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Effects of H_2-Blocking Agents on Hepatocytes *in Vitro:* Correlation with Potential for Causing Hepatic Disease in Patients (42373)

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Abstract. The adverse effects on an *in vitro* model of oxmetidine, an H_2-blocking agent which has been shown to produce hepatic injury in 1 to 4% of patients, were compared with those of cimetidine and ranitidine which have led to only rare instances of hepatic injury. Suspensions of hepatocytes, freshly isolated from Sprague–Dawley rats, were exposed to the three drugs. Oxmetidine, in concentrations of 3×10^{-3} *M* or greater, led to leakage of AST into the medium after 4 hr of incubation. Ranitidine and cimetidine, in concentrations up to 5×10^{-3} *M*, produced no identifiabile leakage. Pretreatment of rats with phenobarbital, 3-methylcholanthrene, or SKF 525A resulted in no significant enhancement or inhibition of the oxmetidine effects. These results suggest that the adverse effects of oxmetidine on the hepatocytes are produced by the native compound, not a metabolite. 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 availability of recent extensive clinical experience with two H_2-blocking agents (cimetidine and ranitidine) and experience in clinical trials with another, oxmetidine, provide data that are useful in efforts to correlate *in vivo* toxicity with injury to *in vitro* models. Cimetidine and ranitidine, which have been widely used, have led to rare instances of hepatic injury (8–12). Oxmetidine, however, was found to cause overt injury in 1 to 4% of recipients during clinical trials and was withdrawn from further testing (13). Accordingly, we have compared the effects of these three drugs on freshly isolated rat hepatocytes (14). Since these studies were undertaken, Rush *et al.* (15) and Hall and his associates (16) have reported that oxmetidine has an adverse effect on rat hepatocytes in suspension.

Materials and Methods. Male CD rats (Charles River Laboratories) were placed on a 12-hr light/12-hr dark cycle and permitted a 1-week "equilibration" period in the animal room prior to experimentations. They were given free access to food and water and were not starved prior to sacrifice. The three H_2-blocking agents and SKF 525A were provided by Smith Kline & French Laboratories (Philadelphia, Pa.).

The hepatocytes were prepared by a slight modification of the Berry–Friend method (17). After cannulation of the portal vein, the liver was perfused with Ca-free Hank's solution (pH 7.4) to remove the blood. Then, it was transferred to a perfusion chamber (37 ± 1°C), and perfused for 30 min with Hank's solution containing 0.05% collagenase. The liver capsule was then broken and the cells were suspended in Hank's solution containing 2% bovine serum albumin (BSA). The cells were strained through two layers of nylon, centrifuged at 500*g* for 5 min, and then resuspended in the buffer and centrifuged two more times to remove the enzyme and broken cells. The final pellet was gently resuspended in the buffer containing 2% BSA and diluted to provide 2×10^5 cells/ml.

The hepatocyte suspension (1.0 ml) was mixed with 1.0 ml of the drug solution (buffer only in controls) for a final hepatocyte content of 1×10^5 cells/ml, and the mixture was incubated for periods ranging from 30 min to 6 hr. At each time period, the samples were removed and centrifuged to precipitate the cells. The cells were lysed by alternately freezing (dry ice in ethanol) and thawing (37°C water bath) three times. Aliquots of the medium and cells were taken and aspartate aminotransferase (AST) levels determined by a modification (18) of the method of Karmen (19). The data shown for the three H_2-blockers are for 6-hr

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incubation periods. The paired *t* test (20) was used to compare differences between treated cells and controls.

Results. Exposure of hepatocytes to all three drugs at concentrations below 3×10^{-3} *M* resulted in no enzyme leakage. However, oxmetidine, at concentrations of 3×10^{-3} *M* and above, led to an increase of about 90% in AST activity in the medium and corresponding decrease in cell content (Fig. 1). Leakage became apparent at 4 hr and reached a peak at 6 hr. Cimetidine and ranitidine had no demonstrable effect at concentrations up to 5×10^{-3} *M* (Fig. 1).

Pretreatment of rats with phenobarbital or 3-methylcholanthrene did not appear to enhance the effect of oxmetidine (Fig. 2). Pretreatment with SKF 525A also did not alter the concentration at which leakage of enzyme occurred, but appeared to blunt the development of greater leakage at higher concentrations of the drug.

Discussion. The demonstration that oxmetidine leads to leakage of AST from cells provides evidence that the drug injures the hepatocyte. This effect is drug dependent as shown by the concentration dependence of the leakage and the failure of the two other H_2-blockers to induce leakage at the same or even higher concentrations.

It appears likely that the adverse effect of oxmetidine on hepatocytes is due to the unaltered molecule rather than to a mixed-function oxidase metabolite, despite the evidence

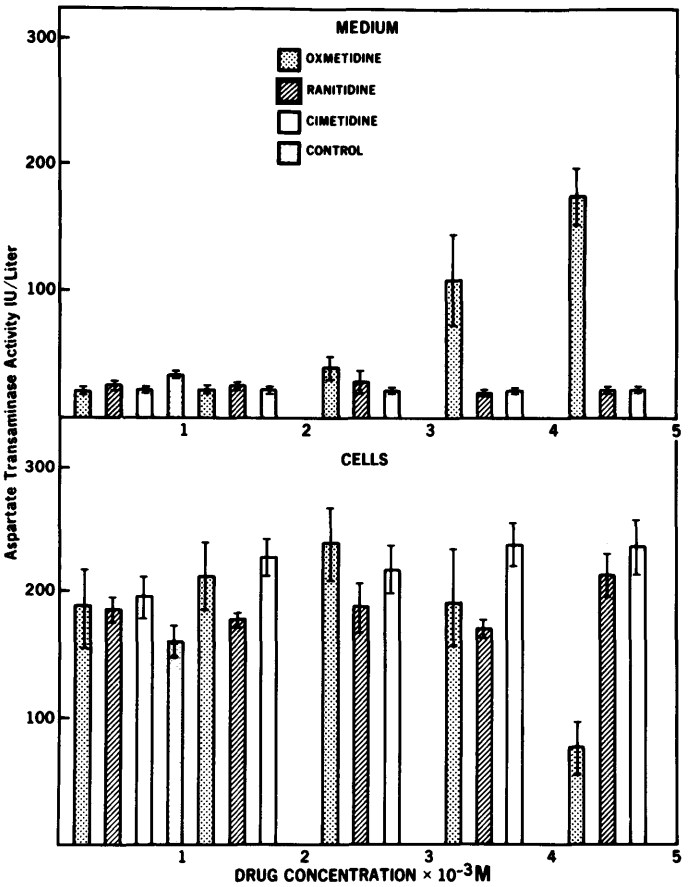

FIG. 1. Levels of AST in medium and cells after 6 hr of exposure to hepatocytes to each of these H_2-blocking agents. Note increase of level in medium at concentrations of oxmetidine of 2×10^{-3} *M* and above. Decrease in cells demonstrable only at concentrations of 10^{-3} *M*. No apparent leakage of enzyme on exposure to ranitidine or cimetidine.

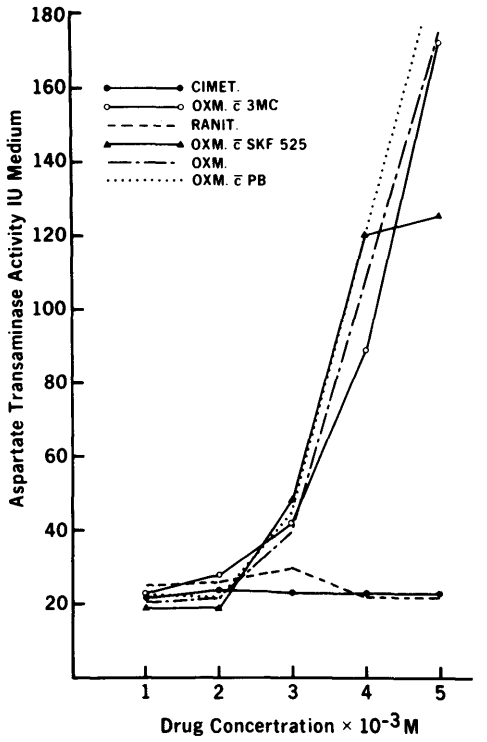

FIG. 2. Effects of oxmetidine, cimetidine, and ranitidine on asparate transaminase leakage from rat liver cells. Lack of effects of induction with 3-methylcholanthrene (3MC) and phenobarbital (PB) and of inhibition (SKF 525) on oxmetidine-induced leakage of enzyme from hepatocytes.

that there is at least some biotransformation of the drug by the cytochrome P-450 system (21). Nevertheless, induction of the cytochrome P-450 group with phenobarbital or the P-448 group with 3-methylcholanthrene did not enhance the leakage. Inhibition of the mixed-function oxidase system with SKF 525A also did not alter the concentrations at which enzyme leakage begins or the degree of leakage at that concentration. The significance of the break in the drug concentration enzyme leakage association seen after SKF 525A pretreatment is obscure.

The adverse effect of oxmetidine occurs at a relatively high concentration of the drug (3×10^{-3} *M*). This is certainly higher than the concentration one would expect to find in the blood. However, it is possible that concentration of oxmetidine by the liver could result in hepatic concentrations in this range. Furthermore, that these observations have biological meaning is supported by the fact that oxmetidine which produces hepatic injury *in vitro* has hepatotoxic effects in man, while cimetidine and ranitidine which do not produce identifiable injury in this *in vitro* model rarely produce identifiable hepatic injury in man.

Injury to the cells by oxmetidine developed after a much longer period than that produced by (CPZ) or (EE). The latter compounds lead

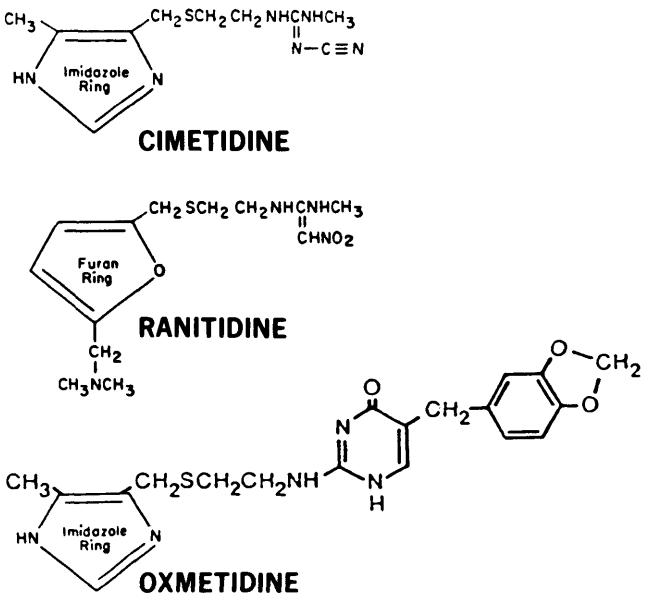

FIG. 3. Structure of three H_2 blockers studied.

to leakage by 30 min (2) while oxmetidine requires at least 4 hr of incubation before leakage becomes apparent. Since the oxmetidine injury appears not to depend on conversion to an active metabolite, some other cellular process must be responsible for the delay in effect.

The recent studies of Rush *et al.* (15) and of Hall and his associates (16) also have demonstrated injury of rat hepatocytes *in vitro* by oxmetidine in approximately the same concentration noted on these studies. The observations of Rush *et al.* (15) led them to infer that the adverse effect of the drug was expressed at the mitochondrial level, perhaps the inner membrane.

The results of this study and others conducted in this laboratory support the view that the hepatotoxic effects of some drugs in man can be inferred from their adverse effects on *in vitro* models. Accordingly, *in vitro* testing may prove to be useful in the evaluation of new drugs. The model also may be useful in examining structural features that affect hepatotoxic potential. Comparison of the structure of the three compounds tested (Fig. 3) suggests that the toxic effects are not attributable to the imidazole ring which is also in cimetidine, but to the side chain which differs from that of cimetidine and ranitidine.

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