absorption spectrometry (AAS 4/EA, Carl Zeiss Jena GmbH, Germany) with the platform technique after mineralization with nitric and perchloric acid. The intra-assay coefficient of variation was 5.1% (mean 70 mmol l^{-1}, 11 replicates). The concentrations were related to grams dry weight. Total urinary protein were measured with the Coomassie-blue dye-binding method according to Bradford.[13] The BUN concentration was determined spectrophotometrically.[14]

Statistics

Data were expressed as mean values ± SEM. Statistical evaluations were done with Student's *t*-test ($n = 6$, $P \leqslant 0.05$).

Table 1. Influence of 0.4 mmol BSO 100 g^{-1} body wt., 100 mg ac-cys 100 g^{-1} body wt., 5 mg B2 100 g^{-1} body wt. and 2 mg Tl_2SO_4 100 g^{-1} body wt. on GSH and GSSG concentrations in renal cortex and medulla[a]

	Control	4 h after BSO	12 h after ac-cys	3 h after B2	48 h after Tl
GSH (μg g^{-1} wet wt.)					
Cortex	1586 ± 32	824 ± 43*	2030 ± 86*	1618 ± 5	1417 ± 48
Medulla	907 ± 50	558 ± 23*	1173 ± 60*	936 ± 55	788 ± 45
GSSG (μg/g^{-1} wet wt.)					
Cortex	267 ± 13	200 ± 9*	238 ± 4	195 ± 14*	246 ± 10
Medulla	91 ± 9	66 ± 4*	98 ± 3	71 ± 5*	99 ± 9

[a] Determinations were performed corresponding to the time interval between pretreatment and Tl administration in toxicity studies and 48 h after Tl (maximum of damage).
* Statistically significant differences in comparison to control ($P \leq 0.05$, $n = 6$).

RESULTS

Buthionine sulphoximine decreased and ac-cys increased the GSH concentration in renal cortex and medulla (Table 1). As a consequence of the inhibition of γ-glutamylcysteine synthetase by BSO, the GSSG concentration decreased too. Riboflavin pretreatment decreased the concentration of GSSG in renal cortex and medulla (Table 1) as a consequence of increased activity of the GSSG reductase.[15] Thallium did not influence significantly the concentration of GSH and GSSG in renal cortex and medulla (Table 1) at the time of maximal damage of kidney function (second day after Tl; see Figs 1 and 2).

Thallium administration led to significant oliguria (1–3 days after Tl) and proteinuria (2–3 days after Tl; Fig. 1). On the 5th day after Tl, BUN was enhanced significantly (27.2 ± 3.0 mmol l^{-1}) in comparison to control rats (6.7 ± 0.4). Pretreatment with B2 did not influence the pattern of Tl nephrotoxicity (Fig. 1) in comparison to the control. BUN was significantly higher in all Tl-treated rats, but there was no influence of B2 pretreatment (data not shown). Thallium concentration in renal cortex and medulla was not influenced by B2 pretreatment (data not shown).

Proteinuria was significantly lower in BSO-treated rats (Fig. 2). *N*-Acetylcysteine did not change Tl-induced oliguria or proteinuria. Neither BUN in ac-cys/Tl (24.1 ± 4.3 mmol l^{-1}) nor in BSO/Tl (30.1 ± 3.6 mmol l^{-1}) was significantly different from Tl-treated rats (25.7 ± 1.0 mmol l^{-1}).

As shown in Fig. 3 (upper part), in all experimental groups the concentration of Tl is significantly higher in renal medulla than in cortex. The concentration of Tl was not changed by ac-cys or BSO treatment in the cortex, but in the medulla of BSO/Tl-treated rats it was significantly lower for 72 h. This correlates with significantly higher urinary Tl excretion during the first 12 h in BSO-pretreated rats (Fig. 3, lower part).

DISCUSSION

Thallium intoxication is considered to be one of the most frequent causes, on a worldwide scale, of purposeful or accidental human poisoning.[16] Its treatment is difficult and there is no consensus regarding management.[4] Many theories have been proposed to account for Tl toxicity on a molecular level, such as alterations of potassium-dependent processes, Tl–sulphydryl group and riboflavin interactions.[3,4,17,18]

Conflicting results have been obtained with respect to the effects of Tl on Na^+/K^+-ATPase. The Tl^+ ion had been shown to replace K^+ in the activation of Na^+/K^+-ATPase in rabbit kidney.[5] However, when it was administered *in vivo*, Tl decreased significantly the ATPase activity in rat liver.[19] The authors suggest that Tl inhibition of the enzyme *in vivo* may be an indirect effect on its microenvironment rather than a direct inhibitory effect. In previous investigations[10] we found no inhibition but a significantly enhanced activity of this enzyme in renal medulla 2 days after the administration of Tl. There was a correlation between the concentration pattern of Tl in the kidney and the activity of Na^+/K^+-ATPase. Significantly higher Tl concentrations were detected in renal medulla (Fig. 3), where the enzyme activity was significantly higher than in cortex.[10] Therefore, we concluded that Na^+/K^+-ATPase is an indirect factor of nephrotoxicity by influencing the concentration of Tl in renal tissue.

Cavanagh[8] compared the clinical symptoms of three types of peripheral neuropathies (thiamine deficiency and arsenic and Tl poisoning). He suggested that Tl neuropathy is caused by a tissue deficiency of available B2, thus leading to significant disturbance of metabolic reactions depending on flavoproteins; this was quoted recently in a couple of reviews.[3,4] In our experiments no schedule of B2 treatment was able to influence Tl nephrotoxicity (Fig. 1). Riboflavin is converted to flavin–adenine dinucleotide (FAD) in the tissue, where binding to specific flavoproteins occurs. Liver and kidney are the major sits of B2 storage.[20] We did not determine the concentration of B2 directly in renal tissue but the activity of glutathione reductase is well established as a measure of the B2 state of the body.[21,22] The B2 dose administered was effective in decreasing the concentration of GSSG in kidney cortex and medulla (Table 1), reflecting the enhanced activity of glutathione reductase activity in renal tissue, which was shown to last at least 24 h.[23] If any shortage of B2 was involved in Tl toxicity, the administration of B2 should diminish the toxic effects.

It has been shown that Tl(I) forms complexes with sulphur-containing ligands.[9] In certain regions of the

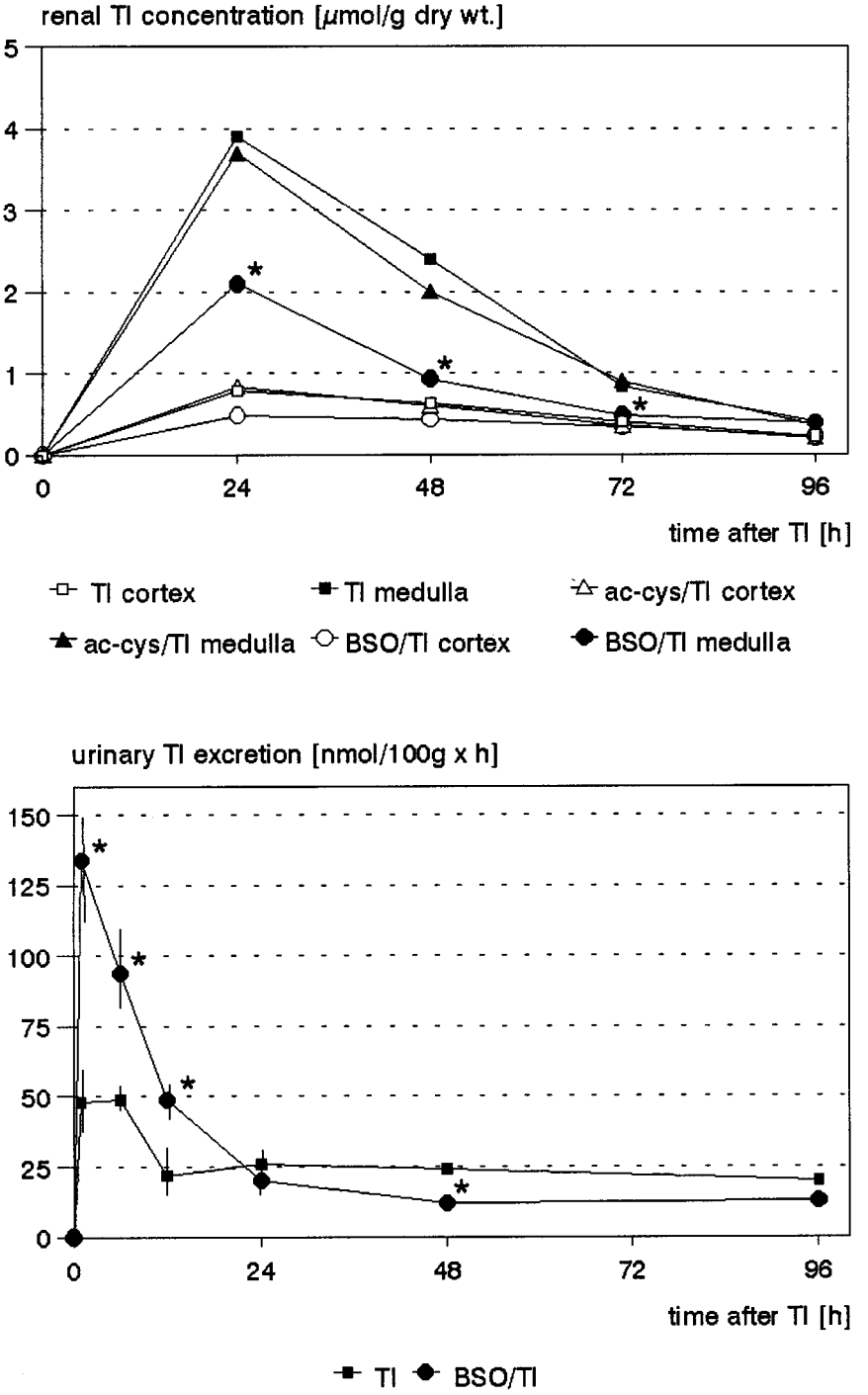

Figure 3. Influence of ac-cys and BSO on Tl concentration in renal cortex and medulla (SEM in-between the symbols) and urinary Tl excretion in Tl- and BSO/Tl-treated rats (for details, see legend to Fig. 2). * Statistically significant differences in comparison to Tl-treated rats. ($P \leqslant 0.05$, $n = 6$).

rat brain,[24] Tl significantly decreased the concentration of GSH. These results support the hypothesis that toxic effects of Tl are related to binding with SH groups and a decrease in GSH.[3,4] On the other hand, in renal tissue no decrease in GSH concentration was detectable (Table 1). The enhancement of GSH in renal tissue by the administration of ac-cys did not influence Tl nephrotoxicity (Fig. 2). In contrast to the hypothesis that a decrease in GSH is connected with Tl toxicity, BSO (an inhibitor of γ-glutamylcysteine synthetase) was very effective in decreasing the nephrotoxic effects of Tl (Fig. 2). This protective effect must be attributed to the significantly lower Tl concentration in renal medulla (Fig. 3), which is the locus of morphological damage.[10] As shown in Fig. 3, Tl excretion via urine is much higher in BSO-treated rats. Buthionine sulphoximine itself is not significantly metabolized and excreted via kidney,[25] but there is nothing in the literature about the interference between Tl and BSO excretion via urine.

If the binding of Tl to GSH played a significant role in Tl nephrotoxicity, the administration of ac-cys should ameliorate the toxic effects at least in part. Complexes formed by Tl(I) are slightly weaker than

those formed by Tl(III) with the same sulphur-containing ligand.[9] Therefore, both ions should react to the same extent with SH groups *in vivo*. Thallium(III) salts are much less toxic than Tl(I) salts,[16] supporting our findings that it is unlikely that toxic Tl effects could be due to interference with SH groups.

In conclusion, our results show that: B2 administration did not influence Tl-induced nephrotoxicity, although it was administered in a dose that was effective in enhancing glutathione reductase activity; Tl nephrotoxicity did not decrease the GSH concentration in renal cortex and medulla, and ac-cys did not influence Tl nephrotoxicity; and BSO decreases Tl nephrotoxicity significantly by decreasing Tl concentration in renal medulla, caused by enhanced urinary excretion of Tl.

With regard of the problems existing in the treatment of Tl poisoning in humans, investigations will be continued to clarify the mechanism of enhanced urinary Tl excretion in BSO-treated rats.

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