Studies on the *in Vitro* Cytotoxicity of Erythromycin Estolate (37676)

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Erythromycin estolate (EE) has been found to produce hepatic injury and jaundice in some patients (1–4), an adverse reaction attributed to hypersensitivity to the drug (5, 6). Studies of chlorpromazine-induced hepatic injury, also assumed to reflect hypersensitivity to that drug, have led us (7–10) to suggest that this form of drug-induced hepatic injury is the result of slight intrinsic toxicity which when coupled with generalized hypersensitivity leads to clinically evident liver disease.

Several *in vitro* models have been employed in the effort to demonstrate intrinsic toxicity of drugs incriminated in the production of hepatic injury. Phenothiazine derivatives have been found to lead to injury of rabbit liver slices (3) and Chang cells (1, 2), and to lead to impaired function of the *ex vivo* perfused liver (10, 11). Previous studies have shown that perfusion of the isolated liver with erythromycin derivatives also leads to inhibition of bile production and to impaired bromsulfophthalein excretion (12). The present studies were undertaken to examine the relative cytotoxicity for Chang "liver" cells of EE, a drug known to produce hepatic injury in patients, with that of erythromycin base (EB) and erythromycin propionate (EP), which have not been observed to cause liver injury in humans (13).

Materials and Methods. Chang human liver cells, harvested from tissue culture layers were obtained from Microbiological Associates, Bethesda, MD. The experiments were carried out within 1 hr after harvesting of cells, using methods previously described (9). To 10^6 cells suspended in 1.0 ml of normal saline was added 1.0 ml of saline (controls); 1.0 ml of ethanol in saline (USP) calculated to give a concentration of 1.7 mM/ml (ethanol controls); or 1.0 ml of an ethanol solution of one of the three drugs (EE, EP and EB) in concentrations ranging from 5.0×10^{-6} to 5.0×10^{-4} M. The ethanol concentration of the solutions of drugs was kept constant and identical to that of controls. Following 30 min incubation of samples at 37°, the cells were centrifuged and the supernate was assayed for enzyme activities. Asparate aminotransferase (GOT), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were determined according to standard methods (9).

Data were analyzed for statistical variability and Student's t was used to test significance of differences between means (14).

Results. Incubation of cells in 1.7 mM concentrates of ethanol resulted in only slightly greater leakage of enzymes than that which occurred on incubation in saline (Fig. 1). At this concentration accordingly, it has been regarded as an "indifferent" vehicle of the drugs. At a high concentration (5.0×10^{-4} M) all three erythromycin derivatives led to significant "leakage" of intracellular enzymes into the medium. However, the effect of EE as measured by leakage of GOT and MDH, was significantly, and much, greater than that of the other derivatives. For GOT and MDH the effect of EE was dose-related. Even at a concentration of 5×10^{-6} M of EE there was a small but significant, adverse effect as reflected by the MDH leakage into the medium. "Leakage" of LDH was obscured by the inhibitory effect of EE at the 5×10^{-4} M concentration. (In separate experiments, the inhibition of LDH by this concentration of EE was demonstrated to be approximately 35%, employing puri-

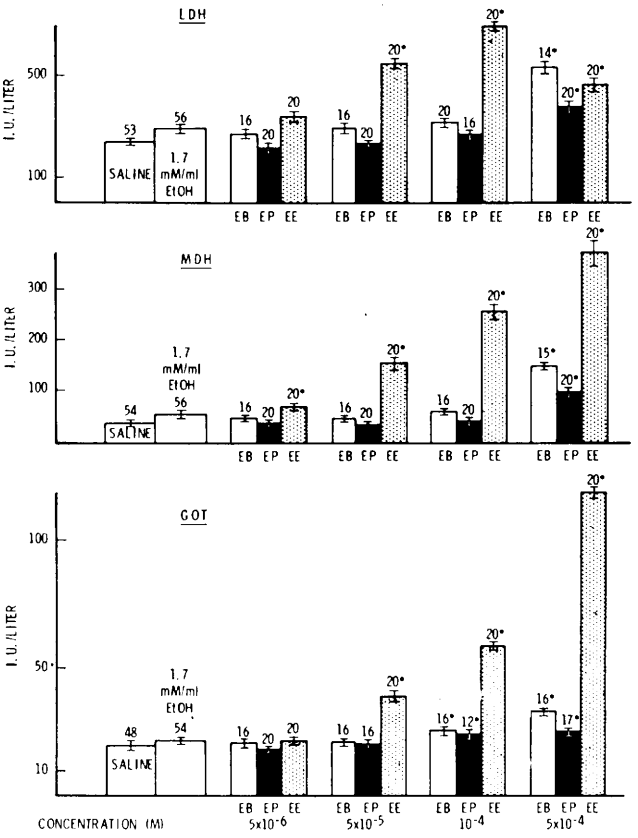

FIG. 1. Effects of erythromycin estolate (EE), erythromycin propionate (EP) and erythromycin base (EB) on suspensions of Chang liver cells as reflected by leakage of enzyme into medium. Asterisk at top of bar indicates significant differences from saline and ethanol (ETOH)-exposed controls.

fied LDH preparations obtained from Worthington Co.). No adverse effects on the cells were observed using EP or EB in concentration lower than 5×10^{-4} *M*.

Discussion. Leakage of cellular enzymes into the surrounding medium has been employed extensively as evidence of cellular injury. Previous studies from this laboratory have demonstrated adverse effects of phenothiazine derivatives on Chang cells (8, 9) and on the *ex vivo* perfused liver (10, 11) which appear to parallel hepatotoxicity for humans. The demonstration in the present study, that EE has more cytotoxic potential than the other two erythromycin derivatives for Chang cells is in agreement with the reports of Dujovne *et al.* (13). It also parallels the clinical observation that EE is the only erythromycin derivative known to produce jaundice in patients and the one that interferes most with function of the perfused rat liver (12).

These results are consistent with the hypothesis (15) that hepatic injury that appears attributable to hypersensitivity also depends on a directly adverse effect of the respective drug on the liver and that this adverse effect can be demonstrated in *in vitro* models.

Summary. Erythromycin estolate (EE) in concentration as low as 5×10^{-6} *M* led to the leakage of enzymes from Chang liver cells exposed to the drug. Two other erythromycin derivatives (propionate and base), which differ from EE in that they do not produce hepatic injury in patients, did not lead to leakage of enzyme from the Chang cells until concentrations of these two de-

rivatives reached 5×10^{-4} *M*.

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