Biochemical Pharmacology, Vol. 35, No. 24, pp. 4519–4522, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

EFFECTS OF THREE H_2 ANTAGONISTS ON THE ISOLATED PERFUSED RAT LIVER

CORRELATION OF BILE FLOW CHANGES WITH POTENTIAL FOR CAUSING HEPATIC DISEASE IN PATIENTS

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(*Received* 11 *December* 1985; *accepted* 9 *June* 1986)

Abstract—The adverse effects of oxmetidine, an H_2 blocking agent which has been shown to produce hepatic injury in 1–4% of patients, on an *in vitro* model were compared with those of cimetidine and ranitidine which have led to only rare instances of hepatic injury. Bile flow was measured in the isolated perfused rat liver (Wistar rats), comparing the effects of each of the three drugs with control perfusions. Oxmetidine in concentrations of 3×10^{-3} M or greater led to a decrease in bile flow within 15 min and, at a concentration of 5×10^{-3} M, to complete cessation of flow within 5 min. Lower concentrations (5×10^{-4} M) led to a marked choleresis. Ranitidine and cimetidine in concentrations up to 5×10^{-3} M produced no decrease in bile flow. Ranitidine, however, led to a choleresis at a concentration of 5×10^{-3} M. The positive correlation between *in vivo* and *in vitro* toxicity supports the view that *in vitro* testing may prove to be of use in predicting the hepatotoxic potential of a drug.

The overall frequency of liver injury in humans following the use of histamine receptor antagonists of the H_2 type, that are in clinical use, appears to be low [1, 2]. Widespread use of cimetidine and ranitidine for the treatment of patients with duodenal ulcer, gastrinomas and other hypersecretory states has led to few relatively convincing reports of hepatic injury [3–14]. Oxmetidine during clinical trials, however, has been found to lead to significant elevations of the AST‖ and ALT in 1.4 to 4.0% of the treated patients [15] and to overt hepatocellular injury, leading to its withdrawal from clinical trial.

The differing potential for hepatic injury of these three drugs in patients, reflected by these data, is paralleled by the effect of the drugs in an *in vitro* model [16, 17]. Studies in our [16] and other laboratories [17, 18] have shown that oxmetidine leads to hepatic injury in isolated rat hepatocytes, whereas cimetidine and ranitidine do not produce injury in this model [16].

Several other drugs that are known to cause hepatic injury in humans as the result of idiosyncratic reaction also have been shown to injure the liver in the *in vitro* experimental models, liver cells and isolated perfused rat liver [19]. For several classes of drugs (phenothiazine, erythromycin), the *in vitro* toxicity has seemed to parallel the toxicity for humans [20].

Accordingly, the present study was undertaken to evaluate the adverse effects of these three H_2 receptor antagonists in another *in vitro* model, measurement of bile flow in the isolated perfused rat liver.

MATERIALS AND METHODS

Male, Wistar rats (275–325 g) were used as donors of livers. The animals were maintained under standard environmental and dietary conditions and were not fasted prior to the experiments.

The method used for perfusing the liver was a modification of that described by Penhos *et al.* [19]. Cannulation of the common bile duct and the portal vein was performed using a PE-10 tube (i.d.—0.010 in., o.d. = 0.024 in.) for the common bile duct and a PE-205 (i.d. = 0.062 in., o.d. = 0.082 in., Intramedic, Clay-Adams) for the portal vein. Rats were anesthesized with chloropenten (Chloropent, Fort Dodge Laboratories).

The perfusion medium was wholly synthetic and consisted of 150 ml Krebs–Henseleit buffer, pH 7.4. It contained, per 100 ml of total volume, 2.0 g of bovine albumin (95–99% Albumin, Sigma), 240 mg glucose and 3000 USP units of heparin (Calbiochem, Hoechst).

The thermostatic chamber was maintained at a temperature of 38 ± 0.5° during the experiment. The flow of the circulating medium was measured through a calibrated bypass and kept at a rate of 50–60 ml/min. The hydrostatic pressure of the portal vein was maintained at 27 cm water. Oxygenation of the buffer was maintained by a constant flow of O_2:CO_2 (95:5 vol.%) and continual mixing was provided with a magnetic stirrer. Sodium taurocholate

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‖ Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; and IPRL, isolated perfused rat liver.

(0.5 μmole/min) was infused during the entire experiment to compensate for the lack of an enterohepatic circulation of bile acids.

Oxmetidine, cimetidine and ranitidine were supplied by Smith Kline & French Laboratories. For the first 20 min, the perfusion was performed with 75 ml of the above-described perfusate for equilibration. Then, an additional 75 ml of perfusate with or without the drug was added, and the perfusion was continued for an additional 70 min. The rate of bile flow was determined by timing collections of bile into 20 μmole pipettes and calculated as μmoles/min. All results are expressed as means ± SE. Student's *t*-test was used in comparing the means, and a level of 5% or less was taken as significant.

RESULTS

The perfusate flow persisted at a constant rate (66–54 ml/min) during all experiments and was not altered by addition of any of the three drugs. Addition of oxmetidine after equilibration of bile flow for 20 min in a 10^{-3} M concentration led to decreased bile flow within 15 min. It then significantly reduced the flow by approximately 69% to 4.5 ± 0.4 μmoles by 35 min after the drug had been added. At a 5×10^{-3} M concentration, oxmetidine led to an abrupt cessation of bile flow within 5 min (Fig. 1).

Conversely, a lower concentration, 5×10^{-4} M, led to an immediate increase in bile flow, reaching a value of 28.1 ± 1.29 μl/min ($P < 0.01$) in 30 min, an 86% increase over the equilibration period flow. The significantly increased bile flow persisted over the entire experiment and was still high at 70 min after the drug had been added (Fig. 1).

Cimetidine in the same concentrations (10^{-3} M, 5×10^{-4} M) led to no significant change in bile flow (Fig. 2). Ranitidine also did not decrease the bile flow in any of the concentrations used, but it did exert a marked choleretic effect at the higher concentration of 5×10^{-3} M. Ten minutes after the drug had been added to the perfusate, the bile flow increased significantly, reaching a maximal flow in 20 min. It then gradually decreased to a flow rate similar to that of the controls (Fig. 3).

Preparation of the rats with phenobarbital for 3 days prior to killing them led to no change in the effects of ranitidine or cimetidine on the IPRL. Phenobarbital-treated rats showed a moderate increase in the bile flow of the IPRL, as predicted by the induction.

Levels of AST and ALT in the perfusate were measured after 30, 60 and 90 min. The enzymatic leakage in the circulating perfusate was similar to those of the control group.

DISCUSSION

Drugs may produce hepatic injury because they are intrinsically toxic or because the exposed individual is unusually susceptible (idiosyncrasy). The idiosyncratic reaction is presumed to be either immunologically mediated (hypersensitivity) or metabolically mediated (metabolic idiosyncrasy) [21]. Despite the importance of host susceptibility in those reactions, the different liver-damaging potential of

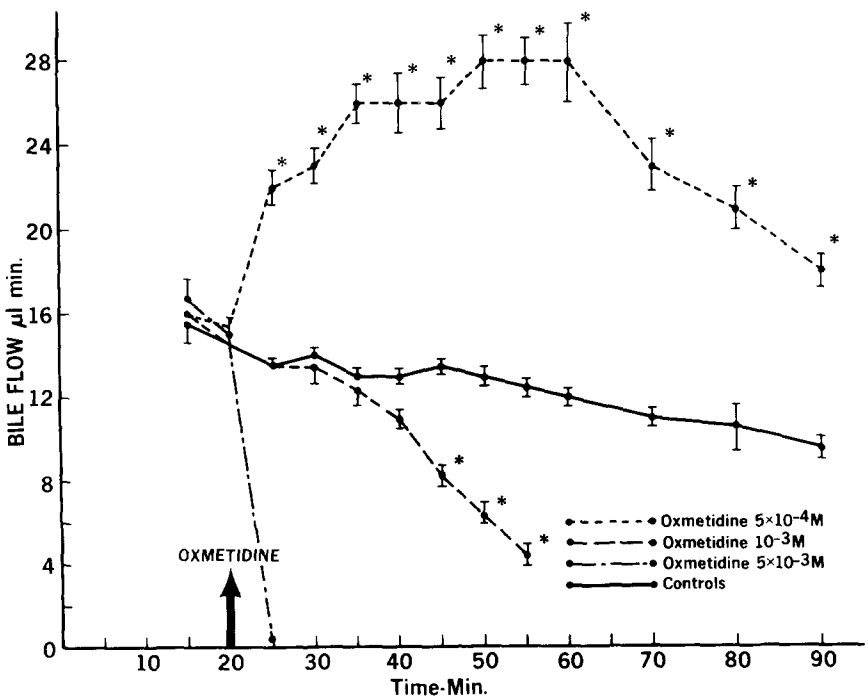

Fig. 1. Effect of oxmetidine on bile flow during perfusion of isolated rat liver (μl/min ± SE). The asterisks indicate values significantly different from controls ($P < 0.01$).

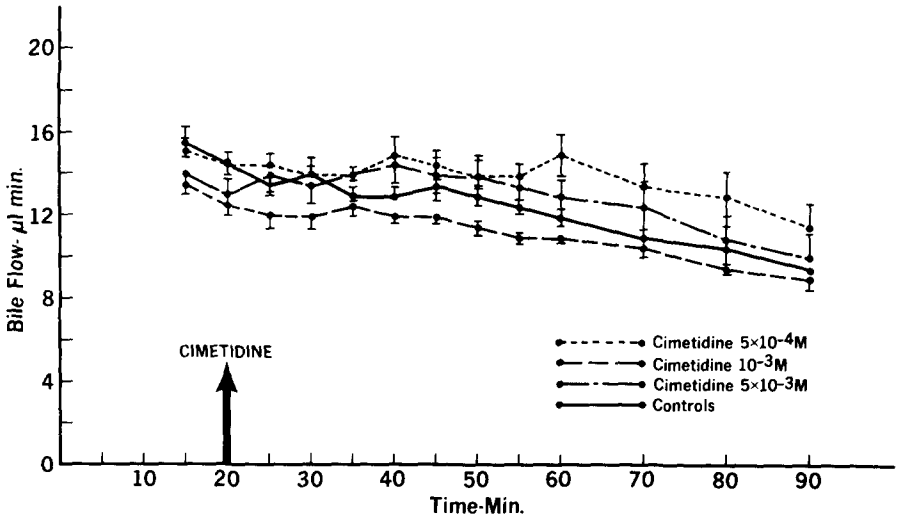

Fig. 2. Effect of cimetidine on bile flow during perfusion of isolated rat liver (µl/min ± SE).

various drugs permits the hypothesis that intrinsic toxicity of the drug plays a role, even in an idiosyncratic reaction [20].

The present study in an *in vitro* model yielded results consistent with this hypothesis. Oxmetidine, the drug most likely to cause injury in humans, had a marked cholestatic effect on the IPRL. At a 10^{-3} M concentration, it had a slight cholestatic effect, while the higher concentration (5×10^{-3} M) caused an immediate cessation of bile flow. That this was meaningful and specific for the drug is shown by the failure of cimetidine and ranitidine to have this adverse effect on the isolated perfused rat liver.

The cholestatic effect of oxmetidine cannot be attributed to altered perfusate flow, which was not affected by the drug. These and other studies [11, 12] indicate that oxmetidine has an intrinsic hepatotoxic effect *in vivo* and *in vitro*, an effect which is at

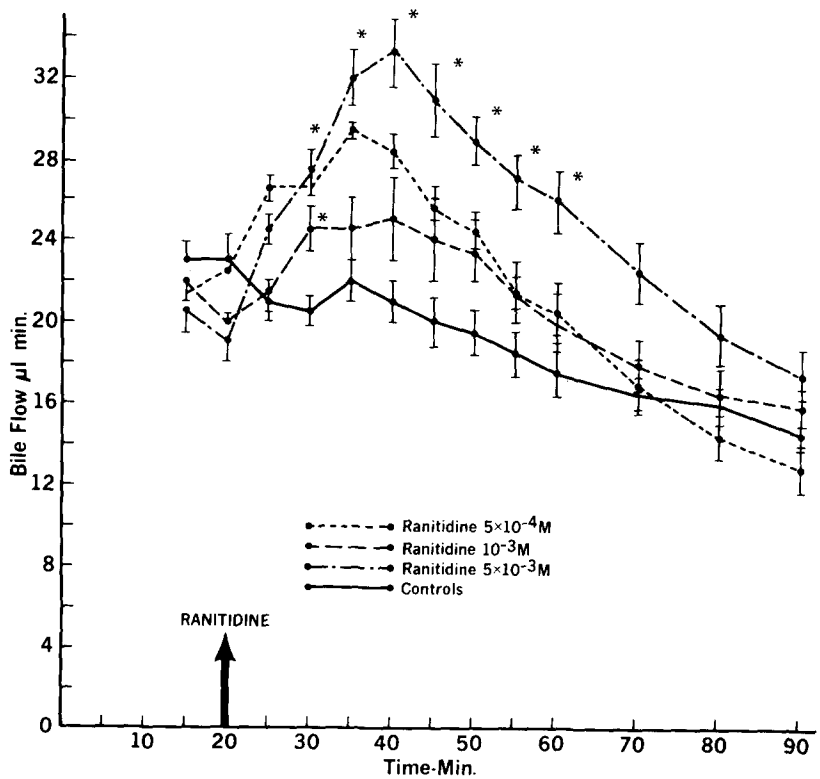

Fig. 3. Effect of ranitidine on bile flow during perfusion of isolated rat liver (µl/min ± SE). The asterisks indicate values significantly different from controls ($P < 0.05$ at 25 min; $P < 0.01$ at all other intervals).

least partially exhibited at the excretory level of the hepatocyte.

An intriguing observation was the effect of the low dose oxmetidine (5×10^{-4} M) on the bile flow. This concentration of drug increased the bile flow promptly after addition, and the increase persisted during the whole experiment. Indeed, the choleretic effect of the low concentation of oxmetidine seemed greater than that of bile acids. Presumably, the drug excreted in the canalicular bile in low concentrations acts as an osmotic agent leading to increased bile flow, whereas high concentration causes canalicular injury leading to a marked decrease of the bile flow.

A significant, choleretic effect of short duration also was produced by ranitidine at a 5×10^{-3} M concentration. While very small amounts of ranitidine are excreted in bile, almost 40% of a dose appears in bile as a metabolite. Reconciliation of this observation with the brief choleretic effect of ranitidine is difficult, however, since the induction of the cytochrome P-450 group by phenobarbital did not alter the bile flow. The relevance of the choleretic effect of oxmetidine to its hepatotoxic effects, accordingly, remains to be determined.

The results of this study support the view that it may be possible to infer potential hepatotoxic effects of some drugs in humans from their adverse effects on *in vitro* models. The available data, however, are not sufficient for *in vitro* testing to be useful, as yet, in the evaluation of new drugs.

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