The effect of colchicine on the development of lithocholic acid-induced cholestasis

A study of the role of microtubules in intracellular cholesterol transport

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The pathogenesis of lithocholic acid (LCA-Na)-induced cholestasis involves a rapid accumulation of cholesterol in the bile canalicular membrane. Since microtubules play an important role in the intracellular transport of many materials, including cholesterol, the present study was undertaken to assess the extent to which they participate in the development of LCA-Na-induced cholestasis. Rats were pretreated with either colchicine (0.2 μ mol/100 g body wt.) or saline solution 90 min before injection with LCA-Na (12 μ mol/100 g body wt.). Colchicine, although not increasing bile flow by itself, significantly reduced the cholestasis caused by LCA-Na (57–32% reduction in bile flow) without affecting its metabolism into less toxic bile acids or its distribution in blood, liver or bile. Bile canalicular membranes isolated from animals treated with a combination of colchicine and LCA-Na contained less cholesterol than those treated with LCA-Na alone. However, membranes obtained from rats treated with colchicine alone contained much less cholesterol than did controls. It was found that the total amount of cholesterol accumulated within the bile canalicular membrane following LCA-Na treatment (LCA-Na + colchicine versus colchicine alone compared with LCA-Na versus controls) was unchanged by colchicine treatment. In view of these findings it is suggested that (i) the total amount of cholesterol present within the bile canalicular membrane determines the extent of LCA-Na-induced cholestasis, (ii) LCA-Na probably moves cholesterol to the bile canalicular membrane via a microtubule independent pathway, and (iii) microtubules are unlikely to function in the transcellular transport of LCA-Na.

INTRODUCTION

Previous work has shown that injection of lithocholic acid (LCA-Na) and its taurine and glycine conjugates can induce intrahepatic cholestasis in experimental animals (Javitt, 1966; King & Schoenfield, 1971; Priestly *et al.*, 1971; Miyai *et al.*, 1975; Layden & Boyer, 1977). Several mechanisms have been suggested to explain the pathogenesis of this cholestasis (Boyer *et al.*, 1977; Miyai *et al.*, 1977; Bovicini *et al.*, 1978; Kakis & Yousef, 1978, 1980; Kakis *et al.*, 1980); however, recent studies indicate that LCA-Na modifies the structural characteristics of the bile canalicular membrane, thereby possibly reducing its permeability to water and ions. These membrane changes are characterized by the binding of LCA-Na to the bile canalicular membrane and a rapid and extensive increase in its cholesterol content (Kakis & Yousef, 1978, 1980; Kakis *et al.*, 1980).

Microtubule-disrupting agents, such as colchicine and vinblastine, interfere with the movement of secretory vesicles within hepatocytes, resulting in an accumulation of secretory materials, e.g. albumin (Le Marchand *et al.*, 1974), fibrinogen (Feldmann *et al.*, 1975) and lipoproteins (Le Marchand *et al.*, 1973; Orci *et al.*, 1973; Reaven & Reaven, 1980), inside the liver cells and diminishing their secretion into the blood (Le Marchand *et al.*, 1974; Feldmann *et al.*, 1975; Le Marchand *et al.*, 1973; Orci *et al.*, 1973; Reaven & Reaven, 1980; Redman *et al.*, 1975; Barnwell & Coleman, 1983). These agents also inhibit

transcellular IgA transport into the bile (Barnwell & Coleman, 1983; Mullock *et al.*, 1980; Godfrey *et al.*, 1982; Goldman *et al.*, 1983), reduce the secretion of biliary lipids (Gregory *et al.*, 1978; Dubin *et al.*, 1980; Erlinger *et al.*, 1980; Barnwell *et al.*, 1984), and have been suggested to play a role in bile salt transport (Gregory *et al.*, 1978; Dubin *et al.*, 1980; Erlinger *et al.*, 1980). In view of the reported effects of microtubule-disrupting agents on the output of biliary components, colchicine was used in the present study to investigate the possible role of microtubules in the cellular transport of LCA-Na and cholesterol to the bile canalicular membrane during the development of LCA-Na-induced cholestasis.

MATERIALS AND METHODS

Materials

Lithocholic acid, sodium salt (LCA-Na), was obtained from Calbiochem-Behring, La Jolla, CA, U.S.A. [14 C]-LCA-Na (sp. radioactivity 55 mCi/mmol) was purchased from Amersham, Oakville, Ont., Canada. Colchicine and other chemicals were supplied by the Sigma Chemical Company, St. Louis, MO, U.S.A.

Animal treatments

Male Wistar rats, 200–250 g (Charles River, Montreal, Quebec), were maintained in an environment of constant temperature (22 °C) and lighting (darkness 19:00 to

07:00) with free access to food and water prior to experimental use.

Animals were anaesthetized with pentobarbital (48 mg/kg body wt., intraperitoneally) and a PE90 catheter was inserted into the jugular vein. The bile duct was cannulated with PE10 tubing and bile was collected for 15 min to ascertain that bile flow was normal. Body temperature was monitored throughout the experiment and maintained at a constant 37 °C by using a rectal probe and a thermostatically controlled i.r. lamp.

After 15 min a second bile sample was collected and either colchicine (0.2 µmol/100 g body wt.) or 1 ml of saline solution (controls) was administered via the jugular vein catheter. This dose of colchicine was previously found to cause a 60% inhibition of biliary IgA secretion and a 2-fold increase in biliary albumin output after 90 min of treatment, and therefore was taken as evidence that colchicine had inhibited microtubular function (Barnwell & Coleman, 1983; Godfrey *et al.*, 1982). After 105 min of bile collection either LCA-Na (12 µmol/100 g body wt.) labelled with 5 µCi of [¹⁴C]LCA-Na and suspended in 2 ml of 7.5% (w/v) bovine albumin and 0.9% (w/v) NaCl, or 2 ml of albumin solution alone (controls) was administered via the intravenous cannula. After a further 60 min of bile collection the experiment was terminated. Blood samples were taken from animals that had received [¹⁴C]LCA-Na and livers were sectioned for electron microscopy. Livers were then flushed free of blood with cold iso-osmotic saline, removed, weighed and homogenized in 1 mM-NaHCO₃ buffer (pH 7.5) with a Polytron homogenizer at speed 7.5 for 3 s.

Preparation of liver plasma membranes

Rat liver plasma membranes enriched in bile canalicular membranes were prepared by a method based on that described by Yousef & Murray (1978). Briefly, homogenates were diluted to 10 times their original volume with 1 mM-NaHCO₃ buffer (pH 7.5), filtered through four layers of gauze, and centrifuged at 600 *g* for 15 min. The resulting crude membrane fraction contained in the pellet was suspended in sucrose, final density 1.22 g/ml. Aliquots of this solution were carefully overlaid with sucrose of density 1.18 g/ml and then with sucrose of density 1.16 g/ml. The pure bile canalicular membrane (BCM) fraction appeared at the 1.18/1.16 g/ml interface after centrifugation at 66000 *g* for 60 min (Song *et al.*, 1969). The fraction at the 1.22/1.18 g/ml interface consisted of the remaining plasma membranes (PM) contaminated with microsomes, mitochondria and other material. Each layer was harvested by syringe and needle and washed with 4 vol. of ice-cold 1 mM-NaHCO₃ buffer (pH 7.5) and centrifuged at 3000 *g* for 10 min. The membranes were then again resuspended and washed with 50 ml of 1 mM-NaHCO₃ buffer (pH 7.5) and recentrifuged at 9000 *g* for 10 min. Finally, BCM and PM pellets were resuspended in 2 ml of 1 mM-NaHCO₃ buffer (pH 7.5) containing 2 mM-CaCl₂ and stored at -70 °C until required.

Analytical methods

Glucose-6-phosphatase (EC 3.1.3.9) activity was measured by the method of Schwartz & Bodansky (1961) and leucine aminopeptidase (EC 3.4.11.1) by the method of Goldbarg & Rutenberg (1958); alkaline phosphodiesterase 1 (EC 3.1.4.1), lactate dehydrogenase (EC 1.1.1.27), 5'-nucleotidase (EC 3.1.3.5) and alkaline

phosphatase (EC 3.1.3.1) were measured as described previously (Godfrey *et al.*, 1981). Protein was determined by the method of Lowry *et al.* (1951).

Bile and membrane fractions were analysed for phospholipid by a similar method to that reported in Barnwell *et al.* (1983) and cholesterol content was determined by g.l.c. (see Kakis & Yousef, 1980). Total biliary bile acids were determined by using 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50) as described by Coleman *et al.* (1979). Bile flow was determined gravimetrically, assuming a density of 1 g/ml.

Radioactivity in blood, bile and liver homogenate samples taken from animals injected with [¹⁴C]LCA-Na was measured in a Beckman liquid-scintillation counter equipped with an external standard. The distribution of [¹⁴C]LCA-Na metabolites in bile was also determined after first separating the individual bile acids by t.l.c. using butan-1-ol/acetic acid/water (10:1:1, by vol.) as a solvent system and by comparison with pure standards of lithocholic acid, tauroolithocholic acid, glycolithocholic acid and the taurine and glycine conjugates of cholic and chenodeoxycholic acids to identify the biliary components.

Ultrastructural methods

Liver samples were fixed in a solution containing 2.5% glutaraldehyde in 0.1 M-cacodylate buffer (pH 7.4) for at least 1 h at 4 °C. After washing with buffer, blocks of tissue were post-fixed with 2% osmic acid in cacodylate buffer for 2 h, dehydrated in graded ethanol and embedded in Araldite. Thin sections were cut from centrolobular zones and were stained with aryl acetate and Reynold's lead citrate. At least three blocks of tissue from each liver were sectioned and later examined.

Statistical analysis of the data was carried out using Student's *t* test. Values of *P* < 0.05 were assumed to be significant.

RESULTS

Bile flow

Table 1 shows that, following the injection of LCA-Na, there was a significant decrease in bile flow. However, the reduction in bile flow was much less (32%) following

Table 1. Effect of colchicine on the extent of the cholestasis induced by LCA-Na

Values are means ± S.D. of the bile flows from six animals. Colchicine was injected at 15 min and LCA-Na at 105 min. Values marked ^a are significantly different from control values (*P* < 0.001); values marked ^b are significantly different from the treatment group receiving colchicine and LCA-Na (*P* < 0.001). ^{NS} means that no significant difference was observed.

Treatment	Bile flow (µl/min per g liver wt.)	
	15–105 min	105–165 min
Control	2.28 ± 0.46	2.14 ± 0.43 ^b
Colchicine	2.09 ± 0.33 ^{NS}	2.02 ± 0.31 ^{b,NS}
LCA-Na	2.05 ± 0.43 ^{NS}	0.92 ± 0.38 ^{a,b}
Colchicine + LCA-Na	2.09 ± 0.26 ^{NS}	1.53 ± 0.49 ^a

Table 2. Distribution of radioactivity in bile, liver and blood 1 h after injection of [¹⁴C]LCA-Na

Values are expressed as a percentage $\pm$ s.d. of the total [¹⁴C]LCA-Na radioactivity injected based on the results from six animals in each group. ^{NS} No significant differences in the distribution or recovery of the radioactivity administered was found between the two groups.

Treatment	Total recovery (%)	Bile (%)	Liver (%)	Blood (%)
LCA-Na	96.1 $\pm$ 10.2	54.9 $\pm$ 15.0	29.7 $\pm$ 7.8	12.5 $\pm$ 3.5
LCA-Na + colchicine	97.5 $\pm$ 11.3 ^{NS}	65.0 $\pm$ 8.8 ^{NS}	17.8 $\pm$ 11.0 ^{NS}	14.0 $\pm$ 4.5 ^{NS}

pretreatment with colchicine than in animals injected with LCA-Na alone (57%). Colchicine treatment by itself did not affect bile flow at any time after treatment.

Distribution of the injected [¹⁴C]LCA-Na in liver, plasma and bile

The values in Table 2 indicate that in control animals 30% of the injected LCA-Na or its metabolites were retained in the liver, 55% in the bile and 12% in the serum. In colchicine-treated animals 65% of the injected dose was secreted in the bile and less retained within the liver (18%), while 14% was present in the serum. Differences in the distribution of [¹⁴C]LCA-derived radioactivity in the various tissues did not reach statistical significance. In all cases the recovery of [¹⁴C]LCA-Na was at least 95%.

Secretion of [¹⁴C]LCA-Na metabolites in the bile

When [¹⁴C]LCA-Na metabolites in the bile samples from colchicine-treated and control animals were separated by t.l.c. and then compared with authentic standards (see Table 3), it was found that there was no significant difference in the proportion of unchanged lithocholic acid and taurine-conjugated lithocholic acid (the bile acids known to cause cholestasis). However, there was a significant reduction in the proportion of dihydroxy taurine-conjugated bile acids secreted by colchicine-treated animals.

Table 3. Comparison of the [¹⁴C]LCA-Na metabolites secreted into bile by colchicine-treated and control rats

Values represent means $\pm$ s.d. of the percentage of ¹⁴C radioactivity secreted into the bile during the period 105–165 min after injection of [¹⁴C]LCA-Na. Individual biliary bile acids were separated by t.l.c. as described in the Material and methods section. ^a Significantly different ($P < 0.001$); ^{NS} not significant.

Bile acid type	Radioactivity (% of total)	
	LCA-Na	Colchicine + LCA-Na
Lithocholate	4.9 $\pm$ 3.0	3.6 $\pm$ 2.9 ^{NS}
Taurolithocholate	36.1 $\pm$ 13.9	46.4 $\pm$ 15.1 ^{NS}
Taurine-conjugated dihydroxy bile acids	35.2 $\pm$ 2.0	18.5 $\pm$ 4.2 ^a
Taurine-conjugated trihydroxy bile acids	6.6 $\pm$ 1.9	7.9 $\pm$ 5.7 ^{NS}
Glycine-conjugated mono- + di-hydroxy bile acids	18.2 $\pm$ 15.6	23.4 $\pm$ 9.3 ^{NS}

Biliary phospholipid and cholesterol

Colchicine administration caused a decline in both biliary phospholipid and cholesterol secretion when compared with saline-treated controls (see Tables 4 and 5). The reduction in biliary cholesterol secretion (48%) during the second sample period (105–165 min) was considerably more marked than the reduction in phospholipid output, which was not significantly different from controls.

A bolus injection of LCA-Na reduced both biliary phospholipid (55%) and cholesterol secretion (48%) (see

Table 4. Effect of colchicine and LCA-Na on the biliary secretion of phospholipids

Values are means $\pm$ s.d. Each test group contained between six and eight samples, and all determinations were carried out in duplicate. Colchicine was injected at 15 min and LCA-Na at 105 min. Values marked ^a are significantly different from controls ($P < 0.001$); those labelled ^{NS} are not.

Treatment	Secretion (nmol/min per g of liver)	
	15–105 min	105–165 min
Control	9.76 $\pm$ 3.00	8.26 $\pm$ 1.30
Colchicine	7.74 $\pm$ 1.58 ^{NS}	7.35 $\pm$ 1.46 ^{NS}
LCA-Na	9.44 $\pm$ 3.40 ^{NS}	4.52 $\pm$ 2.29 ^a
Colchicine + LCA-Na	8.34 $\pm$ 1.04 ^{NS}	4.79 $\pm$ 2.29 ^a

Table 5. Effect of colchicine and LCA-Na on the biliary secretion of cholesterol

Values are means $\pm$ s.d.; each group contained six samples per group. Colchicine was injected at 15 min and LCA-Na at 105 min. Values marked ^a are significantly different from the control group ($P < 0.001$). No significant difference was detected in those marked ^{NS}.

Treatment	Secretion (nmol/min per g of liver)	
	15–105 min	105–165 min
Control	0.84 $\pm$ 0.20	0.81 $\pm$ 0.18
Colchicine	0.57 $\pm$ 0.21 ^a	0.40 $\pm$ 0.19 ^a
LCA-Na	0.83 $\pm$ 0.20 ^{NS}	0.39 $\pm$ 0.15 ^a
Colchicine + LCA-Na	0.55 $\pm$ 0.24 ^a	0.44 $\pm$ 0.09 ^a

Tables 4 and 5). However, the cholesterol output was not significantly different from the group treated with colchicine alone (no LCA-Na).

Plasma membrane enzyme activity in bile

Alkaline phosphodiesterase 1 activity in bile (Table 6) was not significantly affected by colchicine or LCA-Na

Table 6. Effects of colchicine and LCA-Na on the biliary output of alkaline phosphodiesterase 1

Values are means $\pm$ S.D., for six animals. Enzyme units are nmol of substrate hydrolysed/h at 37 °C. Output of enzyme was calculated by dividing the total activity present in a given sample by the number of minutes for which the bile collection was made. The activity of the other plasma membrane enzymes alkaline phosphatase and 5'-nucleotidase were also measured. These enzymes showed an increase in activity (10-fold) in the colchicine + LCA-Na group similar to that shown by alkaline phosphodiesterase 1. The activity of this enzyme in bile was only found to be significantly different from controls in the treatment group which received both colchicine and LCA-Na ($P < 0.001$). This value is marked ^a.

Treatment	Secretion (units/min)	
	15–105 min	105–165 min
Control	3.35 $\pm$ 0.30	3.30 $\pm$ 1.80
Colchicine	3.13 $\pm$ 0.34	3.15 $\pm$ 0.29
LCA-Na	3.25 $\pm$ 0.27	4.15 $\pm$ 0.87
Colchicine + LCA-Na	3.40 $\pm$ 0.33	33.50 $\pm$ 3.50 ^a

injection when compared with untreated controls. However, a combination of colchicine and LCA-Na caused a 10-fold increase in alkaline phosphodiesterase 1 activity. A similar effect was also observed with two other plasma membrane enzymes, alkaline phosphatase and 5'-nucleotidase (results not shown). These results could not be due to biliary enzyme activation by an interaction of these enzymes with LCA-Na and colchicine together since no difference in activities were observed when these agents were incorporated into any of the enzyme incubations at levels similar to those found in sample bile (LCA-Na, 0–25 mM; colchicine, 0–50 nM).

Composition of liver plasma membranes

Table 7 shows that the isolated BCM contained high specific activities of the plasma membrane enzymes 5'-nucleotidase and leucine aminopeptidase. The microsomal enzyme, glucose-6-phosphatase, and the cytosolic enzyme, lactate dehydrogenase, were absent from the purified BCM, indicating little contamination was present. These characteristics of the purified membrane BCM fraction and the total homogenate activities of all the liver enzymes used in this study were not significantly affected by any of the treatments used (results not shown).

The results in Table 8 indicate that LCA-Na caused an almost 2-fold increase in BCM cholesterol content when compared with controls. Colchicine treatment alone resulted in a significantly decreased membrane cholesterol content (52%). Injection of LCA-Na into colchicine-treated animals caused a significant increase in total liver plasma membrane cholesterol content; however, this was not as extensive as with LCA-Na injection alone. No

Table 7. Enzyme activities of rat liver membranes

Values are means $\pm$ S.E.M. of activities measured in six samples using determinations in triplicate for each enzyme assay. Enzyme activity is expressed as μ mol of NADH oxidized/h per mg of protein (lactate dehydrogenase), μ mol of inorganic phosphate released/h per mg of protein (glucose-6-phosphatase and 5'-nucleotidase) or μ g of β -naphthylamine released/h per mg of protein (leucine aminopeptidase) at 37 °C. Samples in which no enzyme activity was detected are labelled ND. No significant statistical variation was found between any of the treatment groups with respect to total enzyme activities in homogenate, BCM or PM.

Fraction	Lactate dehydrogenase	Glucose-6-phosphatase	5'-Nucleotidase	Leucine aminopeptidase
Homogenate	10.5 $\pm$ 1.23	10.1 $\pm$ 1.62	0.9 $\pm$ 0.08	0.5 $\pm$ 0.01
PM	ND	0.4 $\pm$ 0.05	20.2 $\pm$ 1.93	0.6 $\pm$ 0.05
BCM	ND	ND	85.5 $\pm$ 6.13	8.3 $\pm$ 1.10

Table 8. Liver cell plasma membrane composition

All values are means $\pm$ S.E.M. for five or six samples per group. When the protein and phospholipid composition of the isolated BCM was compared by statistical analysis no significant variation between the treatment groups was observed. Statistical analysis of BCM cholesterol content showed that there was significant variation between the treatment groups. The P values in all cases were <0.001 where ^a represents a comparison with the control group, ^b comparison with the colchicine treatment group, ^c comparison with the colchicine and LCA-Na treatment group and ^d comparison with the LCA-Na treatment group.

Treatment	Protein (mg/g of liver)	Phospholipid (nmol/mg of membrane protein)	Cholesterol (nmol/mg of membrane protein)
Control	1.79 $\pm$ 0.79	523 $\pm$ 42	350 $\pm$ 9 ^{b,c,d}
Colchicine	1.79 $\pm$ 0.39	454 $\pm$ 70	182 $\pm$ 20 ^{a,c,d}
Colchicine + LCA-Na	1.86 $\pm$ 0.29	435 $\pm$ 46	392 $\pm$ 27 ^{a,b,d}
LCA-Na	2.48 $\pm$ 0.48	421 $\pm$ 50	615 $\pm$ 66 ^{a,b,c}

significant change in membrane phospholipid or protein content was detected among the various groups (Table 8).

Electron microscopy

LCA-Na injection caused morphological alterations in bile canalicular membrane structure similar to those already published (Javitt, 1966; King & Schoenfield, 1971; Priestly *et al.*, 1971; Miyai *et al.*, 1975; Layden & Boyer, 1977). These changes consisted of canalicular dilation, partial loss of microvilli and the appearance of lamellar structures within the canalicular lumen. Colchicine treatment caused an accumulation of many multivesicular bodies within hepatocytes as previously observed (Barnwell & Coleman, 1983). Liver samples taken after treatment with both colchicine and LCA-Na showed hepatocytes packed with multivesicular bodies yet containing fewer LCA-Na-induced lesions.

DISCUSSION

Previous studies indicate that LCA-Na-induced cholestasis is associated with a retention of this bile salt within the liver (Kakis & Yousef, 1978, 1980; Kakis *et al.*, 1980). Cytochemical staining techniques (Bovicini *et al.*, 1978; Jung *et al.*, 1982) together with cell fractionation studies (Layden & Boyer, 1977; Bovicini *et al.*, 1978; Kakis & Yousef, 1980) have shown that cholestasis is also accompanied by an increase in the cholesterol content of the bile canalicular membrane and an increased binding of LCA-Na. The precise mechanism(s) by which bile flow is impaired is as yet unclear; however, it is possible that disruption of the bile canalicular membrane structure with large quantities of cholesterol may render the membrane less permeable to water and ions (Kakis & Yousef, 1978, 1980; Kakis *et al.*, 1980).

In the present study colchicine was found to reduce significantly the cholestatic effect of LCA-Na (Table 1) and it is of note that this effect is accompanied by a reduction in the total cholesterol content of the BCM when compared with LCA-Na alone. However, it is noticeable (see Table 8) that colchicine alone reduces BCM cholesterol content to a value well below that measured in controls. Interestingly, however, the amount of cholesterol accumulated in the BCM following LCA-Na injection appeared to be similar irrespective of colchicine or saline pretreatment [LCA-Na versus control, 245 ± 22 (S.E.M.) nmol/mg of membrane protein; LCA-Na + colchicine versus colchicine alone, 210 ± 35 (S.E.M.) nmol/mg; *P* value not significant]. Together these observations suggest that an important determinant in the pathogenesis of LCA-Na is the total amount of cholesterol accumulated in the bile canalicular membrane. Further support for this idea is derived from studies in which inhibitors of cholesterol biosynthesis reduced both the accumulation of cholesterol in the bile canalicular membrane and any extensive decline in bile flow (Kugelmass *et al.*, 1982).

Since LCA-Na administration results in a similar quantity of cholesterol being transported to the BCM, both in saline- and colchicine-treated animals, and given that colchicine treatment by itself reduces BCM and biliary cholesterol content, it is suggested that the reduced cholestasis with LCA-Na after pretreatment with colchicine is the result of inhibition of a normal physiological and microtubule-dependent transport of cholesterol into the BCM and bile. Thus, in view of these

results it is likely that LCA-Na can mobilize cholesterol by a completely microtubule-independent transport mechanism. This alternative cholesterol (and/or LCA-Na) transport mechanism may involve binding to cytosolic protein, since studies *in vitro* have shown that LCA-Na induced incorporation of microsomal cholesterol into isolated bile canalicular membranes is enhanced in the presence of a protein-containing fraction derived from liver cytosol (Yousef *et al.*, 1984; Yousef & Tuchweber, 1984). Furthermore, other studies have shown that inhibition of protein synthesis with cycloheximide prevents LCA-Na-induced cholestasis and diminishes LCA-Na binding to cytosolic protein (Yousef *et al.*, 1983).

An alternative explanation for the reduced cholestatic effect of LCA-Na following colchicine treatment could be that this agent in some way allows the LCA-Na to be converted to a less toxic metabolite(s). However, the results in Table 3 show that colchicine caused no significant change in the proportion of LCA-Na excreted as lithocholic acid or tauro lithocholic acid (bile acids known to cause the cholestatic effect). The only significant change caused by colchicine was a reduction in the amount of [^{14}C]LCA-Na excreted as dihydroxy taurine-conjugated bile acids. It is likely that this change can in part be accounted for by an increase in the excretion of glycine-conjugated bile acids.

Yet another alternative explanation for the reduced cholestatic effect of LCA-Na following colchicine treatment is that this agent prevents the uptake and transcellular transport of LCA-Na by the liver, thereby preventing the full development of cholestasis. However, as the amount of LCA-Na remaining in the circulation was similar in both colchicine-treated and control animals, this suggestion is unlikely (see Table 2). Furthermore, since the uptake of LCA-Na remained unaffected, and the biliary secretion was slightly increased, it is also unlikely that microtubules play a significant role in the transcellular transport of this bile salt.

The increased activity of plasma membrane enzymes in the bile following LCA-Na administration to colchicine-treated animals is difficult to explain, since this bile salt is not a detergent and does not normally promote the biliary secretion of these enzymes into bile (see Table 8 and Reichen *et al.*, 1979).

In a number of studies in which bile salts, other than LCA-Na, have been infused into bile fistula rats or isolated perfused rat livers treated with microtubule-disrupting agents the expected increase in biliary phospholipid secretion was virtually abolished (Gregory *et al.*, 1978; Barnwell *et al.*, 1984; Berken *et al.*, 1982; Fukumoto *et al.*, 1982). The present study demonstrated that the reduction in biliary phospholipid secretion by colchicine-treated rats did not reach statistical significance (Table 4), an observation which agrees with the findings of Stein *et al.* (1972). However, in these investigations the measurement of biliary phospholipid secretion was made when the enterohepatic circulation had been interrupted and the endogenous bile salt pool substantially depleted. Furthermore, in addition to these observations, it has been noted that disruption of the hepatocellular microtubule network results in abnormal biliary secretion of newly synthesized plasma proteins normally destined for delivery to the sinusoidal face of the hepatocyte (Barnwell & Coleman, 1983). In view of these findings it

is possible to envisage that disruption of the resupply of biliary phospholipids and an increase in the delivery of abnormal membrane phospholipids, in the form of misdirected secretory vesicles, to the bile canalicular membrane following colchicine treatment could result in qualitative changes to its composition. These changes in structure, combined with the increase in membrane cholesterol content caused by LCA-Na, may therefore render the plasma membrane enzymes more easily detached by the membrane-perturbing (non-detergent) effect of this agent (Cooper *et al.*, 1972). That it is LCA-Na itself which causes the release of the plasma membrane enzymes is made more plausible by the observation that colchicine does not enhance the conversion of LCA-Na into more hydrophilic (detergent) bile acids (Table 3).

In summary, therefore, it can be said that microtubules probably do not play a role in the transcellular transport of LCA-Na, or in LCA-Na-induced cholesterol transport. However, since colchicine interferes with the physiological transport of cholesterol to the bile canalicular membrane, thereby reducing the absolute amount present in the membrane, it is suggested that the total cholesterol content of the bile canalicular membrane is the important determinant in LCA-Na-induced cholestasis.

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