*Department of Pharmacology
Uniformed Services University
Bethesda, MD 20814; and
†Laboratory of Molecular
Carcinogenesis
National Cancer Institute
Bethesda, MD 20892, U.S.A.

TZUU-HUEI UENG*
FRED K. FRIEDMAN†
HARUKO MILLER†
SANG S. PARK†
HARRY V. GELBOIN†
ALVITO P. ALVARES*‡

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‡Author to whom correspondence should be sent.

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Josamycin and troleandomycin increase hepatic glutathione turnover in rats

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The concomitant administration of troleandomycin with a number of other drugs is associated with a high incidence of adverse side-effects [1, 4] that result from the inhibition of the hepatic monoxygenase system by the antibiotic [3, 5, 6]. A metabolite of troleandomycin forms a complex with the iron of cytochrome P-450 thereby inactivating the monooxygenase [7]. This metabolic activation is associated with a decrease in hepatic glutathione (GSH) suggesting that GSH might play a role in the detoxification of the reactive metabolite [8]. However, inhibition of the synthesis of GSH, as well as loss of GSH by formation of a troleandomycin–glutathione adduct, or by some other mechanism, could be responsible for the observed depletion of hepatic GSH.

In order to better understand the mechanism underlying the depletion of GSH we studied the effect of troleandomycin and josamycin on hepatic GSH turnover in rats. Josamycin does not form cytochrome P-450 complexes [9] and does not inhibit drugs metabolism [10] but its effect on hepatic GSH is not known. If this compound affected GSH homeostasis without forming a metabolite reacting with cytochrome P-450 it might provide new insights into the relationship between depletion of GSH and inactivation of cytochrome P-450 following the administration of macrolide antibiotics.

Material and methods

Male Sprague-Dawley rats (Charles River, Wiga GmbH, F.R.G.) weighing 130–250 g had free access to food and water and were kept in an air-conditioned room with a 12 hr light–dark cycle. Josamycin and troleandomycin (Pfizer Laboratories) were dissolved in 1 ml of arachis oil, and 400 μmol/100 g b.w. were administered by gavage. Control animals received the vehicle only. At 24, 48, and 120 hr, portions of the liver were obtained for the measurement of hepatic GSH [11].

To determine GSH turnover [12, 13] identical doses of troleandomycin, josamycin, and vehicle were administered to three other groups of rats. The rats were fasted after administration of the drugs and were studied 24 hr later under pentobarbital anaesthesia (50 mg/kg i.p.). The common bile duct was cannulated with a PE-10 catheter. Throughout the experiment the temperature of the animals measured rectally was maintained between 37 and 38° with a heating lamp. At time zero 30 μCi of ^{35}S-L-cysteine (300 Ci/mmol, NEN, U.S.A.) were injected i.v. Bile samples were then collected into preweighed tubes containing 0.1 ml of 4% sulphosalicylic acid at timed intervals for the determination of the biliary excretion of GSH. For the subsequent determination of the specific activity of GSH, aliquots of bile were collected into 5 mM monobromobimane in acetonitrile which derivatizes GSH for HPLC analysis [14]. At the end of the experiment a portion of the liver was removed for the determination of hepatic GSH.

Analytical methods. For the determination of the specific activity of GSH, the bile samples collected into monobromobimane were analyzed by HPLC using a 7 μm R.P-18 column (E. Merck, Darmstadt, F.R.G.) and water/methanol/acetic acid (89.75 : 10 : 0.25, v : v, pH 3.9) at a flow rate of 1.5 ml/min as the mobile phase [14]. The column effluent was monitored at 254 nm and the GSH peaks were collected and assayed for radioactivity by liquid scintillation spectrometry. The specific activity of GSH was calculated from the radioactivity and the mass of GSH, which was obtained by comparing the area of the GSH peak with a standard curve. Hepatic glutathione (GSH plus GSSG) was assayed by the enzymatic recycling method of Tietze [15].

Calculations. The slope of the declining phase of the specific activity–time curve of GSH in bile was calculated by least-square regression analysis following logarithmic transformation of the measured values. The calculated slope of the monoexponential phase of each curve corresponds to the fractional rate of turnover of hepatic GSH.

Statistics. All values are expressed as mean ± SD. Group means were compared using Student's *t*-test.

Results and discussion

As shown in Fig. 1 single doses of josamycin and troleandomycin resulted in a substantial and prolonged depletion in the liver of small molecular sulfhydryl over 90% of which is GSH.

In the study designed to assess hepatic GSH turnover where, in contrast to the first study, the animals were fasted for 24 hr, the hepatic concentrations of total glutathione, i.e. GSH and GSSG, were not significantly different in the three groups (Table 1). After reaching a peak, the specific activity of GSH excreted in bile declined monoexponentially in all experimental groups. The slopes of the specific activity–time curves reflecting the fractional rate of hepatic GSH turnover were significantly higher ($P < 0.01$) in the animals treated with josamycin and troleandomycin than in control rats (Fig. 2, Table 1). Under steady-state conditions the fractional rate of turnover multiplied by the hepatic concentration of glutathione yields the rate of hepatic synthesis of GSH. The estimated hepatic synthesis was significantly ($P < 0.05$) higher in the animals treated with the macrolide antibiotics (Table 1).

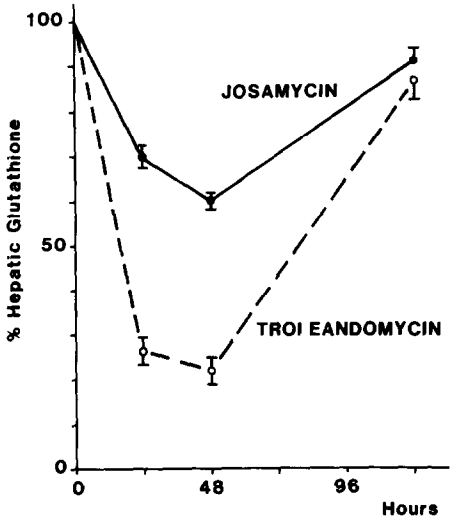

Fig. 1. Concentration of GSH in liver expressed as percentage of control values following the administration of 400 μmol/100 g of josamycin and troleandomycin, respectively. The values at 24 and 48 hr are significantly ($P < 0.05$) lower in both drug-treated groups (mean ± SD, N = 3).

Thus, the two compounds do not decrease hepatic synthesis of GSH. The constellation of an increased fractional rate of turnover not associated with an increased hepatic concentration of GSH can only be explained by an increased consumption and a compensatory increase in the turnover of GSH [14]. The estimated increase in GSH synthesis above the basal rate of synthesis in control animals amounted to approximately 0.5 μmol/g·hr or 60μmol/100 g b.w. in 24hr. An increased synthesis, however, can only be maintained as long as sufficient substrate amino acids are available. The availability of cysteine in particular may become rate-limiting [16]. The observation that a high rate of GSH synthesis was maintained for at least 24 hr after administration of large doses of josamycin and troleandomycin, therefore, suggests that the hepatic GSH was not lost in the form of a stable adduct with the macrolide antibiotics but rather in the form of free GSH or GSSG from which the constituent amino acids can be recovered via gamma glutamyl transferase.

Two mechanisms, formation of a labile adduct with GSH and stimulation of the efflux of GSH from the liver, could account for a loss of GSH but not its constituent amino acids. Circumstantial evidence indicates that a reactive metabolite of troleandomycin forms an adduct with GSH

Table 1. Hepatic GSH turnover 24 hr after administration of 400 μmol/100 g of josamycin and troleandomycin, respectively to rats starved for 24 hr (mean ± SD, N = 5)

	Josamycin	Troleando-mycin	Controls
Hepatic GSH (μmol/g)	1.69 ± 0.38	2.34 ± 0.17	2.65 ± 0.92
Fractional rate of turnover (hr^{-1})	0.601 ± 0.142	0.564 ± 0.050	0.221 ± 0.080
Estimated synthesis of GHS (μmol/g.hr)	1.05 ± 0.42	1.32 ± 0.21	0.55 ± 0.17

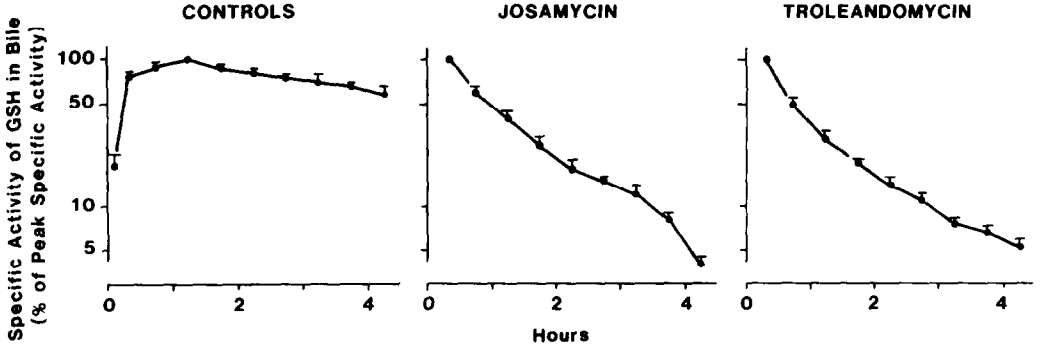

Fig. 2. Specific activity of GSH excreted in bile following the administration of ^{35}S-cysteine at time zero to rats starved for 24 hr that had received 400 μmol/100 g of josamycin and troleandomycin, respectively, 24 hr prior to the study. The specific activities are expressed as percentage of the peak specific activity. The slope of the specific activity-time curve reflecting the fractional rate of turnover of hepatic GSH is significantly higher ($P < 0.01$) in the animals treated with josamycin and troleandomycin (mean ± SD, N = 5).

[8]. Although no complexes of josamycin with cytochrome P-450 have been described [10], a nitroso derivative may be formed which reacts with GSH but not with the iron of cytochrome P-450 because of steric hindrance. It is conceivable that a labile adduct with GSH may be split by free GSH to the hydroxylamine and GSSG [17]. Since the capacity of the hepatocyte to reduce GSSG is limited and excess GSSG is actively extruded from the cell, this mechanism could result in the depletion of intracellular GSH. If the adduct of troleandomycin with GSH was somewhat less labile, the re-utilization of the constituent amino acids would be less efficient, this accounting for the more severe depletion of GSH in fed rats administered troleandomycin. Alternatively, josamycin and troleandomycin might increase the efflux of GSG from the liver. Additional studies are required to clarify the mechanism of the increased consumption of hepatic GSH.

In summary, our study demonstrates that josamycin which does not form cytochrome P-450-nitroso complexes *in vitro* or *in vivo* nevertheless decreases hepatic GSH and markedly increases hepatic GSH turnover like troleandomycin which is known to form a cytochrome P-450-metabolite complex. In spite of an increased utilization following the administration of josamycin and troleandomycin the synthesis of GSH is maintained for a prolonged period of time suggesting that the constituent amino acids are re-utilized for the synthesis of GSH. Thus, a substantial portion of the increased consumption of hepatic GSH may be due to the loss of free GSH or GSSG from the liver rather than a loss by formation of a stable GSH-adduct with the macrolide antibiotic.

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Department of Clinical Pharmacology
University of Berne
CH-3010 Berne
Switzerland

SRINATH P. DORE
BERNHARD H. LAUTERBURG

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In vivo renal tubular secretion of trimethoprim without metabolism

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Because it is potent and concentrates in the renal cortex, the anti-folate agent trimethoprim (TMP) is commonly used as a urinary tract anti-infective drug. There is evidence that this drug enters the urine via a secretory process [1, 2]. Recently, we examined the mechanisms by which TMP is taken up by incubated slices of rat renal cortex [3]. The results indicated the participation of active transport, probably via the organic cation system.

Although TMP is metabolized by the liver [4] and some metabolites are reported active as antibacterials [5], no metabolism of TMP by the slices was evident. The experiments reported here were designed to extend these *in vitro* results by quantifying TMP excretion *in vivo* using the Sperber chicken preparation, a model for the study of tubular transport and metabolism in an unanesthetized animal.

Materials and methods

The theoretical details of the Sperber method have been described previously [6, 7]. Briefly, the method takes advantage of two anatomical peculiarities of birds: lack of a urinary bladder (each ureter draining separately to the outside) and a functional venous portal circulation supplying the peritubular circulation but not the arterial glomerular vessels. Since the renal portal vessels to each kidney are supplied separately with blood by veins draining the ipsilateral leg, it is possible to infuse a test substance into the saphenous vein of one leg and expose that substance to the tubules but not the glomeruli of the same-side kidney before it enters the general circulation. First-pass tubular extraction of the test substance by the infused-side kidney can be quantified as an "apparent tubular excretion fraction" (ATEF), calculated as: $^{I-C}/_{Inf}$ where I = total amount excreted in infused side urine/min, C = amount excreted in control side urine/min and Inf = amount infused/min. In practice, the ATEF of the test substance must be corrected for variable shunting of leg blood away from the portal veins which reduces the amount of infused substance actually entering the kidney. This is accomplished by factoring the ATEF of the test substance by the ATEF of a co-infused blood flow marker such as the anion *p*-aminohippuric acid (PAH) or the cation tetraethylammonium (TEA), both of which are nearly completely extracted in a single pass through the portal vessels. The resulting corrected ATEF