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EFFECT OF COLCHICINE ON HEPATOBILIARY FUNCTION IN CCl_4 TREATED RATS

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Abstract—A number of toxic chemicals affect the biliary excretory function of liver. Organochlorines and halomethanes are known to enhance bile flow. Despite the demonstration that a diversity of agents modify biliary function, the mechanism by which these chemicals manifest this effect is not fully understood. This study was designed to assess the effect of colchicine (0.1, 1.0, or 2.5 mg/kg, i.p., in saline) administration on biliary excretory function 6 and 24 hr later. Additionally, the effect of colchicine (1 mg/kg, i.p. in saline) pretreatment in rats 2 hr prior to the administration of a single low dose of CCl_4 (100 μL/kg, i.p., in corn oil) or corn oil alone (1 mL/kg, i.p.) on hepatic biliary excretory function was also assessed at 6 and 24 hr after the last treatment. The hepatotoxicity was evaluated by serum enzymes, alanine and aspartate aminotransferases, and histopathological alterations of the liver. Biliary excretion of intravenously administered phenolphthalein glucuronide (PG) was assessed in bile duct cannulated anesthetized rats. Only the highest dose of colchicine (2.5 mg/kg) resulted in detectable liver injury as revealed by elevations of serum transaminases. While the lowest dose of colchicine (0.1 mg/kg) did not influence bile secretion, the two higher doses caused a slight choleretic effect at 24 hr. The highest dose caused a transient inhibition of bile flow, but this effect was no longer evident at 6 hr. Biliary excretion of PG was inhibited significantly by colchicine within 6 hr after administration, an effect that was also persistent at 24 hr. Colchicine at a 1 mg/kg dose did not cause any adverse effect on hepatobiliary function. Therefore, for the interactive toxicity study with CCl_4, 1 mg colchicine/kg was chosen as a moderate dose which did not cause any significant adverse effect on hepatobiliary function. Biliary excretion of PG was significantly lower in rats at 6 and 24 hr after the combination treatment with colchicine + CCl_4 than in rats receiving either CCl_4 or colchicine alone. In contrast, rats receiving CCl_4 alone or colchicine + CCl_4 showed a significant increase in cumulative bile flow at 6 hr, whereas, at 24 hr, the bile flow was increased significantly in rats receiving colchicine regardless of CCl_4 treatment. The data suggest that colchicine pretreatment leads to significant inhibition of hepatobiliary excretion in CCl_4 treated rats. Serum alanine transaminase and aspartate transaminase levels were elevated significantly after the colchicine + CCl_4 combination, indicating hepatic injury. Histological examination of the liver revealed that although CCl_4 alone caused observable liver injury at 6 hr, these rats recovered from liver injury at 24 hr. In contrast, a marked increase was evident in the number of necrotic and swollen hepatocytes in colchicine + CCl_4 treated rats, which persisted even at 24 hr after CCl_4 treatment. In conclusion, a single administration of colchicine at 0.1 or 1 mg/kg did not cause any hepatic toxicity and did not affect hepatobiliary function significantly. At 2.5 mg/kg, colchicine caused detectable liver injury and adversely affected hepatobiliary function. The combination of colchicine (1 mg/kg) and CCl_4 (100 μL/kg) at individually nontoxic doses resulted in prolongation of CCl_4 toxicity.

Colchicine has been employed in the treatment of a number of liver and other ailments [1–4]. In particular, there are reports that colchicine may be beneficial in cases of alcoholic cirrhosis [5] and possibly in primary biliary cirrhosis [6, 7]. Rojkind and Kershenobich [2] reported that oral treatment with colchicine inhibits collagen synthesis and deposition during liver malfunction in rats suffering from CCl_4-induced liver cirrhosis. Its mechanism is related to inhibition of collagen synthesis and enhancement of biosynthesis and secretion of collagenase [5, 8–10]. There is considerable interest in the disposition of colchicine in healthy and diseased liver on the one hand, and the effect of colchicine on liver function on the other. Hunter and Klaassen [11] reported that 24 hr after the administration of colchicine to rats, 56% of the administered dose is excreted in the feces as the parent compound. Experimentally induced liver injury in the rats causes impairment in colchicine elimination from the body [12]. This suggests that colchicine is processed predominantly through the liver and excreted in the bile. Colchicine is a well known microtubule inhibitor [13, 14], and there is ample evidence to indicate the need for retaining the integrity of microtubules and microfilaments for normal bile flow [15–22]. Hepatocellular microtubules and microfilaments play an important role in bile formation and biliary excretory function [21, 23, 24] as well as in maintaining the integrity of hepatocellular plasma membrane [16]. In this regard, colchicine has been shown recently to inhibit exocytosis and diacytosis at the bile canalicular level [25, 26] as well as bile canalicular motility in isolated cultured hepatocytes [22].

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Carbon tetrachloride is a hepatotoxic halomethane, capable of causing hepatocellular fatty degeneration and centrilobular necrosis [27, 28]. CCl_4-induced infliction of hepatocellular damage involves the bioactivation of CCl_4 to $\cdot CCl_3$ and $CCl_3O_2^{\cdot}$ radicals and consequent lipid peroxidation [29, 30] and other events leading to cell death. It has been reported that colchicine pretreatment ameliorates CCl_4-induced hepatic injury due to the pleiotropic effect of colchicine at different levels of cellular physiology, biochemistry and function [31–35].

Administration of colchicine (1 mg/kg, i.p.) 2 hr prior to a low dose of CCl_4 (100 μL/kg, i.p.) results in a selective ablation of the early-phase (6 hr) stimulation of hepatocellular proliferation [36, 37] without affecting the second phase (36–48 hr) of cell division [38]. Under these conditions, we found that colchicine pretreatment leads to prolongation of the limited toxicity of a low dose of CCl_4 (100 μL/kg, i.p.). Furthermore, CCl_4 autoprotection, in which the same low dose of CCl_4 given 24 hr before the administration of a large killing dose (2.5 mL/kg, i.p.) results in complete protection against the large dose, is abolished by colchicine antimitosis [38, 39]. The selective ablation of the early-phase cell division and tissue repair after the administration of a low dose of CCl_4 results in a prolongation of otherwise recoverable injury, but after an additional 24 hr complete recovery occurs owing to the unperturbed second phase (36–48 hr) of cell division and tissue repair [38]. Recent evidence indicates that CCl_4 autoprotection is due to augmented and sustained cell division and tissue repair by the protective dose of CCl_4 [40]. The fact that ablation of the early-phase cell division stimulated by the protective dose results in a denial of autoprotection [38] has provided additional support to the new mechanism advanced for CCl_4 autoprotection [40]. There is significant evidence regarding the protective effect of colchicine on alcoholic liver injury [5–7], CCl_4-induced liver cirrhosis [31–35], and galactosamine-induced hepatitis [41]. In the present study, we wished to assess the effect of colchicine at different doses on hepatobiliary function as a way of determining if and at what dose colchicine may affect liver function.

The primary objective of this investigation was to study the effect of colchicine alone at low to moderate doses on hepatobiliary function in rats. Additionally, we were interested in the effect of colchicine pretreatment at 1 mg/kg on any toxicity and hepatobiliary dysfunction caused by the administration of a low dose of CCl_4. An anionic transport model compound, viz. phenolphthalein glucuronide (PG*), which does not require any further metabolism for biliary excretion was used in order to assess hepatobiliary excretory function.

MATERIALS AND METHODS

Materials

Animals. Male Sprague–Dawley rats (200–250 g) obtained from Harlan Sprague–Dawley Inc. (Houston, TX) were maintained for 10 days on a 12-hr photoperiod in the central animal facility over untreated corn cob bedding at 21 ± 1° and 50–80% relative humidity. Water and rat food (Rodent Laboratory Chow No. 5001, Ralston Purina Co., St. Louis, MO) were available at all times *ad lib.*

Chemicals. CCl_4 was obtained from E.M. Science (Gibbstown, NJ), and colchicine and phenolphthalein glucuronide were from the Sigma Chemical Co. (St. Louis, MO). NaOH, HCl and glycine were purchased from Fisher Scientific (Baton Rouge, LA).

Methods

Treatment. In the first set of experiments, groups of four rats received colchicine at doses of 0.1, 1 or 2.5 mg/kg, i.p., in saline. The control rats received saline (1 mL/kg, i.p.). In the second set of experiments, groups of four rats were used for various treatments. CCl_4 (100 μL/kg, i.p.) was administered in corn oil (1 mL/kg) and the controls received only the corn oil vehicle. This low dose of CCl_4 is known to be subtoxic [42, 43]. Colchicine (1 mg/kg, i.p. in saline) or saline (1 mL/kg) was administered to rats 2 hr prior to the administration of corn oil or CCl_4. Colchicine (1 mg/kg) is known to be nontoxic and quite effective in blocking liver cell division, without causing any adverse side-effects [44].

In both experiments, at 6 and 24 hr after the last treatment, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Preliminary experiments established that liver function was not affected 2 hr after the administration of colchicine, while at 24 hr the highest dose did cause hepatic dysfunction. Therefore, the time points of 6 and 24 hr were chosen for detailed studies. A midventral incision was made in order to locate the bile duct at the duodenal region. The bile duct was isolated and cannulated with polyethylene (PE-10) tubing. A femoral vein was cannulated with PE-50 tubing and was used for injecting a bolus dose of PG (3 mg in 0.3 mL saline) [45, 46]. The rectal temperature of the animal was monitored throughout the duration of the experiments and maintained at 37° by means of heating pads and by means of a thermostatically controlled heating lamp adjusted to maintain the rectal temperature at 37° by means of a thermistor probe.

Bile samples were collected in 2.5-mL graduated centrifuge tubes at 15-min intervals over a period of 1 hr. The volume of bile excreted was recorded [45, 46]. At the end of the study, blood was collected from the dorsal aorta of the rats. The blood was used for the estimation of serum enzymes, alanine and aspartate aminotransferase (ALT and AST), by the method of Reitman and Frankel [47], using Sigma Kit 505-OP. The liver was excised and cut into slices in buffered fixative and processed for histopathology.

Histopathology. For histopathology, liver sections were fixed in 10% formaldehyde, embedded in paraffin wax, and sectioned. The sections were stained with hematoxylin and eosin (H & E) for light microscopy. Sections (5 μm) from two separate lobes

* Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; and PG, phenolphthalein glucuronide.

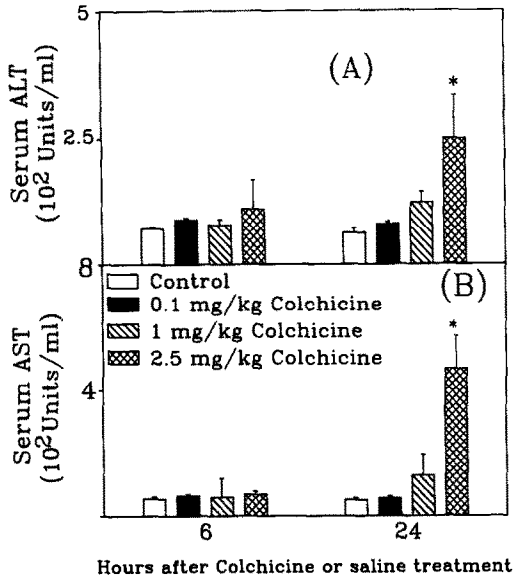

Fig. 1. Serum alanine (A) and aspartate (B) aminotransferase levels at 6 and 24 hr after administration of either colchicine (0.1 or 1.0 or 2.5 mg/kg, i.p., in saline) or saline (1 mL/kg, i.p.) alone to rats. Values are means ± SEM for four rats. Key: (*) significantly ($P < 0.05$) higher value compared to control as well as to 0.1 and 1 mg/kg colchicine treated rats.

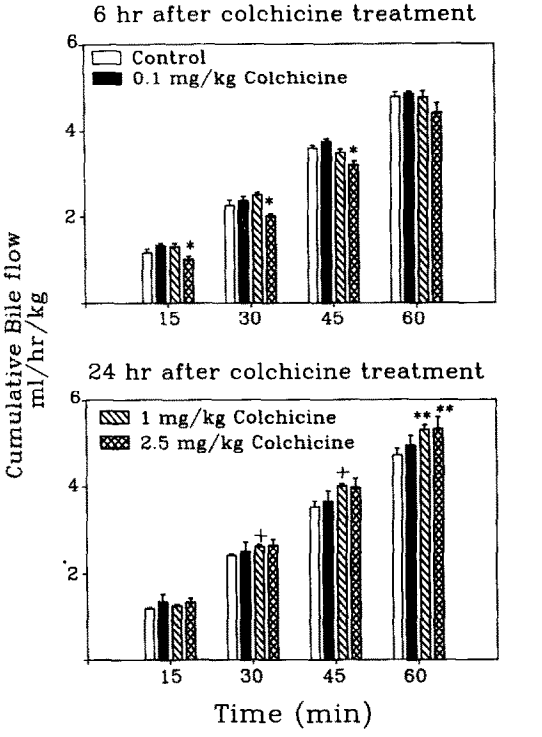

Fig. 2. Effect of colchicine administration (0.1 or 1.0 or 2.5 mg/kg, i.p., in saline) or saline (1 mL/kg, i.p.) alone on cumulative bile flow at 6 and 24 hr after treatment. Values are means ± SEM of four rats. Key: (*) significantly ($P < 0.05$) lower value compared to control, 0.1 and 1 mg/kg colchicine; (+) significantly ($P < 0.05$) higher value compared to control at the same time point, and (**) significantly ($P < 0.05$) higher value compared to control and 0.1 mg colchicine/kg.

of liver tissue from each rat were examined for histopathological alterations including ballooned (swollen) and necrotic hepatocytes.

Estimation of PG in bile. PG was assayed according to the method of Gustafson and Benet [48] as previously described [45, 46]. A 0.1-mL aliquot of bile from each sample was treated with 0.1 mL of 8 N HCl heated in a boiling water bath at 100° for 1 hr to release the aglycone phenolphthalein from the glucuronide conjugate. Upon cooling, 0.1 mL of 5 N NaOH was added to partially neutralize the hydrolysate. Alkalinization was performed by the addition of 5 mL of 0.4 N glycine-NaOH buffer, pH 10.4. After a 24-hr incubation in the dark, absorbances were read at 550 nm in a Gilford-Response™ 2200 model spectrophotometer.

Statistics. The values in the figures are the means ± SEM of four rats. Differences between any treatment group and its appropriate control were determined using the Newman–Keuls test and the Duncan multiple-range test. A statistical criterion of $P < 0.05$ was used for significance of differences between means.

RESULTS

To assess the dose-dependent effect of colchicine and the influence of colchicine in CCl_4 toxicity, the serum enzymes ALT and AST, liver histopathological changes and biliary excretory function were determined. In the first set of experiments, we evaluated the effect of 0.1, 1.0 or 2.5 mg colchicine/kg at 6 and 24 hr after i.p. administration.

Effect of colchicine treatment

Serum enzymes. The effect of a single i.p. administration of colchicine on liver injury was assessed at 6 and 24 hr by measuring serum ALT and AST levels (Fig. 1). Neither of the two low doses of colchicine caused any liver injury at 6 or at 24 hr. However, at 2.5 mg colchicine/kg, some liver injury was evident through elevations of both ALT and AST at 24 hr. The increases ranged from 4- to 6-fold for ALT and AST, respectively. Such increases are indicative of a modest level of liver injury.

Bile flow. At 6 hr the highest dose of colchicine caused a significant, but not substantial decrease in bile flow (Fig. 2). However, by 24 hr this effect was overcome and indeed a slightly enhanced bile flow was evident (lower panel, Fig. 2). Neither of the two lower doses of colchicine influenced bile flow at 6 hr and only a very modest choleretic effect was observed for 1 mg colchicine/kg at 24 hr. Although a statistically significant ($P < 0.05$) increase in bile flow was observed, any biological significance for such modest increases in bile flow is doubtful.

Biliary excretion of PG. At 6 hr there was a slight, but statistically significant ($P < 0.05$) decrease in the excretion of PG at the lowest dose of colchicine

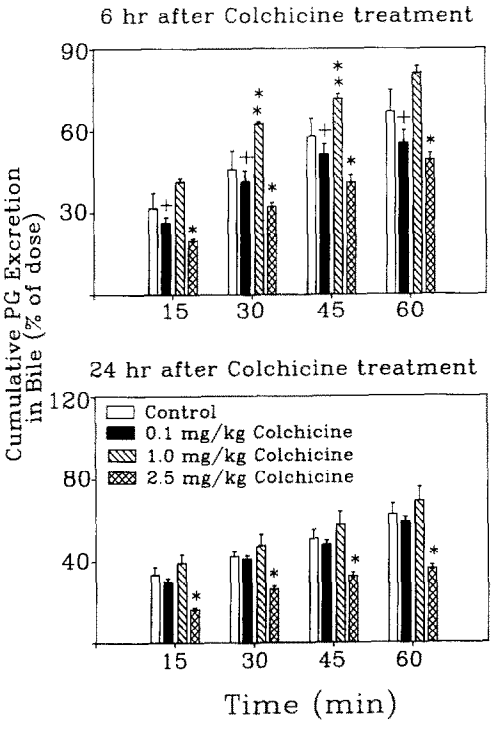

Fig. 3. Effect of colchicine administration (0.1 or 1.0 or 2.5 mg/kg, i.p., in saline) or saline (1 mL/kg, i.p.) alone on cumulative biliary excretion of PG in rats at 6 and 24 hr after the treatment. Values are means ± SEM of four rats. Key: (*) significantly ($P < 0.05$) lower value compared to control, 0.1 and 1 mg colchicine/kg; (+) significantly ($P < 0.05$) lower value than the control value at the same time point, and (**) significantly ($P < 0.05$) higher value compared to all others at the same time point.

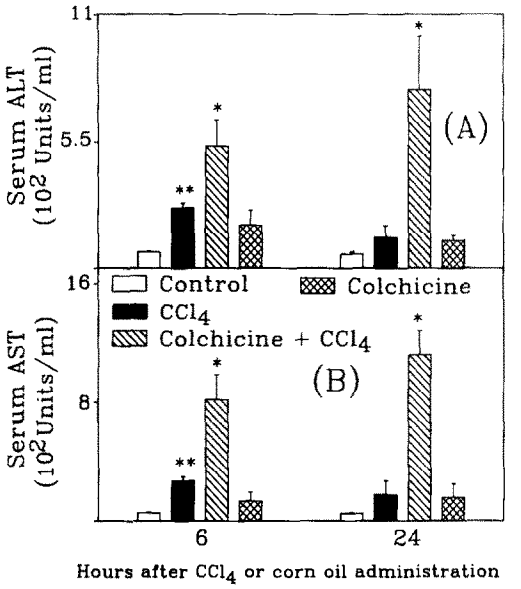

Fig. 4. Serum alanine (A) and aspartate (B) aminotransferase levels at 6 and 24 hr after the administration of CCl_4 (100 μL/kg, i.p.) in corn oil (1 mL/kg) alone to control or colchicine treated rats. Values are means ± SEM for four rats. Key: (*) significantly ($P < 0.05$) higher value compared to control, CCl_4 and colchicine at the same time point; and (**) significantly ($P < 0.05$) higher value compared to control and colchicine at the same time point.

(Fig. 3). However, at 24 hr this decrease in the excretory function was no longer evident. A 1 mg colchicine/kg, biliary excretion of PG was enhanced at 6 hr. This effect was not statistically significant at 24 hr. A more substantial and decisive effect was observed at the highest dose of colchicine. Biliary excretion was inhibited significantly at both 6 and 24 hr, indicating an impairment of hepatic function at this high dose. Interestingly, bile flow was increased slightly (Fig. 2) and hence this effect appears to be a specific interference with hepatobiliary excretory function.

Since these experiments indicated that colchicine was not toxic up to a 1 mg/kg dose, for the second series of experiments this dose of colchicine was employed in combination with a low dose of CCl_4. Colchicine has been employed as an antimitotic agent at this nontoxic dose in a variety of experimental protocols [31–35, 38, 39, 41, 44].

Effects of colchicine, low dose of CCl_4 alone, and the combination

Serum enzymes. Administration of CCl_4 (100 μL/kg) alone resulted in a significant, but very modest increase in ALT and AST at 6 hr (Fig. 4). This elevation in serum enzymes was no longer evident at 24 hr. Administration of colchicine (1 mg/kg) 2 hr prior to corn oil vehicle injection did not cause any significant elevations of serum transaminases (Fig. 4). However, the combination of colchicine and CCl_4 resulted in a substantial elevation of both transaminases (ALT and AST) at 6 hr and this effect was progressive at 24 hr (Fig. 4), indicating persistence of liver injury. Thus, the limited and transient liver injury caused by a low dose of CCl_4 was enhanced and the enhanced injury was persistent at 24 hr as well.

Bile flow. Administration of CCl_4 (100 μL/kg) caused a slight choleretic effect at 6 hr (Fig. 5). This effect was also evident in colchicine treated rats at 6 hr. Both colchicine alone and in combination with CCl_4 caused increased bile flow at 24 hr (Fig. 5). Although statistically significant ($P < 0.05$), these moderate effects in and of themselves are unlikely to be of much biological significance.

Biliary excretion of PG. The combination of colchicine and CCl_4 resulted in significant depression of bilary excretory function as assessed by excretion in PG in bile (Fig. 6). The effect was progressive between 6 and 24 hr. Neither colchicine alone nor CCl_4 alone affected biliary excretion of PG. These findings are consistent with the extent of liver injury as evidenced by elevation of the serum transaminases (Fig. 4).

Histopathology. Histopathology changes were assessed by observing swollen and necrotic hepatocytes in the liver sections (Figs. 7 and 8). Swollen cells (ballooned cells) and necrotic hepatocytes were

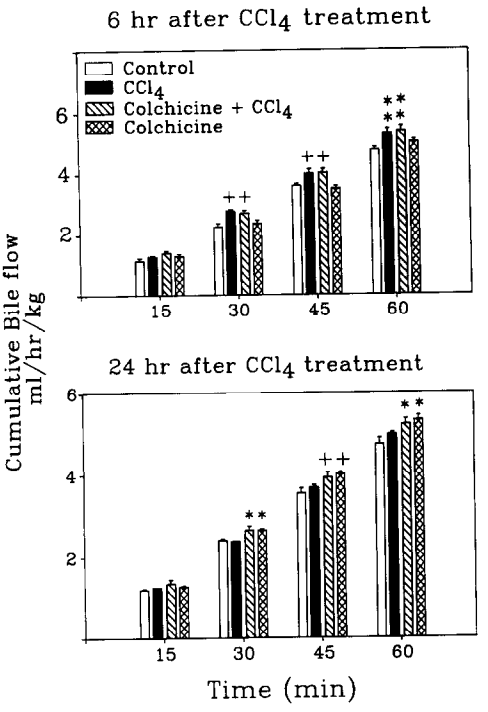

Fig. 5. Effect of colchicine pretreatment on cumulative bile flow at 6 and 24 hr in rats treated with CCl_4 (100 μL/kg, i.p., in corn oil) or corn oil (1 mL/kg, i.p.) alone. Values are means ± SEM of four rats. Key: (*) ($P < 0.05$) higher value compared to control and colchicine; (+) significantly ($P < 0.05$) higher value compared to control; and (*) significantly ($P < 0.05$) higher value compared to control and CCl_4 treated groups at the same time point.

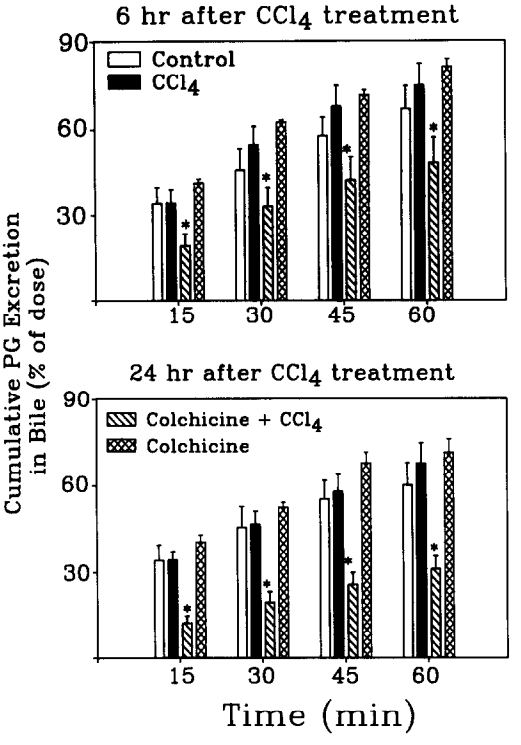

Fig. 6. Effect of colchicine pretreatment on cumulative biliary excretion of PG at 6 and 24 hr in rats treated with CCl_4 (100 μL/kg, i.p., in corn oil) or corn oil (1 mL/kg, i.p.) alone. Values are means ± SEM of four rats. Key: (*) significantly ($P < 0.05$) lower value compared to control, CCl_4 and colchicine at the same time point.

present in significantly larger numbers in the centrilobular region at 6 and 24 hr after CCl_4 administration in colchicine pretreated rats (Figs. 7 and 8). It should be noted that there was no significant difference in the incidence of ballooned cells in the livers at 6 hr after CCl_4 administration regardless of the colchicine treatment (Fig. 7). This becomes apparent if one examines the entire photomicrographs of Fig. 7 rather than just the insets. The rats treated with CCl_4 alone exhibited centrilobular lesions consisting of necrotic and swollen (ballooned) hepatocytes in significant numbers at 6 hr but these changes were no longer evident at 24 hr, indicating recovery from this injury. Persistence of liver injury in colchicine + CCl_4 treated rats even at 24 hr (Fig. 8) was the only major finding that could be attributed to colchicine pretreatment of CCl_4 treated rats. There were no histological alterations in the livers of control rats receiving only the vehicle and colchicine (1 mg/kg, i.p.). The rats receiving the high dose of colchicine (2.5 mg/kg, i.p.) alone showed vacuolation, limited necrosis and damage to the plasma membrane at 24 hr after the treatment (data not shown). There were no histological alterations in these livers at 6 hr after the high dose of colchicine. There were no histological alterations in the livers of rats receiving saline vehicle or lower doses of colchicine. Even at 1 mg/kg, colchicine did not cause any liver injury (Figs. 7 and 8).

DISCUSSION

There is a wide range of evidence regarding the beneficial effect of colchicine on alcoholic liver injury [5–7], CCl_4-induced liver cirrhosis [31–35] and galactosamine-induced hepatitis [41]. The primary objective of this study was to investigate the dose-dependent effect of colchicine on hepatobiliary function by using bile duct cannulated intact animal preparations. Secondly, the effect of a colchicine + CCl_4 combination on hepatobiliary function was also examined. To study the biliary excretion, an anionic transport model compound, viz. phenolphthalein glucuronide (PG), which does not require biotransformation for biliary excretion, was used.

The present studies demonstrated that a single administration of colchicine at 0.1 or 1 mg/kg does not cause any significant adverse effects in the liver. This conclusion was supported by preserved hepatic functional integrity, lack of serum enzyme elevations and any significant histopathological alterations. A slightly decreased excretion of PG observed at 6 hr after the administration of colchicine at 0.1 mg/kg was no longer evident at 24 hr (Fig. 3). Therefore,

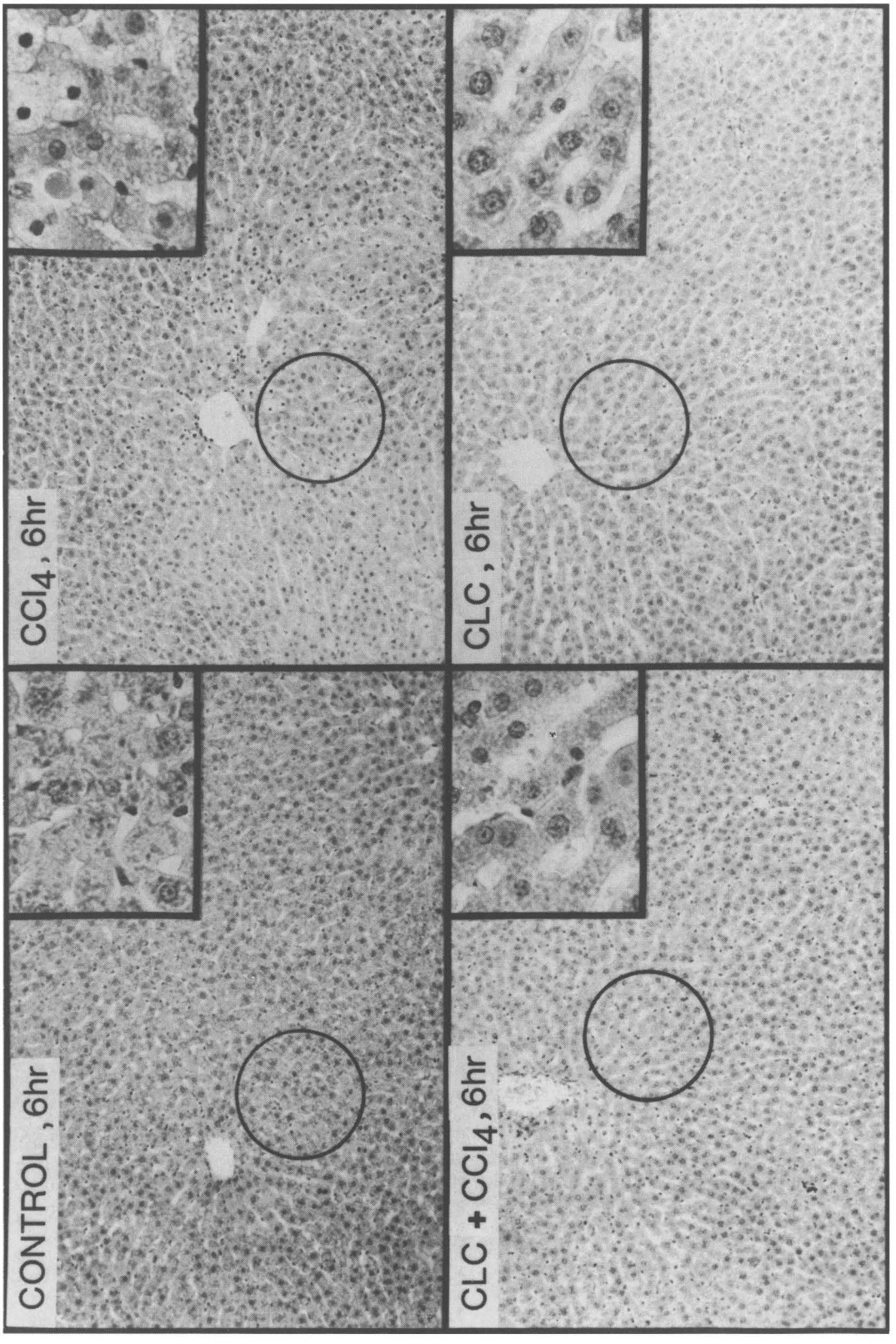

Fig. 7. Typical photomicrograph of liver sections at 6 hr after the administration of CCl_4 (100 μL/kg, i.p., in corn oil) or corn oil (1 mL/kg, i.p.) alone to control or colchicine (1 mg/kg, i.p.) treated rats. H & E stain; magnification, 100×. Inserts represent higher magnification, 400×. CLC = colchicine.

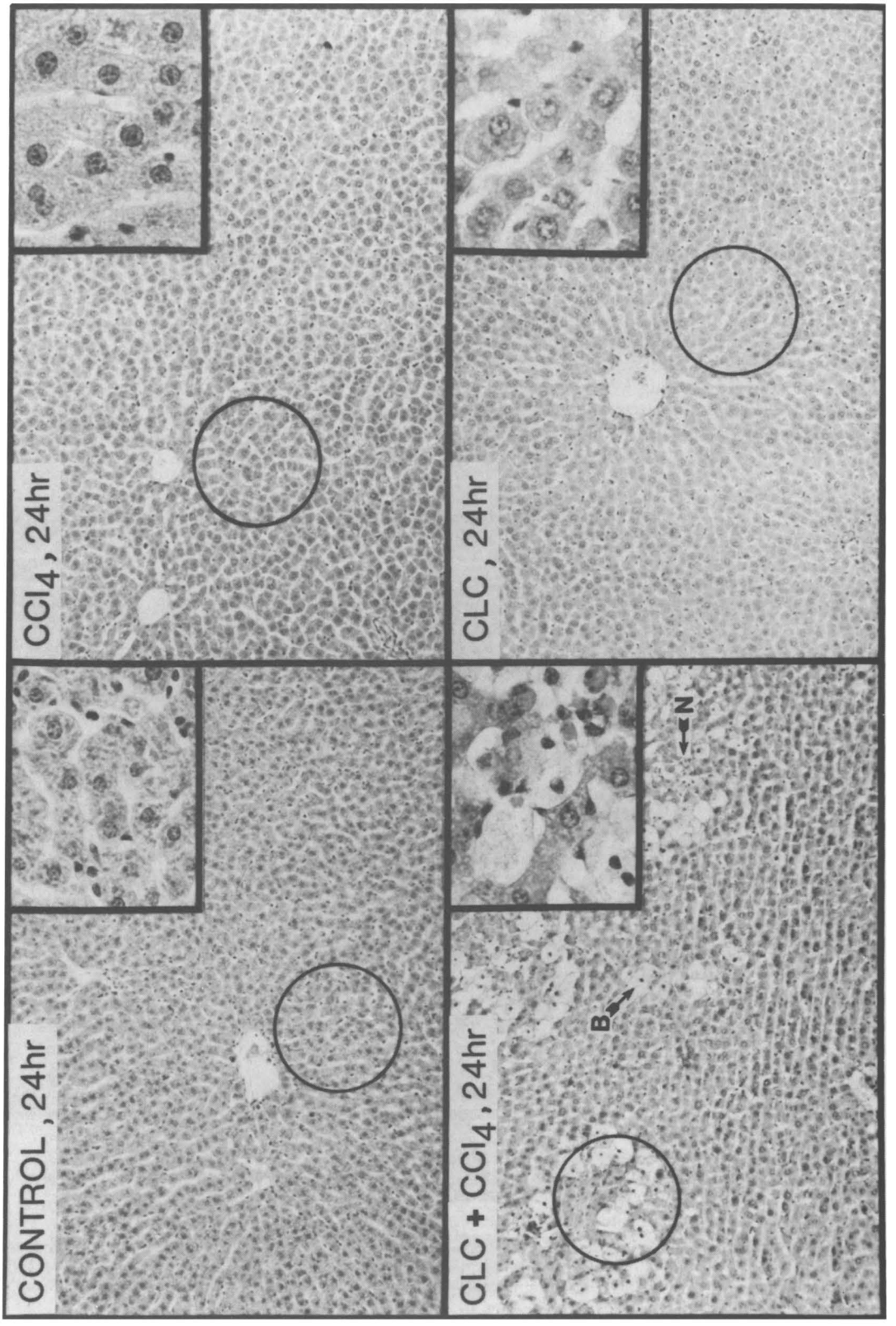

Fig. 8. Typical photomicrograph of liver sections at 24 hr after the administration of CCl_4 (100 μL/kg, i.p., in corn oil) or corn oil (1 mL/kg, i.p.) alone to control or colchicine (1 mg/kg, i.p.) treated rats. H & E stain; magnification, 100×. Inserts represent higher magnification, 400×. N = necrosis; B = ballooned (swollen) cells; CLC = colchicine.

this effect is unlikely to be of any biological significance and appears to be a transient influence on the biliary excretion of the anionic excretory model, employed in these studies. Likewise, a modest choleretic effect of 1 mg colchicine/kg observed at 24 hr (Fig. 2) is unlikely to be of any biological significance. These findings are consistent with a previous report indicating that bile flow was unaffected after administration of 0.5 mg colchicine/kg [23]. Neither of the two serum transaminases (ALT and AST) was elevated by these treatments indicating lack of liver injury. These observations were confirmed by lack of any significant histopathological alterations. Finally, biliary excretion of PG was not affected significantly by either of these two doses of colchicine. These findings indicate that colchicine employed at 1 mg/kg for antimitotic effects in the liver [38, 39] does not cause any detectable or measurable liver injury.

However, at the dose of 2.5 mg/kg, colchicine caused detectable liver injury accompanied by hepatobiliary dysfunction. Both of the serum enzymes were elevated and histopathological observations were consistent with measurable liver injury elicited by this higher dose of colchicine. A closer examination of the parameters of hepatobiliary function revealed that colchicine interferes with the hepatobiliary excretory function and that this effect is independent of any effect on bile flow. While the bile secretory function was enhanced slightly through a choleretic effect, biliary excretion of PG was decisively depressed. Colchicine is known to interfere with microtubule and related membrane functions [13, 14]. While there is evidence to suggest that microtubular and microfilamental integrity is essential for bile secretion [15–22], it is generally assumed that such membrane integrity is also essential for biliary excretion of bilirubin or exogenous model compounds [46]. Stein *et al.* [23] reported that while colchicine interferes with the hepatic release of proteins and lipoprotein into the serum within 2 hr after the administration of 0.5 or 5 mg colchicine/kg, these effects are recoverable at the lower dose within 6–7 hr. These effects persist at 7 hr at the higher dose of colchicine. These effects were not accompanied by decreased bile flow nor was the biliary content of phospholipids and cholesterol affected when examined 2–4 hr after the administration of colchicine [23]. Because of the differences in doses, and more importantly the time of bile flow measurements, a direct comparison of our present findings with those of Stein *et al.* [23] is not possible. Our findings indicate that both bile flow and biliary excretion of PG are adversely affected at 6 or 24 hr after the administration of colchicine at a 2.5 mg/kg dose. Although our present studies do not permit us to directly evaluate the finer aspects of any effects on hepatocellular microtubules and microfilaments in the absence of electron microscopic observations, it appears that colchicine at higher doses is capable of interfering with biliary excretory function, as assessed by the excretion of PG in bile, without interfering with bile secretory function, as assessed by the volume of bile flow. Whether and to what extent any microtubular and microfilamental injury may be associated with these effects remain to be investigated.

Administration of a single low dose of CCl_4 (100 μL/kg) alone does not cause significant injury [36, 37]. In the present study, these findings are supported by additional parameters of liver function. While there was a transient increase in serum enzymes at 6 hr after CCl_4 treatment, liver injury was not evident at 24 hr (Fig. 4). These findings are also supported by lack of any measurable dysfunction in the form of bile secretion or of biliary excretion of PG (Figs. 5 and 6). Hepatic functional integrity was unperturbed by the administration of CCl_4 alone. Therefore, biliary disposition of colchicine would not be expected to be altered in CCl_4 treated rats. The same dose of CCl_4 elicited a significantly greater liver injury in colchicine pretreated rats (Figs. 4, 7 and 8). In these rats, liver injury was also prolonged in comparison to that elicited by CCl_4 alone. Hepatobiliary excretory function assessed as PG excretion was compromised significantly at both 6 and 24 hr. These findings are also supported by histopathological observations of liver sections. While the liver injury observed was consistent with the classic lesion caused by CCl_4, the centrilobular necrosis was much greater at 6 hr (Fig. 7) and was still persistent at 24 hr (Fig. 8).

Colchicine pretreatment led to a significant depression of biliary excretion of PG at 6 and 24 hr after CCl_4 administration. This impairment of biliary excretory function by colchicine in combination with CCl_4 was accompanied by a choleretic effect on the bile flow. A careful examination of these data suggests a dichotomy between the secretory and the excretory functions of bile. While there was a choleretic effect on bile flow, biliary excretion of PG was depressed significantly after this combination treatment, indicating that such an effect was not secondary to decreased bile flow.

From our earlier study [38] and the present investigation, it is apparent that during CCl_4-induced hepatic injury, colchicine pretreatment leads to elevation in serum enzyme levels (Fig. 4) and increased hepatic injury (Figs. 7 and 8) which is accompanied by severe impairment in biliary excretory function (Fig. 6). Therefore, colchicine treatment of patients with severe hepatic dysfunction or liver disease may not be feasible as it was thought earlier and would need more careful consideration.

The mechanism by which colchicine pretreatment results in enhancement and prolongation of CCl_4 toxicity is of interest for several reasons. First, colchicine has been reported to be hepatoprotective against alcoholic liver injury [5–7], galactosamine hepatitis [41] and CCl_4-induced liver cirrhosis [31–35]. While these are all beneficial effects observed on chemically induced liver disease of the chronic type, our present study deals only with the effect of colchicine on acute liver injury. Since the underlying mechanisms may be entirely different in acute versus chronic exposures, it will not be possible to relate the implications of our present findings to these earlier observations directly. However, the seemingly contradictory finding of enhanced CCl_4 liver injury by a single administration of colchicine is of interest. This effect is not due to enhanced CCl_4 uptake,

retention or bioactivation of CCl_4 as revealed by *in vivo* metabolism studies with $^{14}CCl_4$ [39]. It is known that colchicine under conditions employed in this study interferes with the early-phase (6 hr) stimulation of hepatocellular regeneration and tissue repair [36, 37], without affecting the later phase (36–48 hr) of cell division [38, 39]. It is also known that early-phase (6 hr) stimulation of cell division and tissue repair by a low dose of CCl_4 [36, 37] is critical for recovery from that injury [49]. Therefore, it is possible that interference with the early phase of cell division normally stimulated by a low dose of CCl_4 as a result of colchicine antimitosis may simply prevent tissue healing, thus allowing the limited injury to progress and prolong. This is only a conjectural speculation which can be experimentally verified by additional time–course studies using additional experimental manipulations.

Recent experimental evidence indicates that hepatic injury significantly alters colchicine pharmacokinetics in the rat [12] which in turn affects biliary excretion and secretion as has been observed in α-naphthylisothiocyanate- and cimetidine-induced liver injury [12, 13]. For the colchicine + CCl_4 experiments, we employed a dose of CCl_4 (100 μL/kg, i.p.), which did not cause any hepatobiliary dysfunction. Neither bile flow (Fig. 5) nor biliary excretion of PG (Fig. 6) was affected adversely by CCl_4 alone. Therefore, hepatic functional integrity was unperturbed in rats receiving CCl_4 alone, suggesting that it is unlikely to have been a factor in the interaction of colchicine + CCl_4 combination studies. It is believed that hepatocellular microtubules and microfilaments play an important role in bile secretion and bile formation [21, 24] and for normal bile flow [15–22]. There is also evidence to indicate the necessity of microtubules and microfilaments in maintaining hepatocellular plasma membrane integrity [16]. It is well known that CCl_4 causes lipid peroxidation [29, 30], which is one of the events leading to loss of membrane integrity and cell death and colchicine is a known microtubule and microfilament poison [13, 14]. From the present study, it is evident that the colchicine + CCl_4 combination severely affects the biliary excretory function but not secretory function. The loss of exertory function is probably due to loss of membrane integrity and intercellular communication. On the other hand, there is still controversy as to whether microtubules take part in bile formation [21–24]. From our study it is apparent that colchicine alone at 1 mg/kg did not hamper biliary secretory or excretory function. Indeed, both the processes were enhanced (Figs. 2 and 3) at this low dose of colchicine. Moreover, biliary excretory function was impaired significantly in colchicine + CCl_4 and also by colchicine alone at the high dose (2.5 mg/kg). This indicates that microtubules and microfilaments by themselves do not play a significant role in bile secretion or bile flow, but the loss of membrane integrity due to lipid peroxidation or by other means in combination with loss of cytoskeletal support to the membrane leads to impairment in biliary excretory function.

In conclusion, a single administration of colchicine at 0.1 or 1.0 mg/kg did not cause any measurable untoward effects on hepatobiliary function. These doses were also dissociated from any detectable liver injury at the light microscopic level. However, at a higher dose (2.5 mg/kg), colchicine elicited histopathological alterations in the liver accompanied by serum enzyme elevations and hepatobiliary dysfunction. Pretreatment of rats with a nontoxic dose of colchicine (1 mg/kg), known to prevent the early-phase (6 hr) mitosis, ordinarily stimulated by a low dose of CCl_4 (100 μL/kg), resulted in enhanced and prolonged CCl_4 toxicity as evidenced by biochemical, histopathological and hepatic functional perturbations.

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