

Cephalosporin-induced Alteration in Hepatic Glutathione Redox State

A Potential Mechanism for Inhibition of Hepatic Reduction of Vitamin K₁,2,3-epoxide in the Rat

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Abstract

Hypoprothrombinemia is a serious adverse effect of antimicrobial therapy that occurs after administration of some second- and third-generation cephalosporins which contain the methyltetrazole-thiol (MTT) group. Previous studies have shown that in vitro MTT directly inhibits microsomal γ -carboxylation of a synthetic pentapeptide. Since MTT is a thiocarbamide, a type of compound that can increase oxidation of glutathione, the present studies were carried out to determine whether alterations in hepatic glutathione redox state might interfere with vitamin K metabolism.

Dose-related increases in biliary efflux and hepatic concentration of oxidized glutathione (GSSG) occurred after intravenous administration of MTT or MTT-containing antibiotics to rats. This finding suggested that these compounds could alter the hepatic glutathione redox state in vivo. Microsomal reduction of vitamin K epoxide occurred in the presence of 100 μ M dithiothreitol (DTT), but was inhibited by preincubation with GSSG at concentrations as low as 10 μ M. At higher concentrations of DTT (1.0 mM) inhibition by GSSG persisted, but higher concentrations were required, suggesting that the thiol/disulfide ratio, rather than the absolute concentration of GSSG was important. By contrast, GSSG did not effect microsomal γ -carboxylation of a pentapeptide, using either vitamin K₁ or its hydroquinone as a cofactor. These findings suggest a novel mechanism for the hypoprothrombinemia occurring after administration of MTT-containing antibiotics. (*J. Clin. Invest.* 1990. 86:1589-1594.) Key words: γ -carboxylation • drug metabolism • methyltetrazole thiol • prothrombin time • thiocarbamides

Introduction

Hypoprothrombinemia occurs in some patients treated with β -lactam antibiotics containing the methyltetrazole-thiol

(MTT)¹ group (1). This group is contained in cefamandole, moxalactam, cefoperazone, and other second- and third-generation cephalosporins (Fig. 1). In some instances, clinically significant bleeding episodes have occurred, particularly in patients who are fasting, malnourished, immediately postoperative, or have decreased renal function (1).

The mechanism for development of hypoprothrombinemia after administration of MTT-containing antibiotics is the subject of controversy. Some investigators have speculated that the effect is related to alterations in colonic flora which result in vitamin K deficiency (2). Another hypothesis is that these drugs inhibit the vitamin K-dependent step in clotting factor synthesis, the γ -carboxylation of glutamic acid (3, 4). Failure of carboxylation of the clotting factors could be due either to inhibition of vitamin K metabolism and/or direct inhibition of the carboxylation reaction. Metabolism of vitamin K includes a cycle that involves reduction of vitamin K₁ (a quinone), to a hydroquinone, oxidation of the hydroquinone to an epoxide, and finally the reduction of the epoxide to vitamin K₁ (5).

Previous studies have shown that MTT inhibits in vitro microsomal γ -carboxylation of a synthetic peptide (Phe-Leu-Glu-Glu-Ile) (3, 4). This finding suggests that the MTT group, or an active metabolite of this compound might be responsible for the anticoagulant effects. The finding of increased vitamin K epoxide in plasma of patients treated with moxalactam and cefamandole is consistent with an inhibition of the reduction of vitamin K epoxide (6), an effect which cannot be due to vitamin K deficiency.

MTT is a thiocarbamide, a class of compounds which may be metabolized by a flavin-containing monooxygenase to more reactive intermediates, such as sulfenic acids (7). Krieter and his colleagues (8) have shown that infusion of thiocarbamides into the isolated perfused rat liver causes in nonenzymatic oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) with subsequent release of GSSG into bile (8). Such alterations in the ratio of GSH to GSSG may effect the activity of various enzymes (8-12). Under physiologic conditions, the ratio of GSH to GSSG is tightly controlled, but after pharmacologic intervention, large shifts can occur.

The present studies were carried out to evaluate whether MTT-containing antibiotics could inhibit vitamin K metabolism through effects on the hepatic thiol/disulfide ratio. Changes in the hepatic content of GSSG and vitamin K₁,2,3-epoxide were measured to provide evidence that MTT-containing antibiotics alter both the hepatic thiol redox state and vitamin K metabolism in vivo. The effects of GSSG on microsomal reduction of vitamin K₁,2,3-epoxide were studied to

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1. Abbreviations used in this paper: GSSG, oxidized glutathione; MTT, methyltetrazole-thiol.

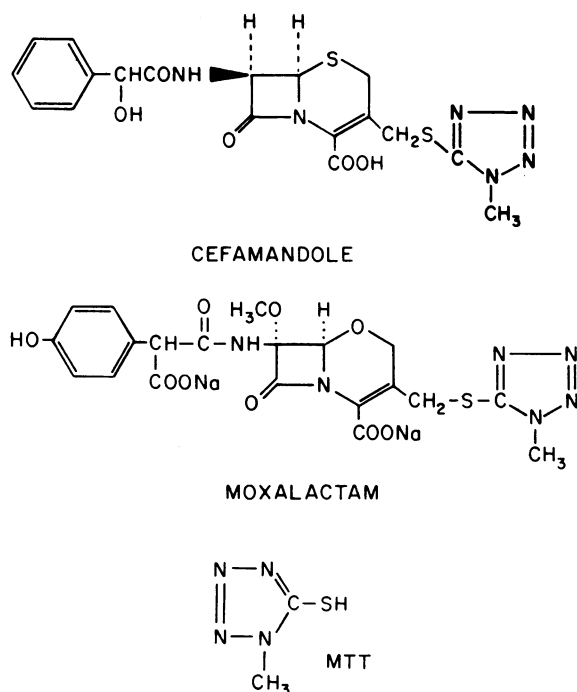


Figure 1. Structures of MTT and the antibiotics cefamandole and moxalactam which contain this side group.

determine whether increases in the ratio of GSSG/GSH could explain the impairment in reduction of vitamin K epoxide observed after administration of cephalosporins containing MTT.

Methods

Microsomal γ -carboxylation of glutamic acid residues. Rat liver microsomes were prepared for determination of the carboxylation of glutamic acid by the method of Houser et al. (14) with the previously described modifications (3). In brief, reactions were carried out in buffer containing 25 mM imidazole, 250 mM sucrose, and 20 mM KCl, pH 7.4, which contained the following substrates: L-phenylalanyl-L-leucyl-L-glutamyl-L-glutamyl-L-isoleucine, 1.45 mM, and 10 μ Ci of $\text{NaH}^{14}\text{CO}_3$ (43 mCi/mmol; final concentration, 0.4 mM); 500 μ l of the solubilized microsomal preparation (average 3.5 mg of protein), and 2 mM NADH. 10 μ l of antibiotic or thiol group was added at the concentration given in the text to give a final volume of 580 μ l. The pentapeptide, NADH, and drugs were added as solutions in the imidazole buffer. Reactions were initiated by the addition of 25 μ l of vitamin K_1 , phyloquinone (used as Aquamephyton B, Merck Sharp & Dohme, West Point, PA), and terminated by the addition of 10% trichloroacetic acid. After centrifugation, the supernatant solution was purged with unlabeled CO_2 . The incorporation of radioactive carbon dioxide into the pentapeptide was determined by liquid scintillation counting of an aliquot of the supernatant solution.

Biliary efflux of GSSG after administration of MTT and antibiotics to intact rats. Male Sprague-Dawley rats weighing 250–300 g were allowed free access to food and water until the morning of each experiment. Animals were anesthetized with pentobarbital 50 mg/kg i.p., and a midline laparotomy performed. The common bile duct was isolated and cannulated with PE-10 tubing. After closure of the incision, the rats were allowed to recover for 10–30 min. Timed aliquots of bile were collected into tared microfuge tubes containing 5% metaphosphoric acid for 1 h. Antibiotics and MTT were dissolved in 0.9% saline and injected into the femoral vein. Bile was collected for 80 min

after administration of test compounds, and flow was determined gravimetrically. After centrifugation to remove precipitated protein, bile was assayed for GSSG and “total” GSH (reduced GSH + GSSG) as described (15). A specific assay for GSSG monitored the oxidation of NADPH at 340 nm in the presence of glutathione reductase. Total GSH was measured using the NADPH glutathione reductase coupled oxidation of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) monitored at 412 nm.

Hepatic concentrations of glutathione after administration of cefamandole. Male Sprague-Dawley rats weighing 200–250 g, allowed free access to food and water, were anesthetized with sodium pentobarbital 50 mg/kg of i.p. The abdomen was opened and the liver was rapidly freeze-clamped with aluminum tongs precooled in liquid nitrogen. Frozen tissue was pulverized under liquid nitrogen and extracted with 5–10 vol of perchloric acid, 2 mM EDTA for total glutathione (reduced + oxidized) or 1.0 M perchloric acid containing 2 mM EDTA and 50 mM *N*-ethylmaleimide for GSSG. For analysis of total glutathione, extracts were diluted into 100 mM sodium phosphate buffer, pH 7.5 and assayed as described above. For analysis of GSSG, the acid extract was carefully neutralized with 2.0 M KOH containing 0.3 M 3-[*N*-morpholino]-propanesulfonic acid as described (15). Neutralized supernatants were extracted on solid-phase C18 columns (Bakerbond, J. T. Baker, Inc., Phillipsburg, NJ). Columns were washed with 1.0 ml of 100 mM sodium phosphate buffer, pH 6.0. Removal of *N*-ethylmaleimide was verified by checking the optical density at 315 nm. GSSG was assayed using the DTNB recycling assay as described above. Recovery was > 90% routinely.

Effects of MTT-containing antibiotic on hepatic vitamin $\text{K}_{1,2,3}$ -epoxide. Male Sprague-Dawley rats weighing 200–250 g allowed free access to food and water, were given 3 g/kg of cefamandole by intraperitoneal injection each day for 5 d. 24 h after the last injection, animals were killed under pentobarbital anesthesia. Liver (100 mg) was homogenized in 2 ml of sucrose-imidazole buffer and extracted for 10 min with 2 vol of hexane/isopropanol (1:1, vol/vol). The organic phase was dried under a stream of nitrogen and the residue dissolved in 100 μ l of methanol. 20- μ l aliquots were injected for measurement of vitamin K_1 and vitamin $\text{K}_{1,2,3}$ -epoxide.

Synthesis, purification, and analysis of vitamin $\text{K}_{1,2,3}$ -epoxide and vitamin K_1 by high pressure liquid chromatography (HPLC). Vitamin $\text{K}_{1,2,3}$ -epoxide was synthesized chemically from vitamin K_1 , by the method of Tishler et al. (16). The epoxide was further purified by HPLC separation on a reversed phase C-18 preparative column (30 cm \times 4.8 mm i.d.) (Waters Associates, Milford, MA) using methanol as a mobile phase. For analytical purposes, an Excelapak C-18 column (15 cm \times 4.6 mm i.d.) (R. G. Gourley Co., Laurel, MD) was used to separate vitamin K_1 and vitamin K epoxide. Retention times in 100% methanol were 7.6, and 5.0 min, respectively. Peaks were detected at 254 and at 220 nm using a Spectromonitor III (Lab Data Control, Riviera Beach, FL), or a diode array detector (LKB, Uppsala, Sweden). Quantitation was carried out by integration of peak areas using a recording integrator (Hewlett-Packard Co., Palo Alto, CA).

Preparation of KH_2 by reduction of vitamin K_1 . Vitamin K_1 was reduced chemically by sodium dithionite (hydrosulfite) as described (17). Reduction was confirmed by UV spectral analysis just before use.

Microsomal reduction of vitamin $\text{K}_{1,2,3}$ -epoxide. Rat hepatic microsomes were prepared using a modification of the method described (18) in which the microsomal pellet was suspended in 25 mM imidazole, pH 7.2, containing 0.5% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) and diluted to a concentration of 10 mg of microsomal protein/ml. Vitamin K epoxide reductase activity was assayed by a modification of the method of Wallin and Martin (19). The solubilized microsomal fraction was incubated with varying concentrations of vitamin $\text{K}_{1,2,3}$ -epoxide in the presence of 0.1–5.0 mM DTT at 22°C for 30 min. The reaction was terminated by the addition of 2 vol hexane/isopropanol (1:1, vol/vol). Vitamin K metabolites were extracted by vortexing for 30 s followed by centrifugation at 2,000 g for 10 min. The organic phase was transferred to glass tubes and dried under a stream of N_2 (N-Evap, Organomation Asso-

ciates, Worcester, MA). The residue was redissolved in 100 μ l of methanol and an aliquot (20–50 μ l) was injected into a high-pressure liquid chromatograph (LKB). Vitamin K metabolites were detected as described above. Results are expressed as a ratio of vitamin K₁ to vitamin K epoxide, reflecting the conversion of vitamin K₁,2,3-epoxide to vitamin K₁, since neither compound is endogenously present in the microsomal preparation. Recovery was > 90% in all experiments.

Statistics. Analysis of variance for repeated measures was used with Duncan's test for multiple comparisons to compare changes in biliary efflux of GSSG after antibiotics in each experiment. Student's t-test was used to compare group means.

Results

Inhibition of microsomal γ -carboxylation of glutamic acid. Since previous work had shown that MTT is able to inhibit microsomal carboxylation of glutamic acid (3, 4), concentration inhibition curves for this effect were established for the intact MTT-containing antibiotics, cefamandole and moxalactam, and compared to the previous results obtained with MTT. Neither moxalactam nor cefamandole inhibited γ -carboxylation > 20% when added in concentrations up to 2.5 mM (Fig. 2). Addition of MTT to microsomes inhibited γ -carboxylation of glutamic acid in a dose-dependent fashion as shown in Fig. 2 with an IC₅₀ concentration between 0.5 and 0.6 mM.

The effect of GSSG upon carboxylation was examined. As shown in Table I, GSSG had only minimal effect upon carboxylation. When the hydroquinone (KH₂) was used to initiate the reaction in the absence of NADH, no inhibition was observed, indicating no direct effect of GSSG upon the carboxylation reaction itself.

Effects of MTT and antibiotics on biliary efflux and hepatic concentrations of GSH and GSSG. After i.v. administration of MTT there was a rapid and striking, dose-related increase in both biliary GSSG concentration and efflux (Fig. 3 A) ($P < 0.05$ for 2.5 and 5.0 mmol/kg vs. baseline). Baseline GSSG output was 0.36 ± 0.07 nmol/min per g liver. After 60 min

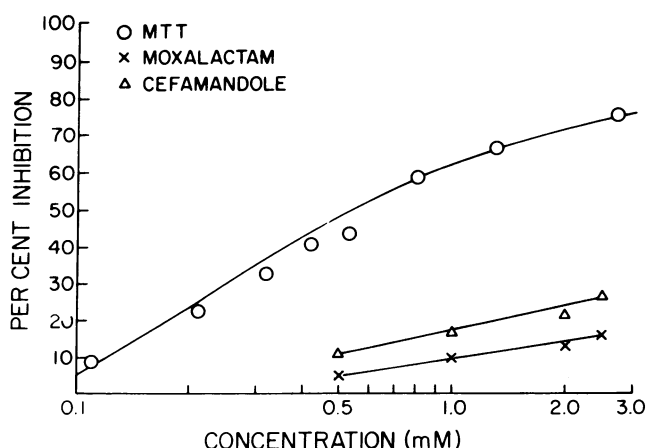


Figure 2. Effects of intact cephalosporins and MTT on microsomal γ -carboxylation of a synthetic pentapeptide. Microsomal γ -carboxylation was performed as described in Methods. Addition of MTT inhibited carboxylation of glutamic acid residues in a dose-related fashion, while neither moxalactam nor cefamandole inhibited > 20% in concentrations up to 2.5 mM.

Table I. Effects of GSSG on Microsomal γ -Carboxylation of a Synthetic Pentapeptide

GSSG	Vitamin K form	NADH	Inhibition
mM		mM	%
1.0	Quinone	2.0	5 \pm 6
2.5	Quinone	2.0	3 \pm 6
5.0	Quinone	2.0	2 \pm 7
5.0	Hydroquinone	0	0 \pm 6

Rat hepatic microsomal γ -carboxylation of a synthetic peptide (Phe-Leu-Glu-Glu-Ile) was determined by the incorporation of ¹⁴CO₂ into the peptide as described in Methods. Varying concentrations of GSSG were preincubated with microsomes for 2 min before addition of vitamin K to initiate the reaction. As shown, GSSG had no statistically significant effect on γ -carboxylation. Data shown are the mean \pm SE results of three experiments.

from the time of injection, the increase in GSSG efflux returned toward baseline.

After administration of cefamandole, but not moxalactam or MTT, there was increased biliary efflux of GSH ($P < 0.01$ vs. baseline), which was not accounted for by the increase in efflux of GSSG alone (Fig. 4). This finding was unexpected since administration of other thiocarbamides such as methimazole does not alter biliary GSH output (10, M.C. Mitchell, unpublished observations).

Administration of the intact antibiotics cefamandole and moxalactam also increased the biliary efflux of GSSG ($P < 0.05$ vs. baseline) to a similar extent as MTT on a molar basis (Fig. 3 B). In addition, both cefamandole and moxalactam significantly increased bile flow: from 1.36 ± 0.22 to 3.74 ± 0.45 μ l/min per g liver and from 1.57 ± 0.16 to 3.40 ± 0.33 μ l/min per g, respectively ($P < 0.05$). Hepatic levels of GSSG were significantly higher 30 min after intravenous injection of 2.5 mmol/kg of cefamandole, confirming the increased biliary efflux of GSSG was accompanied by higher tissue levels. The ratio of GSH to GSSG was significantly lower at the same time, indicating a change in the intracellular thiol/disulfide ratio (Table II).

Effects of cefamandole on hepatic vitamin K₁,2,3-epoxide. Administration of MTT-containing antibiotics to rats has been shown to prolong plasma prothrombin times (20). Cefamandole was given daily to rats for 3 d to determine whether this treatment was also associated with changes in the relative amounts of vitamin K₁ and vitamin K₁,2,3-epoxide. After administration of cefamandole, the amount of vitamin K epoxide expressed as a percentage of vitamin K metabolites was significantly higher, 7.87 ± 1.03 compared to 4.10 ± 0.50 in control animals ($P < 0.05$). These observations confirm that both prolongation of the prothrombin time (20) and inhibition of the reduction of vitamin K₁ epoxide occurs in rats as well as in humans receiving MTT-containing cephalosporins (6).

Effects of GSSG on microsomal reduction of vitamin K₁,2,3-epoxide. Reduction of vitamin K₁,2,3-epoxide to vitamin K₁ in solubilized microsomes required the presence of a thiol as well as K epoxide. The physiological thiol required for this reaction remains unknown. Data in Table III give comparisons of activity for various concentrations of thiols. On a molar basis, the dithiol, DTT, was significantly better than

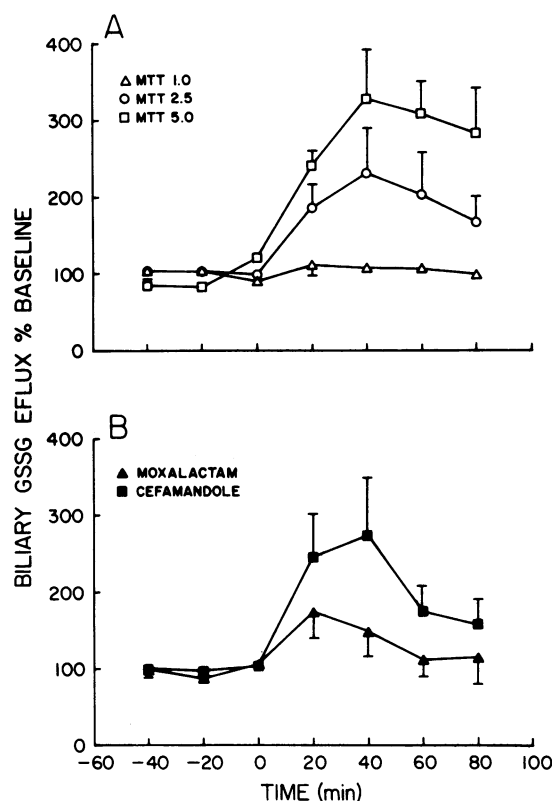


Figure 3. (A) Effects of intravenous administration of MTT on biliary efflux of GSSG. Baseline GSSG efflux was 0.36 ± 0.07 nmol/min per g liver. Administration of MTT—(Δ) 1.0 mmol/kg, (\circ) 2.5 mmol/kg, (\square) 5.0 mmol/kg—resulted in a rapid, dose-related increase in efflux of GSSG which returned toward baseline after 60 min. Results shown are the mean \pm SE as a percentage of baseline for six animals per group. (B) Effects of intravenous administration of intact MTT containing cephalosporins on biliary efflux of GSSG. Administration of both moxalactam (\blacktriangle) 2.5 mmol/kg and cefamandole (\blacksquare) 2.5 mmol/kg increased biliary GSSG efflux to a similar extent as MTT. Results shown are the mean \pm SE as a percentage of baseline for six animals per group.

GSH in supporting this reaction. Similar findings have been reported for the human liver enzyme (18). Some activity could be detected at concentrations of DTT as low as 0.05 mM.

Preincubation of solubilized microsomes for 2 minutes with GSSG blocked the reduction of vitamin K epoxide at 0.1 mM DTT in a concentration-dependent manner (Fig. 5). Under these conditions inhibition was detected at concentrations as low as 0.01 mM GSSG. At a higher concentration of DTT (1.0 mM), a higher concentration of GSSG was required to achieve the same degree of inhibition.

In separate experiments, 0.1 mM GSSG reduced the activity of epoxide reductase to 57% of control in the presence of 0.1 mM DTT. Further addition of DTT to achieve a final concentration of 1.1 mM DTT restored activity to 95% of control, whereas addition of buffer did not alter the inhibition observed at 30 min.

To determine whether GSSG causes nonenzymatic oxidation of DTT, thereby depleting a necessary cofactor for vitamin K epoxide reductase; oxidation of DTT was monitored spectrophotometrically as described by Cleland (21). In the presence of 0.2 mM GSSG, 50% of 0.1 mM DTT was oxidized

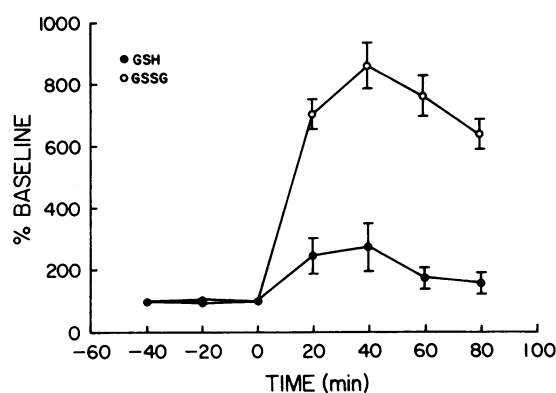


Figure 4. Effects of intravenous administration of cefamandole (2.5 mmol/kg) on biliary efflux of GSH (\bullet) and GSSG (\circ). Cefamandole resulted in a marked increase in biliary efflux of both "total" glutathione (GSH + GSSG) and GSSG. Baseline GSH efflux was 1.68 ± 0.23 nmol/min per g liver. Baseline GSSG efflux was 0.32 ± 0.06 nmol/min per g liver. The larger increase in "total" glutathione (reduced + oxidized) compared to GSSG alone shows that cefamandole increased efflux of reduced GSH as well as GSSG. Neither moxalactam nor MTT increased efflux of reduced GSH (data not shown). Results shown are the mean \pm SE expressed as a percentage of baseline for eight animals.

over 30 min. At this concentration of GSSG, the activity of epoxide reductase was completely inhibited (Fig. 5). If the observed inhibition of the reductase was due entirely to a decrease in the concentration of reduced DTT, one would expect to find no activity in the presence of 0.05 mM DTT. However, activity of the reductase was 63% of that observed in the presence of 0.1 mM DTT. These observations suggest that, although GSSG may cause nonenzymatic oxidation of DTT, its effects upon the activity of epoxide reductase are not due entirely to this mechanism.

Discussion

The results of this study provide new insight into how MTT, a thiocarbamide side group of several β -lactam antibiotics, may cause hypoprothrombinemia. Administration of MTT-containing antibiotics can produce hypoprothrombinemia (20) and alter vitamin K metabolism in the rat (22). Vitamin K

Table II. Effects of Cefamandole on Hepatic Concentrations of Glutathione

Treatment	Total GSH $\mu\text{mol/g liver}$	GSSG nmol/g liver	2 GSH/GSSG
Saline	6.73 ± 0.65	52 ± 8.1	318 ± 62
Cefamandole	6.11 ± 0.44	$71 \pm 5.3^*$	$182 \pm 21^*$

Livers of rats were freeze-clamped 30 min after treatment with intravenous cefamandole (2.5 mmol/kg) or saline. Total glutathione (reduced and oxidized) and GSSG were measured as described in the methods. As shown, hepatic GSSG was increased significantly and the ratio of GSH/GSSG decreased significantly by cefamandole. Data shown are the mean \pm SE of nine animals in each group.

* $P < 0.05$.

Table III. Hepatic Microsomal Reduction of Vitamin K₁,2,3-epoxide in the Presence of DTT and GSH

Concentration of reductant <i>mM</i>	Vitamin K epoxide reduced	
	GSH	DTT
	%	
20	9±1	—
10	0	—
5	0	—
1	0	32±2
0.5	—	36±6
0.1	—	20±6

Microsomal reduction of vitamin K₁,2,3-epoxide was measured in CHAPS-solubilized microsomes in the presence of varying concentrations of DTT or GSH. Reactions were initiated by the addition of thiols and carried out for 30 min at 22°C. Vitamin K₁ and vitamin K₁,2,3-epoxide were measured by HPLC after extraction of microsomes with hexane/isopropanol as described in Methods.

metabolism may be indirectly altered by MTT through formation of GSSG in addition to the previously reported direct effects of MTT upon γ -carboxylation of clotting factors (3–5, 22). The increased amount of vitamin K₁,2,3-epoxide as a percentage of vitamin K metabolites observed after cefamandole is consistent with the increase in plasma vitamin K₁,2,3-epoxide seen in humans receiving these same drugs (6). Both observations strongly suggest that the reduction of vitamin K epoxide to vitamin K₁ is inhibited by these cephalosporins and could contribute to development of hypoprothrombinemia. The similarity of findings in the rat and humans indicates that the rat is an appropriate animal model for exploring further the mechanism underlying these effects.

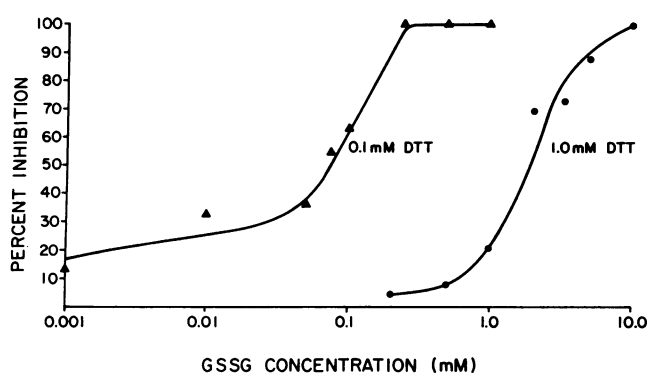


Figure 5. Effects of GSSG on reduction of vitamin K₁,2,3-epoxide in CHAPS-solubilized hepatic microsomes. GSSG was preincubated with microsomes for 2 min before addition of vitamin K epoxide and DTT. GSSG inhibited reduction of the epoxide in a concentration-dependent fashion. At higher concentrations of the reductant, DTT, larger concentrations of GSSG were needed to inhibit the reaction suggesting that the thiol disulfide ratio was important. Incubation of DTT with GSSG in the absence of microsomes did not reduce GSSG to GSH (data not shown). Results shown are the mean of three experiments. At 0.1 mM DTT 20±6% of vitamin K epoxide was reduced in 30 min at 22°C, compared with 32±2% at 1.0 mM DTT.

The increased hepatic concentration and biliary efflux of GSSG after administration of MTT and cefamandole demonstrated in these studies is consistent with previously reported findings with other thiocarbamides (8). Therefore, we investigated the possibility that GSSG, an endogenous disulfide, might interfere with reduction of vitamin K epoxide or directly inhibit carboxylation of glutamic acid or both. Although we did not find evidence for direct inhibition of carboxylation by GSSG, our results show that GSSG can inhibit reduction of vitamin K₁,2,3-epoxide in a concentration-dependent manner. The fact that there is less inhibition seen when GSSG is incubated in the presence of higher concentrations of DTT suggests that the thiol/disulfide ratio rather than the absolute concentration of GSSG may be an important determinant of the activity of the vitamin K epoxide reductase.

The concentrations of GSSG shown to inhibit reduction of vitamin K epoxide are pharmacologically relevant, occurring as low as 10–25 μ M. With increasing thiol concentration, inhibition by GSSG was prevented, perhaps through reduction of mixed disulfides of glutathione and protein. Since the physiologically active thiol in this reaction is unknown, it is difficult to extrapolate this finding to in vivo conditions. Regulation of enzyme activity by the thiol/disulfide ratio has been proposed for several enzymes. Although the regulation of enzymes by physiologic alterations in thiol/disulfide status is a subject of controversy (12), the magnitude of changes in hepatic content of GSSG such as occurs after pharmacologic challenges may be sufficient to alter enzyme activity through such a mechanism. Recent work has also suggested that cellular resistance to cancer chemotherapeutic drugs may be related to alterations in the cellular glutathione redox state (23).

Glutathione can prevent, but not reverse, inhibition of γ -carboxylation in vitro by MTT (using vitamin K₁ as a cofactor) even when MTT is added in 40-fold excess (4). These findings also suggest that the thiol status may be important in maintaining activity of an enzyme(s) involved in γ -carboxylation or the vitamin K cycle. Alternatively, GSH may react directly with a metabolite of MTT to prevent inhibition under in vitro conditions. Rapid oxidation of GSH to GSSG is likely to lower hepatic levels of GSH allowing MTT-related inhibition of γ -carboxylation in vivo. This alteration in the thiol/disulfide ratio could indirectly enhance sensitivity of an enzyme to MTT in vivo. The observation that MTT containing antibiotics as well as MTT cause a disulfiram effect (24) suggests this may occur, since disulfiram reacts with critical sulfhydryl groups on aldehyde dehydrogenase, resulting in loss of activity which can be prevented by thiols (25). Disulfiram has also been shown to inhibit both γ -carboxylation of glutamic acid residues (4) and reduction of vitamin K₁ to the hydroquinone form (22). Furthermore, there is evidence that the disulfiram-like inhibition of aldehyde dehydrogenase by MTT is caused by a more reactive metabolite of MTT (26, 27).

It is of interest that lack of oral intake of food is considered a clinical risk factor for cephalosporin-associated hypoprothrombinemia (1). Although depletion of vitamin K stores has been postulated to explain this enhanced susceptibility, it seems equally attractive to speculate that decreased glutathione levels owing to fasting (28) are important in pathogenesis. At lower hepatic concentrations of GSH, oxidation of GSH to GSSG would result in a GSSG/GSH ratio which would be considerably higher than under normal conditions. In some patients, cephalosporin-associated hypoprothrombinemia is

reversible within 24 h after administration of vitamin K₁ (1). This response is consistent with inhibition of the reduction of vitamin K epoxide, since large doses of vitamin K₁ would overcome the requirement for the intact recycling of vitamin K. However, in other patients, a much longer time is required for reversal of this effect. In the latter situation, impaired reduction of vitamin K epoxide alone would be an inadequate explanation for these findings. Direct inhibition of γ -carboxylation of glutamic acid residues due to a metabolite of MTT would offer a more satisfactory explanation.

In summary, the results presented here show that (a) administration of MTT-containing antibiotics alters both the reduction of vitamin K₁,2,3-epoxide and the thiol redox ratio in rats in vivo, and (b) GSSG can inhibit the microsomal reduction of vitamin K₁,2,3-epoxide in vitro. These observations suggest that the hypoprothrombinemia occurring after administration of MTT-containing antibiotics may be due, in part, to an indirect effect on vitamin K metabolism resulting from oxidation of GSH to GSSG during hepatic metabolism of MTT.

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