Perfusion of the Isolated Rat Liver with Erythromycin Estolate and Other Derivatives (36345)

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The lauryl sulfate salt of the propionyl ester of erythromycin [erythromycin estolate (EE)] has long been known to cause, in some individuals, a reversible cholestatic hepatic injury (1–4). This reaction has been attributed to hypersensitivity to the drug (5–7). Studies with chlorpromazine suggest, however, that intrinsic toxicity of a drug, coupled with hypersensitivity, may be responsible for hepatic injury.

There are no useful experimental models with which one can test the possible mechanism of EE-induced hepatic injury. Early studies in intact animals have shown a very low order of toxicity for both EE and the mother-compound, erythromycin-base (EB) (8, 9), after either chronic oral administration or acute parenteral administration of large doses of the drugs.

Previous studies have shown that the isolated rat liver may be used to demonstrate adverse effects of drugs on hepatic function (10, 11). In the present report, data are presented indicating that EE also impairs function of the isolated, perfused rat liver after brief periods of exposure to the drug. Data comparing effects of several erythromycin congeners on the function of the perfused liver are also presented.

Methods and Materials. Animals. Female rats (Sprague-Dawley, mean body wt 301.8 g $\pm$ 3.6, SEM) were used as liver donors. The animals were kept under standard conditions and were not fasted prior to experimentation.

Preparation. Isolated livers (mean wet wt 10.4 g $\pm$ 0.1, SEM) were obtained according to methods previously described (12) and perfused with wholly synthetic media as reported elsewhere (10).

Drugs. Erythromycin-base (EB), estolate (EE), propionate (EP), and glucoheptonate (EG) were supplied by Eli Lilly Laboratories and lauryl sodium sulfate (SLS) was obtained from Mann Research Co.

The drugs were added to the perfused medium in two different concentrations, 2.5×10^{-4} and 5.0×10^{-6} *M*, exactly 30 min after perfusion was started. Since water solubility of these compounds is low (about 2.5×10^{-3} mole/liter for EB; 2.0×10^{-6} mole/liter for EE, or slightly less for EP) they were administered either (a) as well mixed suspension in a sample of the perfusion medium; or, for comparison, (b) as a clear solution in 0.06 ml of absolute ethanol (USP). In the latter experiments, preparations containing only this concentration of ethanol (15 m*M*) were run as controls. As there were no significant differences between the two methods of preparing the drugs for addition to the perfusate, only results obtained from treatment with a perfusate-suspended drug are reported below.

The glucoheptonate (EG) and SLS were soluble in water and were added to the perfusate as 1.0 ml of saline solutions.

Sulfobromophthalein (BSP)[1] was added 1 hr after perfusion was started in a concentration of 10 mg/100 ml of perfusate. Samples of perfusate (0.2 ml) were obtained at intervals for the determination of BSP removal rate by the liver. The rate of bile flow was measured by timing collection of bile into 20 μl pipettes. BSP assay in the perfusate and in bile was performed colorimetrical-

[1] Supplied by Hynson, Westcott and Dunning, Inc.

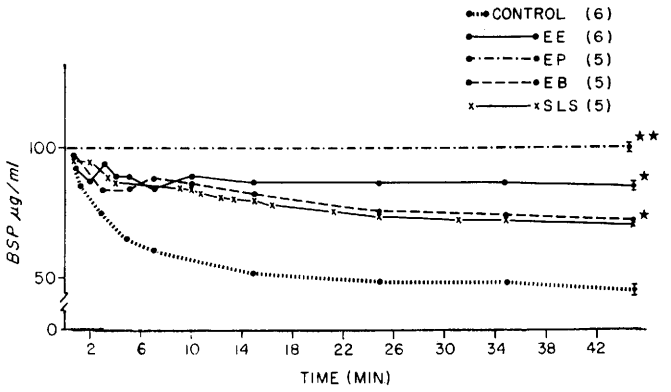

FIG. 1. BSP disappearance from the perfusate of control and drug-treated preparations: (*) significantly different from controls ($p < 0.01$); number of experiments is shown in parentheses.

ly as previously described (10). Analyses of variance and statistical tests of significance were calculated according to standard methods (13).

Results. BSP removal and excretion. Untreated, control liver preparations removed 50% of the added BSP after 20 min of perfusion (Fig. 1). At a concentration of 2.5×10^{-4} *M*, EP, EE, EB, or SLS each interfered with removal of the dye from the perfusate. The greatest inhibition was induced by EP and the least by EB. At a concentration of 5.0×10^{-6} *M*, none of the drugs interfered with dye removal by the liver. The glucoheptonate (EG) essentially acted as EB and separate results are hence not shown for this derivative.

A significant decrease in excretion of BSP also was observed after perfusing livers with all tested compounds (Fig. 2), even at the lower concentration used. At 5.0×10^{-6} *M*, the drugs still reduced significantly ($p < .01$) the biliary concentration of the dye, although these decreases were less striking than those obtained with the higher concentration of drugs (a range of 3.9–5.0 vs 0.1–1.7 mg/ml, respectively). Comparison of the drugs revealed that at 2.5×10^{-4} *M* concentration, EE or EP reduced the biliary concentration of BSP significantly more ($p < .01$) (1.7 vs 0.1 mg/ml bile, respectively) than did EB, while SLS had variable, but insignificant, effects at this concentration (not shown on the diagram).

Bile excretion. During the period of equilibration, a mean rate of 9.3 µ/min (±0.4,

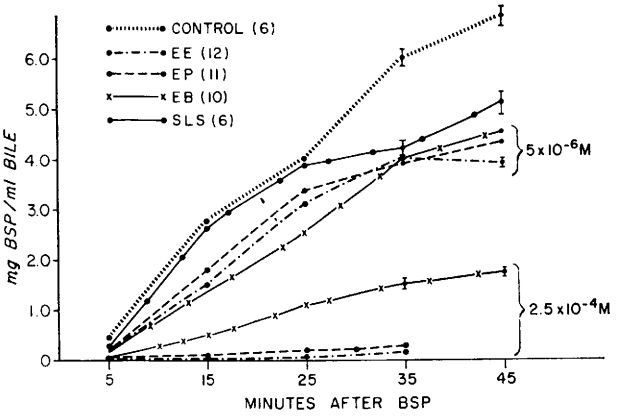

FIG. 2. Effect of two concentrations of erythromycin derivatives on the biliary concentration of BSP: (bars) SE; number of experiments is shown in parentheses.

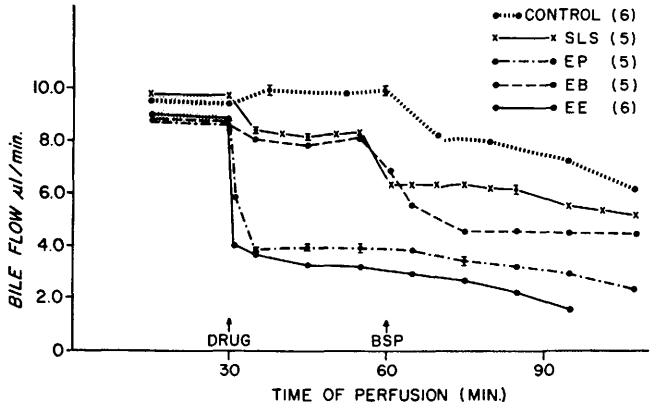

FIG. 3. Effect of erythromycin derivatives (2.5×10^{-4} *M*) on the rate of bile flow excreted by perfused livers: Number of experiments is shown in parentheses.

SEM) was recorded for all livers (Fig. 3). At the concentration of 5.0×10^{-6} *M* none of the drugs caused a significant change in the biliary rate of excretion. A concentration of 2.5×10^{-4} *M* of EE or EP led to an abrupt decrease in excretion. By 1 min after addition of drug, the rate of excretion had dropped to 3-4 µl/min and remained at this low rate of bile flow throughout the experiments. The addition of BSP changed little of this pattern. This concentration (2.5×10^{-4} M) of EB or SLS also led to a significant reduction in bile excretion, but a significantly lesser degree than those recorded for EE or EP. In the experiments with EB or SLS, addition of BSP further decreased significantly ($p < .05$) the bile output.

Rate of flow or perfusate. EP was the only one of the drugs tested which induced a significant change in the perfusate flow (Fig. 4). In a concentration of 2.5×10^{-4} *M*, it decreased the flow from a control rate of 60 to 29 ml/min within 5 min. This effect lasted for about 40 min after which perfusate flow again reached control values. A lower concentration of EP was ineffective in altering the rate of flow.

Discussion. The results of this study show that several erythromycin derivatives and SLS can significantly interfere with the function of the perfused liver. The extent of liver impairment was directly proportional to the concentration of the respective drug in the perfusate. However, the observed hepatic dysfunction was more pronounced after treatment with EP or EE than with EB or EG. This was apparent in the rate of BSP removal from the perfusate and its excretion in bile and in the rate of bile flow. While altered perfusate flow induced by EP at the higher concentration might have contributed to the decrease in excretory ability of the liver induced by this drug, adverse effects of the other drugs (EE and EB) could not be attributed to an effect on perfusate flow. Moreover, at the lower concentration used (5.0×10^{-6} *M*) there were no changes in perfusate flow induced by any of the drugs, yet all caused a reduction of BSP biliary concentrations.

These concentrations of the drugs are in the range (2.5×10^{-6} *M*) of those that have been demonstrated in the serum of patients receiving erythromycin in oral therapeutic doses (14). Animal studies indicate (15) that the concentration in the liver is approximately 150 times that in the serum, *i.e.*, approximately 5.0×10^{-4} *M*. The latter concentration of erythromycin derivatives in the perfusate led to decreased flow of bile, decreased excretion of BSP and decreased clearance of dye from the perfusate. The mechanisms for the adverse effect of erythromycin remain speculative. Since it is excreted in the bile, its effect may be that of interference with excretion of bile constituents and foreign dyes. Interference with transport of bile constituents into the bile would be ex-

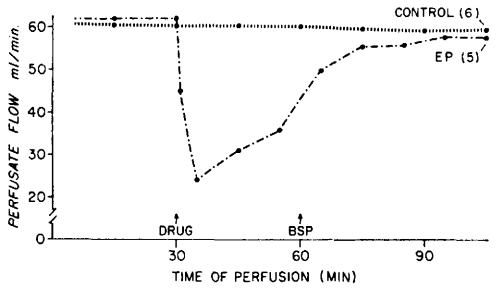

FIG. 4. Rate of perfusate flow in livers treated with erythromycin propionate (2.5×10^{-4} *M*): Number of experiments is shown in parentheses.

pected to lead to decreased bile flow. Differences in the physiochemical characteristics of the erythromycin derivatives may be responsible for the observed differences in adverse effects.

Of the drugs studied in these experiments EE is the only one that has been etiologically related to clinical instances of jaundice (6, 7). EB has not been reported as a causative agent of liver dysfunction. Accordingly, a clinical sensitization mechanism based on the presence of a propionyl radical has been proposed (16).

Studies of hepatic injury caused by other drugs (*e.g.*, chlorpromazine) (11) have led to the suggestion that apparently hypersensitivity-induced liver disease may represent an adverse effect by the respective drug on the liver, that translates generalized hypersensitivity into frank hepatic injury. Observations of the present study are consistent with this suggestion since EE has a greater adverse effect than does EB on function of the perfused liver. Mild hepatotoxicity of EE may be responsible for impaired liver function observed in patients taking the drug. When generalized hypersensitivity occurs, it may intensify the mild injury and lead to jaundice. EP, however, despite its adverse effect on the function of the perfused liver has not been reported to produce jaundice in patients. Accordingly, the parallel proposed and its significance, between the propensity of an erythromycin derivative for the production of hepatic injury in a clinical setting and its effect on the perfused liver, remains to be determined.

Summary. Several erythromycin derivatives were tested for their effect on the function of the isolated, perfused rat liver. Erythromycin estolate inhibited bile output, sulfobromophthalein (BSP) removal from the perfusate and the biliary excretion of this dye. The propionate congener also induced a significant decrease in these parameters, at least as great as that caused by estolate. Other derivatives showed lesser effects on these parameters of liver function. Even at drug concentrations so low that rate of bile flow and removal of BSP were unaffected, all derivatives decreased BSP biliary excretion.

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