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Hepatic disposition of clinically useful 5-nitrofurans

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Since Dodd and Stillman described the antimicrobial activity of nitrofurazone [1], several 5-nitrofurans have been synthesized and are widely used in human and veterinary medicine and as food preservatives and feed additives. Perhaps the most clinically used 5-nitrofurans are nitrofurantoin (NFT), nitrofurazone (NFZ) and furazolidone (FZ). In addition to being mutagenic [2], NFT, NFZ and FZ may all cause adverse side effects ranging from gastrointestinal distress to neurologic, pulmonary, renal and hepatic toxicities [3]. Interestingly, the lung, kidney and liver which are targets of the clinically manifested toxicities are all capable of metabolizing these drugs [4–10]. In a previous study [11], we demonstrated that NFZ is removed rapidly by the isolated perfused rat liver, that it exhibits a significant choleretic capacity, and that glutathione appears indispensable for its efficient clearance and for the choleresis. In this paper we describe the hepatic disposition of two other clinically useful 5-nitrofurans, NFT and FZ, by the perfused rat liver. The effect of glutathione (GSH) depletion on their overall removal is also described.

Materials and methods

Nitrofurantoin, furazolidone and nitrofurazone were provided by Norwich-Eaton (Norwich, NY). Diethyl maleate (DEM) was purchased from the Sigma Chemical Co. (St. Louis, MO). [^{35}S]Methionine (sp. act. 1097 Ci/mmol) was purchased from New England Nuclear (Boston, MA). All the other chemicals were of the highest grade commercially available. Livers were removed under light ether anesthesia from 55- to 60-day-old, male Sprague–Dawley rats (Bantin & Kingman, San Leandro, CA). Where indicated, a group of rats had been injected intraperitoneally (0.6 ml/kg body weight) with DEM approximately 1 hr before starting surgery. After mobilization from the abdomen, the liver was placed in a thermoregulated and humidified cabinet and perfused by a recirculating system via the portal vein as previously described [11]. After 30 min, a bolus dose of 17.5 or 70 μmol of NFT or FZ (in 250 μl dimethyl sulfoxide which has no effect on the liver or bile flow) was added to the reservoir (final concentration 0.35 or 1.4 mM), and perfusate (100 μl) and bile (total volume) were sampled at intervals for the next 40–45 min. In addition, in order to assess the glutathione status of the organ, one of the small caudate lobes was tied off and removed just prior to addition of the drug. It was quick frozen in liquid nitrogen and stored at −70° until analysis. NFT and FZ concentrations in bile and perfusate were determined by high pressure liquid chromatography (HPLC) as previously described [11]. Tissue and bile levels of GSH and its disulfide (GSSG) were determined by the HPLC method described by Reed *et al.* [12] modified by developing the chromatograms isochratically using the final solvent of their gradient system. In order to prelabel a portion of the liver glutathione pool, a group of rats was injected intraperitoneally 90 min before surgery with 0.2 to 0.5 mCi of [^{35}S]methionine diluted with cold methionine and dissolved in normal saline to give a dose of 250 μg/g body weight [13]. Livers from these animals were perfused single pass with either FZ or NFT or NFZ at a concentration of 0.15 mM. Perfusate and bile were collected, and aliquots were added to Scinti Verse E (Fisher, San Francisco, CA) and counted for radioactivity (LS 7800, Beckman Instruments, San Jose CA). In addition, perfusate and bile were assayed for GSH and GSSG as described above; however, a radiochemical detector (Flo-one β, Radiomatic Instruments, Tampa, FL) was used in addition to the usual u.v. monitor. Perfusate concentrations were analyzed by nonlinear least square curve fitting using an exponential function. The area under the curve was calculated as the extrapolated initial concentration divided by k, the slope of the curve; the half-life as $0.693/k$; the clearance as dose/area under the curve per g of liver weight; the extraction ratio as clearance/perfusate flow rate; the presence of NFT or FZ excreted in bile as cumulative amount excreted/dose ×100. Statistical significance was assessed by Student's t-test.

Results and discussion

At a dose of 17.5 μmol, both NFT and FZ were removed rapidly from perfusate (Table 1). At the high dose, the removal rate was significantly slower for both drugs. This phenomenon, however, does not appear to reflect a saturation of the transport system since the concentration versus time curve was best fitted by a single exponential decay in all experiments.

Table 1. Disposition of nitrofurantoin and furazolidone in the isolated perfused rat liver*

	Nitrofurantoin			
	17.5 μmol	70 μmol	17.5 μmol + DEM	70 μmol + DEM
Half-life (min)	7.34 ± 0.96	12.84 ± 1.10 $P < 0.005$†	9.33 ± 1.30 $P < 0.1$‡	NC§
Clearance (ml·min^{-1}·g^{-1})	0.64 ± 0.07	0.37 ± 0.05 $P < 0.025$	0.36 ± 0.07 $P < 0.02$	NC
Extraction ratio	0.23 ± 0.05	0.12 ± 0.02 $P < 0.05$	0.12 ± 0.03 $P < 0.05$	NC
% Excreted unchanged in bile	0.64 ± 0.10	0.79 ± 0.15 $P < 0.10$	1.08 ± 0.06 $P < 0.005$	NC
	Furazolidone			
	17.5 μmol	70 μmol	17.5 μmol + DEM	70 μmol + DEM
Half-life (min)	7.84 ± 1.86	18.43 ± 4.40 $P < 0.025$	9.63 ± 1.47 $P < 0.1$‡	44.7 ± 11.7 $P < 0.025$‡
Clearance (ml·min^{-1}·g^{-1})	0.92 ± 0.04	0.44 ± 0.11 $P < 0.005$	0.67 ± 0.05 $P < 0.005$	0.17 ± 0.01 $P < 0.05$
Extraction ratio	0.31 ± 0.03	0.15 ± 0.04 $P < 0.02$	0.19 ± 0.01 $P < 0.005$	0.06 ± 0.01 $P < 0.05$
% Excreted unchanged in bile	0.47 ± 0.10	0.83 ± 0.10 $P < 0.01$	1.13 ± 0.52 $P < 0.10$	0.57 ± 0.15

* Results are mean ± SEM of four to five experiments each.
† Versus values for the 17.5 μmol dose.
‡ Values are compared with untreated livers.
§ Not cleared.

As with NFZ [11], both NFT and FZ were minimally excreted in bile unchanged (Table 1). Interestingly the percentage of parent compound in bile increased with increasing dose. However, in the case of NFT the increase was not statistically significant. Panels A and C of Fig. 1 show the effect of NFT and FZ administration on bile flow. It can be seen that the bile flow increased from a control value close to 1 $\mu l \cdot min^{-1} \cdot g^{-1}$ to a value close to or greater than 2 $\mu l \cdot min^{-1} \cdot g^{-1}$ between 5 and 10 min after drug administration, remained high for the next 10 min, and then gradually returned to control levels. The choleresis seemed slightly greater after NFT administration and appeared to be dose related. If, however, biliary excretion or biliary concentration values of the parent drug were plotted versus the bile flow, no significant correlation was demonstrable (data not shown). Furthermore, the low concentrations of unchanged NFT and FZ in bile clearly rule out an osmotic choleresis due to the parent compounds. When DEM was given to rats and GSH measured immediately before the experiment, levels decreased to 11.3 ± 3.9% of controls. DEM pretreatment also had a marked effect on the perfusate disposition of the two 5-nitrofurans as shown in Table 1. The effect was most dramatic at the dose of 70 μmol when the clearance decreased over 2-fold for FZ, while NFT was not cleared at all. When compared to the values in untreated livers, the percentage of unchanged NFT and FZ in bile

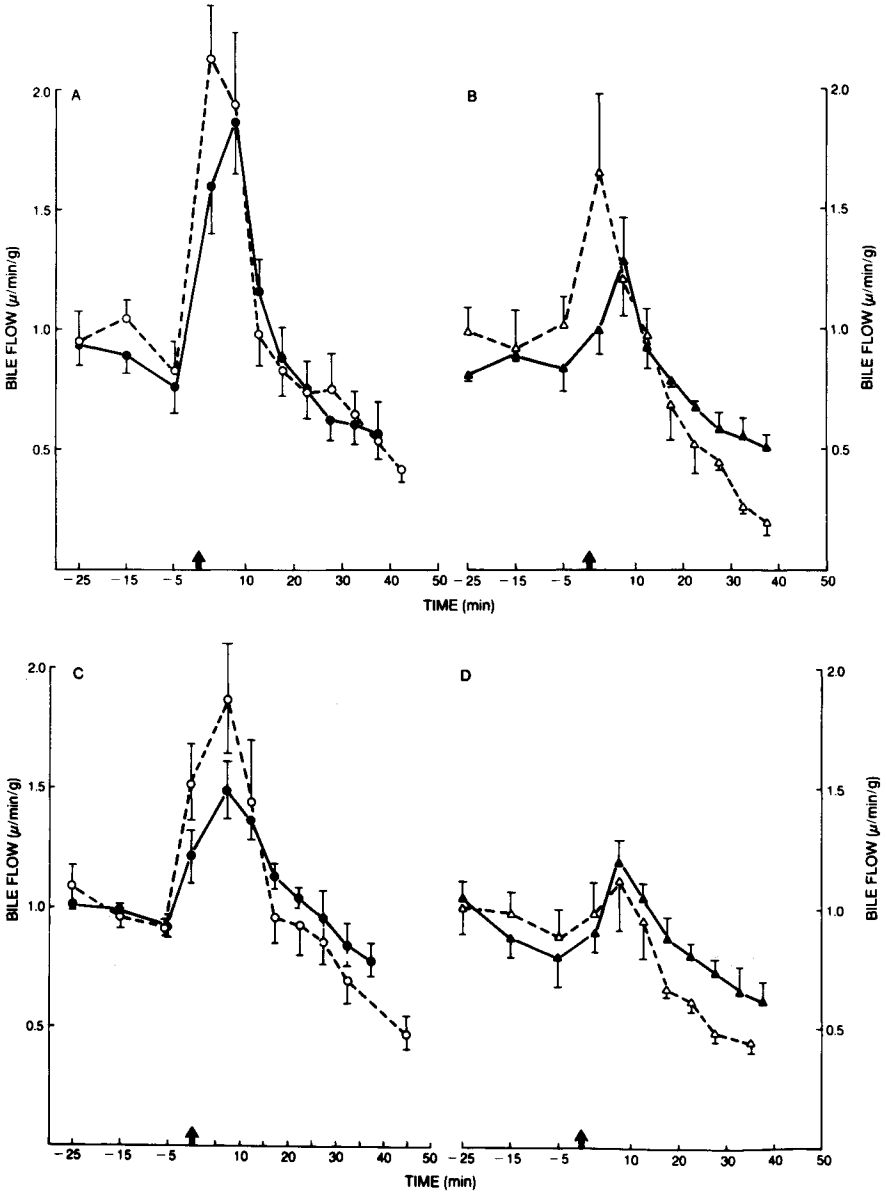

Fig. 1. (A) Bile flow during the control period and after administration (arrow) of nitrofurantoin as a 17.5-μmol (●) or a 70-μmol (○) bolus dose. (B) Bile flow (where μ = μl) during the control period and after administration (arrow) of nitrofurantoin as a 17.5-μmol (▲) or a 70-μmol (△) bolus dose to DEM-pretreated livers. (C) Bile flow during the control period and after administration (arrow) of furazolidone as a 17.5-μmol (●) or a 70-μmol (○) bolus dose. (D) Bile flow during the control period and after administration (arrow) of furazolidone as a 17.5-μmol (▲) or a 70-μmol (△) bolus dose to DEM-pretreated livers. Points are mean ± SEM (N = 4–5).

increased in GSH-depleted livers given the low dose. At the high dose, however, the comparison is probably inappropriate because the experiments were not carried out long enough given the extremely long perfusate half-life (FZ) or the lack of effective removal (NFT). Panels B and D of Fig. 1 show the effect of glutathione depletion on bile flow. The marked hypercholeresis observed in untreated livers was reduced.

To investigate further the role of glutathione in the hypercholeresis, the hepatic glutathione pool of some animals was labeled by administration of [^{35}S]methionine; one of the 5-nitrofurans was then given as an infusion, and the biliary radioactivity was analyzed with a radiochemical detector. Figure 2 shows the results of an experiment with FZ. Prior to the infusion of FZ (panel A), both GSH and GSSG were shown to be excreted into the bile (peak 1 and 2 respectively). After addition of the drug (panel B), there was an increased efflux of GSSG into the bile and a third peak, of unknown nature and retaining the ^{35}S label, appeared. This coincided with the increase in bile flow. The third peak and the excess GSSG excretion disappeared when the FZ infusion was discontinued. Similar results were obtained with NFT and NFZ (data not shown). These observations help to explain the data of Fig. 1 and clearly show that 5-nitrofurans increased the bile flow, at least partially, through an increased excretion of glutathione and its by-products which could be conjugates of the drugs with glutathione itself.

The decreased efficiency of the glutathione-depleted liver to remove 5-nitrofurans may be explained by several reasons. First, because the intracellular mobile nucleophile pool available for covalent binding is decreased, the reactive intermediate metabolites of the 5-nitrofurans could attack vital macromolecules, thus affecting the cell viability and capacity to eliminate the drug. Although diminished, this capacity might be sufficient to eliminate a minor load of drug but it could be inadequate to eliminate the higher amount. This hypothesis assumes that the normal liver can rapidly dispose of 5-nitrofurans because the reactive intermediate metabolites can be bound by GSH. If these metabolites were eliminated in the bile they might contribute to an osmotic choleresis which would be diminished or absent in the DEM-treated livers. The results shown in Fig. 1 support this theory. The glutathione conjugates of 5-nitrofurans can be formed *in vitro* has been shown in previous work [14–19]. Moreover, as shown in Fig. 2, we believe that we have detected a conjugate of glutathione (and/or its by-products) and FZ. A compound with a similar HPLC elution pattern is excreted in the bile of livers given NFT and NFZ (data not shown). While we cannot, at this time, exclude the possibility that this is a metabolite of glutathione itself, known metabolites of GSH and GSSH elute before GSH in this HPLC system [12].

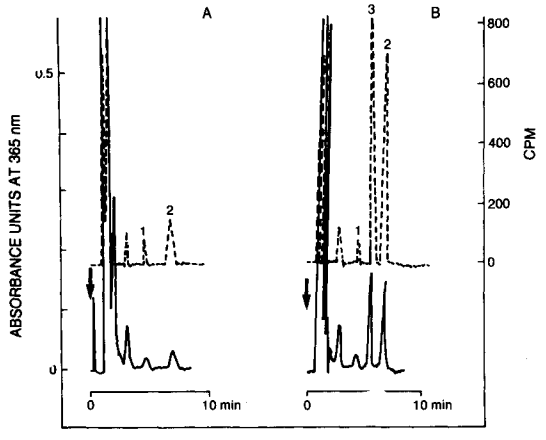

Fig. 2. HPLC trace (see Materials and Methods) of a control period bile sample of a liver from a rat pretreated with [^{35}S]methionine. Both the u.v. absorbance at 365 nm (—) and the radioactivity cpm (---) are shown. The peak labeled 1 is GSH and that labeled 2 is GSSG. (B) HPLC trace of a bile sample from the same liver taken 25 min after a 0.15 mM furazolidone infusion was started. There is an increased biliary efflux of GSSG (peak 2) and a new compound (peak 3) not seen with the drug-free perfusion.

A second explanation for the increased toxicity of NFT and FZ in the DEM-treated livers could be an increase in the lifetime of the reactive oxygen species including OH· and H_2O_2 generated during the redox cycling of the 5-nitrofurans [20]. Without an adequate pool of glutathione, other pathways for detoxification of these reactive oxygen species may be overwhelmed. These reactive species could then attack normal cellular constituents and thus contribute to the toxicity of the drug. In normal livers GSH is oxidized during this detoxification process. The excess GSSG induced by oxidative stress may be excreted in the bile (see Fig. 2) [21, 22]. This increased secretion of GSSG may also contribute to the osmotic choleresis seen in the control but not in the glutathione-depleted livers.

Finally, DEM *per se* could affect the hepatic clearance of 5-nitrofurans either by interfering with their reductive metabolism or by perturbing the hepatocyte homeostasis. However, 5-nitrofuran reductive metabolism in the intact organ is mediated by cytochrome *c* reductase which has been shown to be unaffected by DEM [5, 23–26]. Likewise, DEM has been shown not to alter the hepatocellular function and morphology [27], and our indices of liver viability, including bile flow, do not seem to be affected by DEM treatment at least at the dose and over the time tested. Thus, we conclude that it is improbable that DEM has a direct effect on the disposition of NFT or FZ. We therefore conclude that our first and second hypotheses are both supported by the data. We cannot eliminate either and, in fact, believe that both contribute to our observations.

In summary, we have shown that NFT and FZ, like NFZ, were rapidly cleared by the isolated perfused rat liver. The disposition of each, however, was critically dependent on glutathione. Each also produced a cholerectic effect which appears to be due to the biliary excretion of their metabolites conjugated with GSH and/or GSH by-products and to the excess GSSG excreted because of oxidative stress induced by the redox cycling of the 5-nitrofuran.

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