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SHORT COMMUNICATIONS

Effect of nafcillin on hepatic excretory function

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Considerable evidence has accumulated to support the view that the hepatic transport pathways for bilirubin and bile acids are essentially independent [1]. Part of the knowledge supporting this view is that individuals recognized as having unconjugated hyperbilirubinemia, as occurs in Gilbert's syndrome, and individuals recognized as having conjugated hyperbilirubinemia, as occurs in Dubin–Johnson syndrome, have no evidence of impaired bile acid transport [2, 3]. Further evaluation of this concept can be obtained by analyzing the effects of specific compounds on the hepatic transport pathways for bile acids and bilirubin. Such information can be particularly helpful in evaluating the changes that may occur in the plasma levels of bile acids and bilirubin in patients receiving various medications.

Because nafcillin [6-(2-ethoxy-1-naphthamido)penicillanic acid] therapy requires the administration of 8–12 g a day, and it is excreted mostly in the bile [4], we applied a well-established rat model to specifically evaluate its effect on the transport of bilirubin and bile acids.

Methods

Preparation of animals. During pentobarbital anesthesia, adult male Sprague–Dawley rats were prepared with polyethylene cannulae (PE10, Clay Adams) in the bile duct, femoral artery and femoral veins as previously described [5]. Bile was allowed to drain overnight into graduated centrifuge tubes. Animals were considered to be suitable for infusion studies if at least 10–15 ml of bile had drained overnight, and body temperature had returned to preoperative values. Patency of vascular cannulae was maintained by slow infusion of 5% dextrose in 0.45% NaCl.

Infusions. Sodium taurocholate and bilirubin: 215 mg sodium taurocholate [24-^{14}C] (mol wt. 538, sp. act. 1.03 μCi/nmole) was dissolved in 2 ml of 5% dextrose in 0.45% NaCl; unconjugated bilirubin (23.38 mg, Sigma) was dissolved with warming in 2 ml of 5% dextrose in 0.45% NaCl and 0.1 ml of 5 N NaOH. To this mixture was added 2 ml of 25% human serum bilirubin (Cutter) and the 2 ml of dissolved bile salts. The pH of the solution was adjusted to pH 8.0–8.5 with addition of 0.18 ml of 2 N HCl. Finally the solution was brought to a total volume of 10 ml by addition of 3.7 ml of 5% dextrose in 0.45% NaCl. The final concentration of bile salts was 40.0 mM and that of bilirubin was 4.0 mM. The solution was prepared just prior to each experiment. Nafcillin (mol wt. 436) (Wyeth): 3.4 ml sterile water was added to a vial containing 1.0 g of nafcillin powder. The final concentration was 250 mg/ml. Sulfobromphthalein (BSP): in some experiments, BSP was substituted for bilirubin. For these studies, 1.0 ml of a 50 mg/ml solution of BSP was added to a 40.0 mM solution of ^{14}C-labeled sodium taurocholate and 5% human serum albumin (HSA) in a total volume of 10.0 ml. BSP concentration was 6.0 mM.

Experimental design. (A) Effect of nafcillin on bilirubin and bile acid excretion: The sodium taurocholate and bilirubin or BSP mixture was infused at rates of 2.0 μmoles/min and 0.2 or 0.3 μmole/min respectively. Bile samples were collected quantitatively in tared tubes every 10–15 min, and blood samples were collected every 15–30 min. After 60 min, nafcillin was infused at a rate of 2.0 μmoles/min for 30 min during which the bile acid–bilirubin infusion was continued. The nafcillin infusion was stopped after 30 min, and the bile acid–bilirubin infusion was continued for an additional 60 min. (B) Effect of nafcillin on bilirubin removal from plasma: Animals were given an intravenous injection of 10 mg of unconjugated bilirubin dissolved in 12% HSA, as described. Serum samples were taken at 3, 5, 10, 15, 20, 30, 40 and 60 min. At 30 min an intravenous injection of nafcillin (100 mg) was given. Plasma samples were assayed for conjugated and unconjugated bilirubin.

Analyses. Conjugated and total bilirubin in plasma and bile were determined by the method of Malloy and Evelyn [6]. Bile acid concentration was calculated from the specific activity of the infused sodium taurocholate. Each bile and serum sample (0.05 to 0.1 ml) was dissolved in 4.0 ml of Atomlight (New England Nuclear), and radioactivity was estimated using a liquid scintillation counter. Prior to counting, the solutions were bleached under an ultraviolet lamp, and the samples were corrected to constant efficiency by external standardization. BSP in serum and bile was determined by the method of Seligson *et al.* [7].

Results and Discussion

The results of a typical infusion study are illustrated in Fig. 1. During the control period, bile acid and bilirubin excretion rates were constant. Nafcillin infusion caused a significant decrease in both bilirubin excretion and bilirubin concentration in bile. The decrease in bilirubin excretion rate occured 10–15 min after the start of nafcillin infusion and continued for as long as 40 min after the infusion. The maximum decrease in bilirubin excretion rate was 72%. In contrast, nafcillin produced no significant change in bile acid excretion rate.

The results of three studies in three rats are summarized in Table 1. In each study, a significant fall in the excretion rate of conjugated bilirubin in bile occurred with no change in bile acid output. Bile acid concentration fell 41.6 ± 2.1% from the control period which is attributable to the increase in bile flow induced by nafcillin.

The findings were similar in two studies in which BSP was substituted for bilirubin. One study is illustrated in Fig. 2. BSP concentration and excretion rate in bile fell with the onset of the nafcillin infusion. No change in bile acid excretion occurred.

The effect of nafcillin on plasma bilirubin removal rate is illustrated in Fig. 3. A bolus injection of nafcillin during a curve of bilirubin disappearance from plasma had no apparent effect on the subsequent slope compared to animals not receiving the compound. However, a marked increase in conjugated bilirubin occurred in the animals given nafcillin. At 60 min unconjugated serum bilirubin levels in control and nafcillin-treated rats were 3.85 ± 0.15 (S.E.) mg/100 ml and 3.40 ± 0.2 (S.E.) mg/100 ml, respectively, compared to conjugated bilirubin levels of 1.65 ± 0.6 (S.E.) and 7.0 + 0.3 (S.E.) in control and treated

Table 1. Changes in bile composition after nafcillin infusion*

	Control	Minimum after nafcillin	Mean change	Percent change $\frac{\text{Mean change}}{\text{Control}} \times 100$
Bile flow (ml/min)	0.028 ± 0.005	0.047 ± 0.011	+0.019 ± 0.006	65.9 ± 11.1
Bilirubin concentration (μmoles/ml)	4.35 ± 1.26	0.94 ± 0.17	−3.42 ± 1.17	−77.52 ± 6.2
Bilirubin excretion rate (μmoles/min)	0.123 ± 0.002	0.042 ± 0.019	−0.075 ± 0.025	−61.3 ± 14.8
^{14}C-bile acid concentration (μmoles/ml)	60.96 ± 19.6	35.35 ± 10.4	−25.61 ± 9.26	−41.6 ± 2.14
Bile acid excretion rate (μmoles/min)	1.75 ± 0.62	1.76 ± 0.73	0.01 ± 0.12	−1.97 ± 9.2

* Each value is the mean ± S.D. for three rats.

animals.

It is reasonable to conclude that the specific effect of nafcillin is on the transport of bilirubin conjugates from the liver cell across the canalicular membrane into bile. This transport step is thought to be carrier mediated and separate from the transport of bile acids [8–10]. The independence of the pathways for the canalicular transport of bile acids and bilirubin should be distinguished from events that can cause a severe reduction in canalicular bile flow which, in principle, can lead to secondary effects on organic anion excretion [11].

With increasing use of a variety of therapeutic agents in individuals with complex illnesses, it is important to distinguish competitive effects on transport systems that can lead to elevations in serum bilirubin and/or bile acid levels from liver cell necrosis which can also cause these same changes as part of a generalized loss in cell function. Knowledge of the specific effects of drugs by the techniques used in these and other studies can be useful in analyzing the problem.

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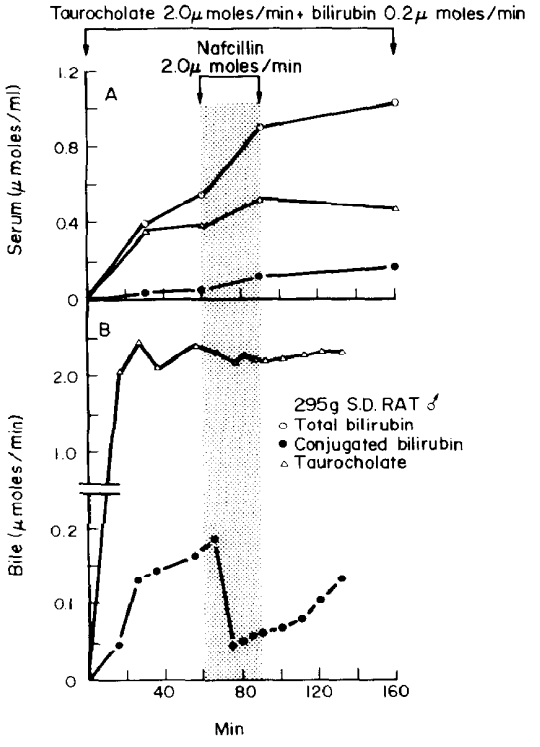

Fig. 1. Effects of nafcillin on bile acid and bilirubin excretion. The upper panel (A) illustrates changes in plasma concentration. The lower panel (B) illustrates rates of excretion in bile during and after infusion of nafcillin.

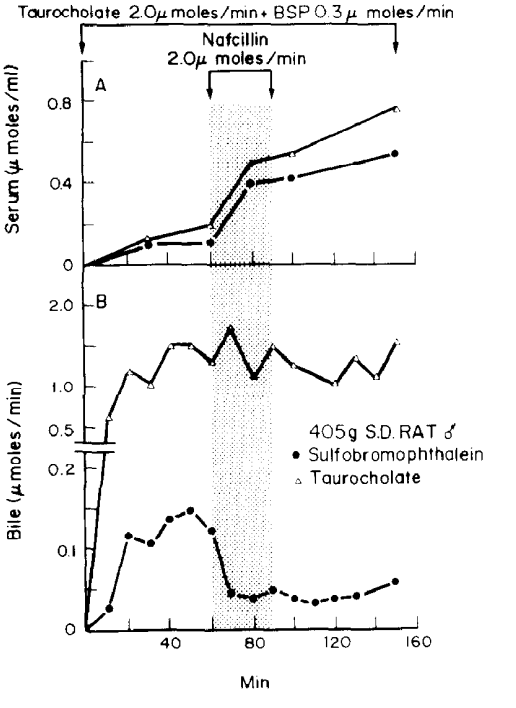

Fig. 2. Effects of nafcillin on bile acid and BSP excretion. The upper panel (A) illustrates changes in plasma concentration. The lower panel (B) illustrates rates of excretion in bile during and after infusion of nafcillin.

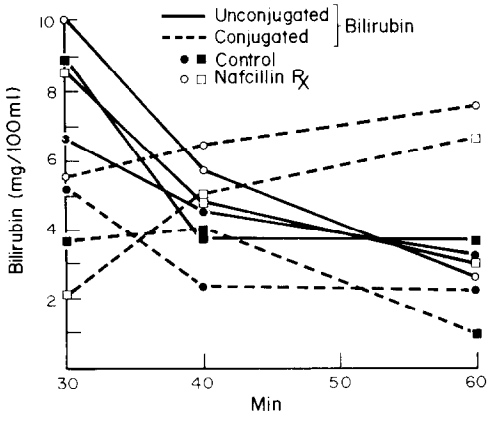

Fig. 3. Effects of nafcillin on plasma bilirubin disappearance rate. Each of two rats (circle and square) received bilirubin as two, 10 mg single intravenous boluses given 24 hr apart. Nafcillin (100 mg) was given on one occasion 30 min following a bilirubin injection.

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REFERENCES

1. P. Berk and N. Javitt, *Am. J. Med.* **64**, 311 (1978).
2. N. B. Javitt, T. Kondo and K. Kuchiba, *Gastroenterology* **75**, 931 (1978).
3. J. G. Douglas, G. J. Beckett, I. A. Nimmo, N. D. C. Finlayson and I. W. Percy-Robb, *Eur. J. clin. Invest.* **11**, 421 (1981).
4. G. Green and J. Geraci, *Proc. Staff Meet. Mayo Clin.* **40**, 700 (1965).
5. N. B. Javitt and S. Emerman, *J. clin. Invest.* **47**, 1002 (1968).
6. H. Malloy and K. Evelyn, *J. biol. Chem.* **119**, 481 (1937).
7. D. Seligson, J. Marino and E. Dodson, *Clin. Chem.* **3**, 638 (1957).
8. B. F. Scharschmidt, J. G. Waggoner and P. D. Berk, *J. clin. Invest.* **56**, 1280 (1975).
9. S. Erlanger and M. Dumont, in *The Liver. Quantitative Aspects of Structure and Function* (Eds. G. Paumgartner and R. Preisig), pp. 306–14. Karger, Basel (1973).
10. J. Barnhart, D. Ritter, D. Ware and B. Combes, in *The Liver. Quantitative Aspects of Structure and Function* (Eds. G. Paumgartner and R. Preisig), pp. 315–25. Karger, Basel (1973).
11. C. A. Goresky, H. H. Haddad, W. S. Kluger, B. E. Nadeau and G. G. Bach, *Can. J. Physiol. Pharmac.* **52**, 389 (1974).

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Differences between cyclopyrrolones (suriclone and zopiclone) and benzodiazepines binding to rat hippocampus photolabelled membranes

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Since the discovery of benzodiazepine (BZD) receptors, it has generally been accepted that pharmacological actions of BZD are mediated through their interaction with their receptors. Some other drugs such as the two cyclopyrrolone derivatives, zopiclone (ZPC) and suriclone (SRC) which possess the same pharmacological profile but are chemically unrelated to BZD, have more recently been shown to have probably a similar mode of action [1, 2]. Moreover Ro 15-1788, a BZD derivative which antagonizes the central effect of classical BZD drugs has been revealed as a very interesting tool for studying interactions of BZD with their receptors. Indeed the nature of interaction is probably quite different in the case of BZD agonists on one hand and Ro 15-1788 on the other: in contrast to agonists, the affinity of the antagonist is neither increased in the presence of GABA nor decreased after photolabelling membranes with flunitrazepam (FLU) [3, 4]. It has, therefore, been assumed that the binding of the antagonist is not influenced by the conformational changes induced by GABA or photolabelling. Similar properties have been found for BZD antagonists of other chemical families such as β-carboline derivatives or the pyrazoloquinolinone CGS 8216 [5, 7]. Consequently it has been postulated that such differences could permit the distinction *in vitro* between agonists and antagonists.

However, Brown [8] recently found that the affinity of the two pyrazoloquinolinones CGS 9896 and CGS 9895, which have BZD agonist and partial agonist properties respectively [9], is not modified after photolabelling. These latter results indicate that affinity changes do not predict reliably pharmacological activity. With these conflicting results at hand it was particularly interesting to examine if the hypothesis linking agonist properties and large changes in affinity after photolabelling could nevertheless be validated using agonist drugs such as ZPC and SRC of a quite new and original chemical family.

Methods and results

Rat hippocampus membranes prepared as previously described [2] were labelled with non-radioactive FLU (3 nM, 10 mn, UV) and thereafter extensively washed (3 times) by centrifugation. in control experiments we made