Biochemical Pharmacology, Vol. 36, No. 6, pp. 915–918, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

NITROFURAZONE DISPOSITION BY PERFUSED RAT LIVER

EFFECT OF DOSE SIZE AND GLUTATHIONE DEPLETION

DARIO SORRENTINO, WILHELMINA BODE and BETTY-ANN HOENER*
Department of Pharmacy, Department of Medicine and the Liver Center, University of California, San Francisco, CA 94143, U.S.A.

(*Received* 17 *February* 1986; *accepted* 15 *August* 1986)

Abstract—The disposition of nitrofurazone was studied in the isolated perfused rat liver using a recirculating system. The drug was administered as a bolus in two different doses (3.5 and 14 mg: initial concentrations 0.35 and 1.4 mM respectively), and its disappearance was monitored by analyzing perfusate samples at various times. Biliary excretion and bile flow were also measured. In all experiments perfusate disappearance was monoexponential, and no significant difference was found between the two doses ($T_{1/2}$: 5.34 ± 2.03 and 6.19 ± 1.47 min for 14 and 3.5 mg respectively). Bile flow increased more than 2-fold 5–10 min after administration of the drug and subsequently returned to control levels. The increase in bile flow was dose-related and paralleled the excretion of the parent drug in the bile; however, of the total dose administered, only 0.27 ± 0.04% was excreted unchanged in bile, thus ruling out an osmotic choleresis due to the parent drug. Since nitrofurazone may be excreted in part as a glutathione conjugate, this or other metabolites could have caused an osmotic choleresis. This hypothesis was tested by administering diethylmaleate which causes glutathione depletion. Although the initial bile flow in treated livers was not different from untreated livers, bile flow did not increase after administration of nitrofurazone. In addition, the perfusate half-life of nitrofurazone was increased (18.18 ± 1.30 min, $P < 0.005$). These results suggest that nitrofurazone is cleared rapidly by the liver and that glutathione plays an important role in its disposition.

The 5-nitrofuran, nitrofurazone, is used clinically as an antibiotic in human and veterinary medicine. In particular, nitrofurazone has proved useful for the therapy of mixed infections of superficial wounds and diseases of the skin, in the treatment of burns, in the prophylaxis of nosocomial infections, in promoting healing of donor skin graft sites, in the prevention of peritoneal adhesions and as an antiseptic lubricant for transurethral resection [1]. Nitrofurazone is used also in the systemic treatment of Gambian and Rodhesian sleeping sickness (African Trypanosomiasis) [2]. Since it can keep animals disease-free, it is used in animal feeds as a growth promoter [1]. Nitrofurazone, however, is toxic, a peripheral neuropathy being a relatively common side effect. Also, it markedly affects liver function and metabolism: nitrofurazone stimulates O_2 uptake in rat liver mitochondria by interfering with the function of the respiratory chain [3]; is a competitive inhibitor of liver monoamine oxidases [4]; interferes with the hepatic metabolism of essential amino acids [5]; and, if given orally or subcutaneously to rats, causes pathologic changes in the liver ranging from swelling and cytoplasmic and nuclear degeneration to severe toxic hepatitis [6]. Despite these pronounced effects, little is known about the hepatic transport and metabolism of this drug. We describe in this paper the disposition of nitrofurazone by the isolated perfused liver and its effects on bile flow. Some speculations on the metabolic fate of nitrofurazone are also given.

* Author to whom correspondence should be addressed: 926 Science, School of Pharmacy, University of California, San Francisco, CA 94143.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (Bantin & Kingman; 55- to 60-days-old, 200–250 g) with free access to standard rat chow and water were used.

Materials. Nitrofurazone (2-[(5-nitro-2-furanyl)methylene]hydrazine carboxamide) and nitrofurantoin (1-[[(5-nitro-2-furanyl)methylene]-amino]-2,4-imidazolidinedione) were gifts from Norwich-Eaton (Norwich, NY). Dimethyl sulfoxide was purchased from Burdick & Jackson (Muskegon, MI), and Fluosol (FC-43 Emulsion) from Alpha Therapeutics (Los Angeles, CA); bovine serum albumin and diethylmaleate (DEM) were from the Sigma Chemical Co. (St. Louis, MO).

Liver perfusion. After surgical excision from the rat, livers were perfused in a humidified and thermoregulated cabinet where the perfusate is circulated from the reservoir by a peristaltic pump (LKB, Bromma, Sweden) through a membrane lung (consisting of a polyethylene tubing, Clay Adams, Parsipanny, NJ), a filter, a bubble trap-pressure gauge and an in-line pH probe, before returning to the liver and mixing reservoir. Liver viability was judged on the basis of gross appearance, bile production, perfusion pressure and oxygen consumption. The pH of the perfusate was kept constant (7.35 to 7.45) throughout the experiment by modifying the pCO_2 and pO_2 as necessary.

Nitrofurazone assay. Nitrofurazone concentrations in perfusate and bile were determined by HPLC, 100-μl aliquots of bile or perfusate were mixed with 100 μl of internal standard, nitrofurantoin, in acetonitrile, and protein and fluosol, if present, were removed by centrifugation (15 min at 20,000 *g*). A standard curve was prepared for each set of samples.

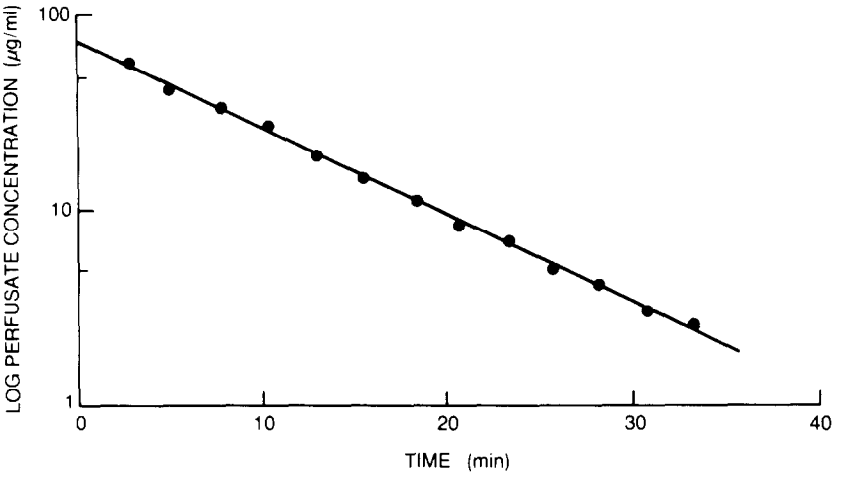

Fig. 1. Concentration of nitrofurazone in the perfusate after administration of a bolus dose of 3.5 mg. The data are plotted on a semilogarithmic scale.

HPLC analysis was performed using a Waters HPLC instrument, with a 6000 A pump, a U6K injector and a model 440 UV detector set at 280 and 365 nm. An Alltech C18 column (10 μm, 4.6 mm i.d. × 25 cm) was used with methanol/water (30/70, v/v; 2 ml/min) as eluent.

Experimental design. The hepatic disposition of nitrofurazone was studied using a recirculating system. Livers were perfused with 50 ml of a 20% emulsion of fluosol (a fluorocarbon oxygen carrier) containing 1% bovine serum albumin at a flow rate of approximately 3 ml · min^{-1} · g^{-1} of liver (range 2.7 to 3.4). After a 30-min equilibration period a bolus dose of 14 or 3.5 mg of nitrofurazone (in 100 μl dimethyl sulfoxide) was added to the reservoir. Perfusate (100 μl) was collected every 2.5 min for 15 min then every 5 min for the next 30 min. Bile was collected in preweighed vials during the equilibration period and after adding the drug. Bile volume was calculated gravimetrically assuming a specific gravity of 1. Bile volume was adjusted to 100 μl with addition of double-distilled water. Bile and perfusate samples were frozen at −20° until analysis by HPLC.

DEM treatment. DEM was given intraperitoneally at a dose of 0.6 ml/kg body weight approximately 1 hr before starting surgery.

Pharmacokinetic calculations and statistical analysis. An exponential function was fitted to perfusate concentrations and biliary excretion rates using a nonlinear least squares procedure. The $T_{1/2}$ was calculated as ln2/k (k = slope of the curve); clearance as dose/area under the curve; extraction as clearance/flow rate; percent nitrofurazone excreted in bile as cumulative nitrofurazone biliary excretion/dose × 100. Student's unpaired *t*-test was used for statistical comparisons.

RESULTS

Perfusate disposition of nitrofurazone. In all experiments, nitrofurazone disappearance from the perfusate conformed to a monoexponential decay pattern (Fig. 1). At the doses tested, the removal of nitrofurazone was virtually complete by 35–45 min. Values for $T_{1/2}$, clearance and extraction ratio are given in Table 1. No statistical difference between these parameters was found for the two doses ($P > 0.1$).

Biliary excretion. Nitrofurazone in bile was detectable 2.5 min after administration and reached maximum concentration at 7.5 min (Fig. 2, lower panel). The decay phase of biliary excretion rate was monoexponential with a half-life of about 10 min (10.46 ± 2.28 and 10.14 ± 2.16 for 3.5 and 14 mg doses respectively). The cumulative biliary excretion of nitrofurazone averaged 0.28 ± 0.04% (3.5 mg) and 0.29 ± 0.03% (14 mg) of the administered dose. Again, there was no statistical difference between values obtained at the two doses.

Effects on bile flow. Nitrofurazone administration increased the bile flow rate markedly. During the equilibration period the bile flow was similar to that in control livers; however, 2.5 min after administration of nitrofurazone it began to increase reaching a peak at approximately 7.5 min. Thereafter, the bile flow gradually returned to basal levels by about 45 min. The basal bile flow in control livers was constant for about 30 min and then decreased because of depletion of the bile acid pool.

The stimulatory effect of nitrofurazone on bile flow was seen at both doses (Fig. 2, upper panel). Figure 2 also shows that the nitrofurazone concentration in bile closely paralleled the behavior of bile flow and that the increase in bile flow was dose dependent.

Recent studies [7] indicate that nitrofurazone is excreted in bile largely as a glutathione or cysteine-glycine conjugate. To determine whether these metabolites, rather than nitrofurazone itself could be responsible for the marked dose-dependent stimulation of the bile flow observed above, the effect of the drug was also studied in rats pretreated with diethylmaleate to deplete hepatic glutathione [8].

The data relative to bile flow and biliary excretion of nitrofurazone in diethylmaleate-treated livers are shown in Fig. 3. Although the bile flow did not differ

Table 1. Perfusate disposition of nitrofurazone

	Nitrofurazone 3.5 mg (0.35 mM)	14 mg (1.4 mM)	P
Half-life (min)	6.19 ± 1.47	5.34 ± 2.03	NS
Clearance ($ml \cdot min^{-1} \cdot g^{-1}$)	0.55 ± 0.11	0.61 ± 0.07	NS
Extraction ratio	0.19 ± 0.04	0.18 ± 0.02	NS

Data represent mean ± SEM of four experiments for each dose. NS = not significant.

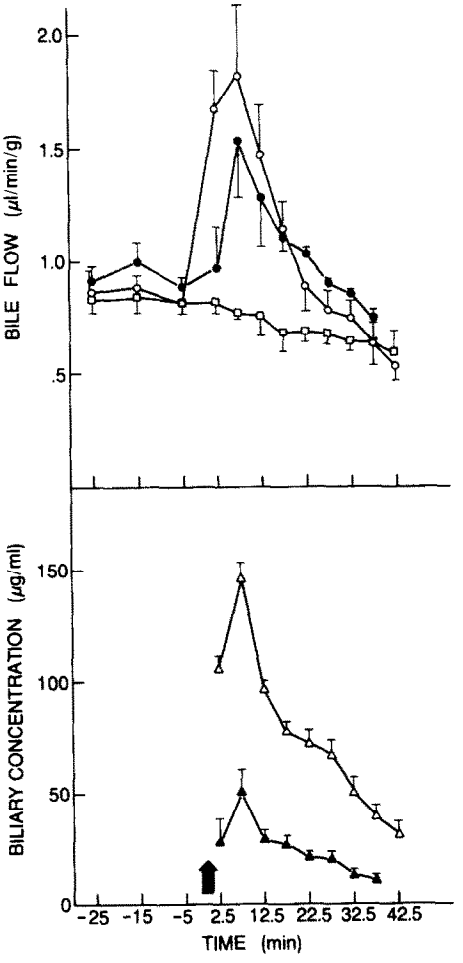

Fig. 2. Upper panel: Bile flow before and after administration (arrow) of nitrofurazone as a 3.5 mg (●) or a 14 mg (○) bolus dose. The bile flow in control (□), nontreated livers is also shown. Lower panel: Biliary concentration of nitrofurazone following the 3.5 mg (▲) or 14 mg (△) bolus doses. Points are mean ± SEM.

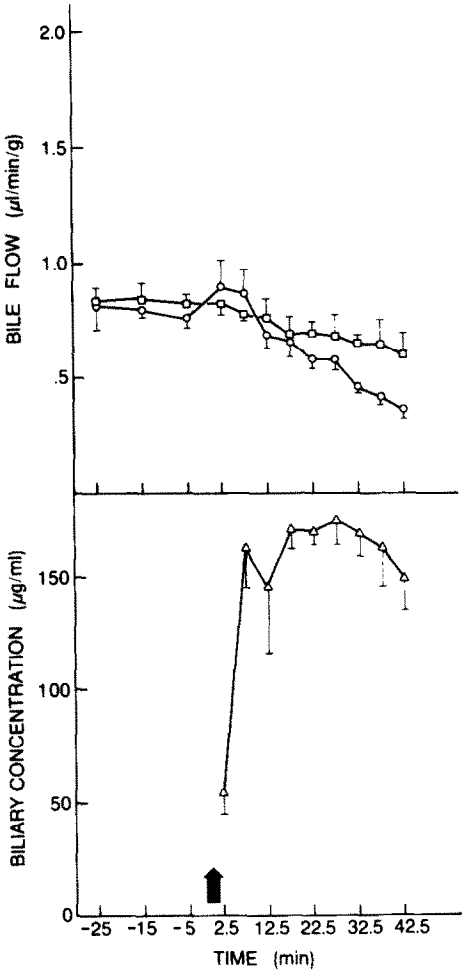

Fig. 3. Upper panel: Bile flow before and after administration (arrow) of a 14 mg (○) bolus dose of nitrofurazone to DEM-pretreated livers. Control values (□) in nontreated livers are shown for reference. Lower panel: Biliary concentration of nitrofurazone after a 14 mg (△) bolus dose to DEM-pretreated livers. All points are mean ± SEM.

from controls during the equilibration period, it did not increase significantly (a small peak is barely appreciable between 2.5 and 7.5 min) after administration of nitrofurazone. The biliary concentration of nitrofurazone was higher than in untreated livers given the same dose. Interestingly, the total amount of drug excreted unchanged increased but the increase was not statistically significant (0.36 ± 0.10 vs 0.27 ± 0.03% for diethylmaleate and untreated respectively). The parameters relative to perfusate disposition are illustrated in Table 2. Half-life was significantly greater in DEM-treated livers, whereas clearance and extraction were reduced.

DISCUSSION

Before the present study the hepatic disposition of nitrofurazone was unknown. These studies show that nitrofurazone was readily taken up by the perfused liver, its half-life being approximately 6 min, it was metabolized and excreted in bile mainly as metabolites. At the two doses tested there were

Table 2. Perfusate disposition of nitrofurazone by DEM-treated liver*

	DEM-treated liver	P
Half-life (min)	18.18 ± 1.30	< 0.005
Clearance ($ml \cdot min^{-1} \cdot g^{-1}$)	0.31 ± 0.01	< 0.01
Extraction ratio	0.09 ± 0.01	< 0.02

* The dose of nitrofurazone given was 14 mg. DEM (0.6 ml/kg body wt) was given i.p. 1 hr before surgery. Results are compared with untreated livers (Table 1) given the same dose of nitrofurazone. Data represent mean ± SEM of four experiments.

no differences in half-life, clearance and extraction ratio, suggesting that the removal process was operating well below its V_{max} at the concentration of 1.4 mM. Unexpectedly, nitrofurazone had a pronounced choleretic effect. Although this effect was dose dependent, it is unlikely that nitrofurazone itself was responsible because of its low concentration in bile. In the rat, bile osmolarity averages 332 mOsm [9]. The maximal concentration of nitrofurazone in bile approximated 1 mM; thus, this would represent a mere 0.3% increase in bile flow, whereas the latter increased up to 2-fold. It has been proposed recently that nitrofurazone is excreted in bile as a glutathione or a glutathione metabolite conjugate [7]. Our results using livers of animals treated with diethylmaleate, a known depletor of the glutathione pool [8], support this hypothesis and also suggest that the increase in bile flow after nitrofurazone administration is, at least partially, due to an osmotic effect of its metabolites. Biliary efflux of oxidized glutathione induced by the redox cycling of the 5-nitro function which has been shown in the case of nitrofurantoin, a chemically similar 5-nitrofuran antibiotic, may also contribute to the choleretic effect [10, 11]. In fact, in diethylmaleate-treated livers no clear increase in bile flow was observable after nitrofurazone administration and the fraction of the dose excreted unchanged tended to increase (0.36 vs 0.27%). Although glutathione depletion causes many derangements in liver function and homeostasis and in the long term may be deleterious to the hepatocyte [12], in our experiments a normal bile flow and function were maintained. It is possible that DEM, in addition to depleting glutathione by direct conjugation, may directly alter the metabolism of nitrofurazone. DEM is known to alter the cytochrome P-450-mediated metabolism of some xenobiotics [13]. However, nitrofurazone is not metabolized by cytochrome P-450 [14]. Its reductive metabolism in the intact organ is mediated by cytochrome *c* reductase [15, 16] which has been shown to be unaffected by DEM [17]. The ring hydroxylation of nitrofurantoin is not phenobarbital inducible in the isolated perfused rat liver [16]. Ring hydroxylation occurs only after pretreatment with 3-methylcholanthrene, but we found no evidence for this metabolite in the bile or perfusate of our preparations. Thus, we conclude that it is improbable that DEM has a direct effect on the disposition of nitrofurazone. One could then speculate that, when challenged with nitrofurazone, the diethylmaleate-treated liver is unable to use alternative pathways to eliminate the drug. We conclude that nitrofurazone is removed rapidly from the perfusate by the isolated perfused rat liver. Although biliary excretion of unmetabolized nitrofurazone is negligible, the drug has a pronounced choleretic effect which is probably caused by biliary excretion of oxidized glutathione induced by the redox cycling of the 5-nitro function together with nitrofurazone conjugates with glutathione or glutathione derivatives.

Acknowledgements—The authors wish to thank Ms. Linda Kendrick for excellent technical help and Dr. V. Licko and Dr. R. A. Weisiger for valuable discussions. This work was supported by National Institutes of Health Research Grant AI 18423 and the Regione Sardegna Assessorato P.I. Italy.

REFERENCES

1. S. C. Harvey, in *The Pharmacological Basis of Therapeutics* (Eds. A. G. Gilman, L. S. Goodman, T. W. Rall and F. Murad), 7th Edn, p. 967. Macmillan, New York (1985).
2. B. M. Greenwood, in *Cecil Textbook of Medicine* (Eds. J. B. Wyngaarden and L. H. Smith), 16th Edn, p. 1727. Saunders, Philadelphia, PA (1982).
3. S. V. Kozhanova, A. T. Basenova and G. G. Buzurbaev, *Vop. med. Khim.* **26**, 605 (1980).
4. A. S. Kines and D. O. Carr, *Biochem. Pharmac.* **31**, 2639 (1982).
5. V. S. Khomenko and N. R. Khomenko, *Veterinariia* **1**, 58 (1982).
6. M. C. Dodd, *J. Pharmac. exp. Ther.* **86**, 311 (1946).
7. B. Hoener, W. Bode and T. R. Krueger, *Acad. Pharm. Sci.* **15**, 157 (1985).
8. F. Varga, E. Fischer and T. S. Szily, *Biochem. Pharmac.* **23**, 2617 (1974).
9. C. D. Klaassen, *Can. J. Physiol. Pharmac.* **52**, 334 (1974).
10. T. P. M. Akerboom, M. Bilzer and H. Sies, *J. biol. Chem.* **257**, 4248 (1982).
11. B. H. Lauterburg, C. V. Smith, H. Hughes and J. R. Mitchell, *J. clin. Invest.* **73**, 124 (1984).
12. J. R. Mitchell, J. A. Hinson and S. D. Nelson, in *Glutathione: Metabolism and Function* (Eds. I. M. Arias and W. B. Jakoby), p. 357. Raven Press, New York (1976).
13. M. W. Anders, *Biochem. Pharmac.* **27**, 1098 (1978).
14. D. R. Feller, M. Morita and J. R. Gillette, *Proc. Soc. exp. Biol. Med.* **137**, 433 (1971).
15. C. Y. Wang, B. C. Behrens, M. Ichikawa and G. T. Bryan, *Biochem. Pharmac.* **23**, 3395 (1974).
16. H. G. Jonen, *Naunyn-Schmiedeberg's Archs Pharmac.* **315**, 167 (1980).
17. R. Reiter and A. Wendel, *Chem. Biol. Interact.* **40**, 365 (1982).