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Induction of Hepatic Alkaline Phosphatase by Colchicine: Role of Cholestasis and Bile Acids (41636)

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Abstract. We studied the induction of hepatic alkaline phosphatase by colchicine, a drug which impairs bile flow. By analogy to bile duct obstruction, where retained bile acids induce alkaline phosphatase, we hypothesized that the mechanism of induction involves the drug's cholestatic property and subsequent intrahepatic retention of bile acids. After administering colchicine to bile-fistula rats, we monitored bile flow. In colchicine-treated rats which did not develop cholestasis, we varied the transhepatic flux of taurocholate 15-fold over the physiologic range in order to study the role of intrahepatic bile acids. Induction of alkaline phosphatase was observed when cholestasis was absent and the bile acid flux was 5% of normal. The magnitude of enzyme induction was not augmented by increasing the transhepatic flux of taurocholate. The results demonstrate that colchicine induces hepatic alkaline phosphatase by a mechanism which operates independently of cholestasis and is not modified by the level of bile acid in the liver.

Colchicine increases serum alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) in patients with cirrhosis (1) and induces hepatic alkaline phosphatase in rats (2, 3). Although the mechanism(s) responsible for these alterations is not known, recent evidence has shown that colchicine is cholestatic—it inhibits bile acid secretion *in vivo* after bile acid challenge (4) and *in vitro* (5). Since bile acids retained in the liver are responsible for induction of alkaline phosphatase observed during the severe cholestasis of bile duct obstruction (6), we hypothesized that elevated intrahepatic levels of bile acids trigger the alkaline phosphatase increase noted after colchicine treatment. Support for this hypothesis would allow generalization to the many other stimuli of liver alkaline phosphatase (7).

Materials and Methods. Fasted, male Sprague–Dawley rats weighing between 200 and 300 g were used. With the rats under light ether anesthesia, we placed a PE-10 catheter via the jugular vein into the superior vena cava, cannulated the bile duct with a 43-cm segment of the tubing, and secured a third piece of tubing with one end free in the peritoneal space. The latter two tubes were tunneled subcutaneously to emerge near the tail. Operations were performed in the morning. After surgery the rats were placed in Bollman-type restraining cages and had free access to water. Bile was drained for 4–6 hr to deplete the bile acid pool (8), and 0.9% NaCl was given iv to replace the volume of bile collected. The experiments were begun after this period of drainage. Each animal was randomized to receive either colchicine (0.1 mg/100 g body weight) or the carrier (0.9% NaCl) ip. The colchicine-treated group was further subdivided to receive a constant iv infusion of taurocholic acid (Calbiochem-Behring, La Jolla, Calif.) at one of three rates: 0, 100, or 200 nmole/min/100 g body weight. Animals not treated with colchicine were given either 0 or 200 nmole/min/100 g body weight. Taurocholate was dissolved in 0.45% NaCl–0.5% albumin, pH 7.5. The taurocholate infusion continued for the next 20 hr during which time all bile was collected. Rats which developed cholestasis, defined as 50% or greater decrease in bile secretion compared to pretreatment flow, were excluded from the study.

After 20 hr, the rats were exsanguinated by cardiac puncture, and the chest was rapidly opened. The intrathoracic inferior vena cava was cannulated. The liver was flushed with 30 ml of ice-cold 0.9% NaCl after we clamped the inferior vena cava above the right renal vein and cut the portal vein. The liver was excised, weighed, and minced over ice. To extract alkaline phosphatase, 1 g of the minced liver was homogenized with 4 ml of 0.25 *M* sucrose–0.01 *M* Tris (pH 7.4) in a ground-

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glass homogenizer. 1.25 ml *n*-butanol (9) was added to the homogenate; the tubes were kept on ice and vortexed every 5 min for 30 min. After centrifugation for 30 min at 20,000*g* and 4°C, the aqueous layers were removed and frozen at −20°C until assayed. Plasma was separated from the heparinized blood by centrifugation and stored at −20°C.

In a companion study using unoperated rats, colchicine (0.1 mg/100 g body weight) or 0.9% NaCl was injected ip. After 20 hr, the animals were exsanguinated, and the livers were prepared as above.

Alkaline phosphatase was assayed by the method of Ohkubo *et al.* (10). One international unit of activity represented the hydrolysis of 1 μmole of substrate/min. The bile acid content of bile was measured enzymatically (11). Protein was determined by the Lowry method, using bovine serum albumin as standard (12). Results are expressed as mean ± SE. The significance of differences among the means for various groups was analyzed with the nonpaired Student's *t* test.

Results. In unoperated rats, colchicine caused an increase in hepatic alkaline phosphatase activity from 8 ± 1 to 34 ± 6 mIU/mg protein ($P < 0.005$, $n = 6$ and 7, respectively). All subsequent results refer to operated rats fitted with bile duct cannulae.

After initial drainage of the bile acid pool, biliary secretion of bile acids remained low during the 20-hr study period (Table I, groups I and III). This output equals about 5% of the normal secretion rate (8) and can be attributed to reabsorption of the small amount of bile acid remaining in the gut as well as to ongoing synthesis of bile acids by the liver. Quantitatively complete secretion of infused bile acid was documented in those groups receiving taurocholate: II, IV, and V. The secretion rates found in groups II and V represent about 70% of the normal bile acid secretion rate (8). Higher infusion rates of bile acid were not investigated because preliminary studies had revealed that, after treatment with colchicine, such rates caused cholestasis. As it was, in the present work, four of nine colchicine-treated rats receiving the largest dose of taurocholate developed cholestasis, as did one of six rats in each of the two other colchicine-treated groups. None of the cholestatic animals was included in the analysis. This decision was made prospectively at the time of designing the study because we anticipated cholestasis (4, 5) and had observed it in our preliminary work. Cholestasis was not observed in control animals treated with colchicine-free carrier.

In rats not treated with colchicine (groups I and II), bile acid treatment alone caused no enzyme induction. In the presence of low rates of bile acid secretion (groups I and III), colchicine caused a threefold rise in hepatic alkaline phosphatase. Among colchicine-treated groups, increasing the rate of bile acid infu-

TABLE I. BILE ACID SECRETION AND ALKALINE PHOSPHATASE ACTIVITIES AFTER ADMINISTRATION OF COLCHICINE[a]

Group	Colchicine	Taurocholate infusion (nmole/min/100 g body wt)	Bile acid secretion (nmole/min/100 g body wt)	Hepatic alkaline phosphatase (mIU/mg protein)	Plasma alkaline phosphatase (mIU/ml)
I	No	0	22 ± 4	24 ± 7	69 ± 10
II	No	200	217 ± 13	21 ± 4	71 ± 8
III	Yes	0	12 ± 3	74 ± 10[b]	189 ± 56[d]
IV	Yes	100	124 ± 4	75 ± 7	188 ± 36
V	Yes	200	196 ± 7	76 ± 16[c]	199 ± 25[e]

[a] Rats were given colchicine ip (0.1 mg/100 g body wt) after the bile acid pool had been drained via biliary cannulae. Taurocholic acid was then infused iv for 20 hr as bile was collected. Animals which developed cholestasis, <50% of pretreatment flow, were excluded. After 20 hr, plasma was obtained, and alkaline phosphatase was extracted from the liver. Each group is composed of five animals; values are the mean ± SE.

[b] Compared to value for group I, $P < 0.005$.

[c] Compared to value for group II, $P < 0.02$.

[d] Compared to value for group I, $P < 0.10$.

[e] Compared to value for group II, $P < 0.005$.

sion did not increase alkaline phosphatase induction (groups III, IV, and V). Plasma alkaline phosphatase behaved similarly: colchicine caused a threefold rise which was unaltered by bile acid treatment.

Discussion. The induction of alkaline phosphatase observed in unoperated rats confirms previous reports (2, 3). To study the mechanism of this phenomenon, we needed to monitor bile flow because a decrease in bile flow (cholestasis) from any cause is known to induce hepatic alkaline phosphatase (13) and colchicine is known to be cholestatic (4, 5). By performing our study on operated rats with bile duct cannulae, we eliminated the uncontrolled variable of drug-induced cholestasis. Dubin and colleagues discuss possible mechanisms of colchicine-induced cholestasis (4); the present studies do not clarify these possibilities.

These results prove that the induction of hepatic alkaline phosphatase by colchicine occurs in the absence of cholestasis. Furthermore, because all bile acids secreted into bile pass through the liver, the intrahepatic level of bile acids during the experiment must have been proportional to their rate of secretion into bile. Thus, the data indicate that intrahepatic taurocholate does not affect the enzyme's induction. We cannot explain the failure of the 15-fold difference in bile acid levels to affect enzyme induction but suspect the explanation involves the maintenance of bile flow in the present study in contrast to the situation with bile duct obstruction (6). Also, the subcellular distribution of intrahepatocytic bile acids was not studied. Current methods such as equilibrium dialysis allow estimation only of such distribution (14). Possibly, bile acids are distributed differently in normal, colchicine-treated, and obstructed rats; this may explain different patterns of alkaline phosphatase behavior. We conclude that colchicine induces alkaline phosphatase activity by a mechanism which is independent of cholestasis and does not require the presence of bile acids.

A prominent effect of colchicine is to disrupt the microtubular network of the cytoskeleton, a process thought to account for impaired hepatic secretion of very-low-density lipoproteins (15, 16). Similarly, and more pertinently, colchicine blocks the translocation of hepatic alkaline phosphatase from the Golgi apparatus to the plasma membrane (17). Thus, during colchicine exposure, alkaline phosphatase activity falls at the canaliculus, the area of membrane with greatest enzyme activity (18). Perhaps, the enzyme induction that is observed represents an overcorrection for this deficiency at the canaliculus, a possibility we did not address in this study.

Both colchicine and bile duct obstruction cause cholestasis. Each stimulus is associated with induction of hepatic alkaline phosphatase activity. It is evident when comparing the present results with those obtained after obstruction (6), however, that the enzyme is induced by distinct mechanisms. Therefore, we must discard the unifying hypothesis that one mechanism, intrahepatic bile acid retention, accounts for all instances of hepatic alkaline phosphatase induction.

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