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Comparative Study of Colchicine and Trimethylcolchicinic Acid on Prolonged Bile Duct Obstruction in the Rat

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Key words: trimethylcolchicinic acid; colchicine; colchicinoids; liver damage; biliary obstruction; cirrhosis; ATPases; erythrocyte; plasma membrane; peroxidation

The aim of this work was to compare the effects of colchicine and trimethylcolchicinic acid (TMCA) on liver damage induced by bile duct ligation (BDL) for 2 months in male Wistar rats. Colchicine was evaluated at a dose of 10 μg rat^{-1} day^{-1}, p.o. only, because higher doses produced diarrhoea and death. Trimethylcolchicinic acid showed no toxic effects at 10, 50 or 100 μg rat^{-1} day^{-1}, p.o. Biliary obstruction resulted in a 65% mortality, colchicine decreased it to 46% and TMCA (10 μg) to 33.3%. Serum markers of liver damage increased by BDL ($P < 0.05$), colchicine prevented it partially ($P < 0.05$) and TMCA did it in a dose-dependent manner. Liver peroxidation increased 10 times by BDL and both drugs prevented it. Hepatic glycogen content decreased 80% by BDL, colchicine and TMCA (10 μg) failed to preserve it and 50 μg of TMCA preserved it completely. Hepatocyte and erythrocyte plasma membrane Na^+/K^+- and Ca^{2+}-ATPase activities decreased after BDL ($P < 0.05$) and 100 μg of TMCA preserved normal ATPase activities. It is concluded that TMCA is better than colchicine for protecting the liver from BDL-induced cirrhosis and, due to its lower toxicity, can be used at higher and more effective doses without the common side-effects of colchicine, thus making TMCA a suitable compound to be studied in other hepatic lesions and in humans.

INTRODUCTION

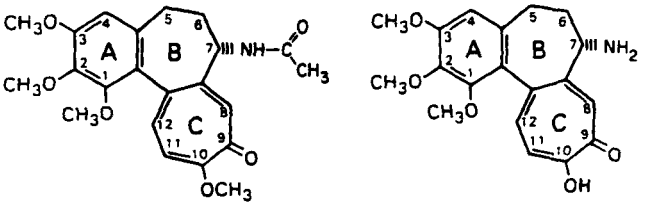
It has been demonstrated that bile duct obstruction for 15 days or more led to cirrhosis, and after 4 weeks this is found in the majority of the aninmals.[1]

The morphological changes are comparable to those in human biliary cirrhosis. The bile duct obstruction has been associated with portal hypertension indicated by splenomegaly[2] and with decompensated cirrhosis indicated by the development of ascites.[1] This obstructive model appears to have some advantages over the CCl_4 model[3] and may prove to be a useful tool for studying human cirrhosis. It is also a good model for the evaluation of drugs.[4]

On the other hand, it has been reported that colchicine treatment protects the liver of experimental animals against galactosamine, CCl_4 and acetaminophen intoxication.[5–8] In addition, Kershenobich *et al.*[9] have shown some beneficial effects of colchicine in the treatment of cirrhotic patients. Warnes *et al.*[10] also showed beneficial effects of colchicine in primary biliary cirrhosis.

However, diarrhoea is a common side-effect of colchicine even at therapeutic doses.[11] The beneficial effects of colchicine on human liver cirrhosis have been modest,[9,10] probably due to the small dose used (1 mg per day, 5 days a week), but higher doses cannot be used due to its toxicity.[11–14] The toxicity of colchicine has been attributed to its ability to bind microtubule protein.[13,14] Thus, an effort was made to obtain colchicine-like molecules which lack the ability to bind microtubule protein but conserve its effects. Trimethylcolchicinic acid (TMCA), a derivative of colchicine (Fig. 1), did not displace [^{3}H]colchicine from microtubule protein.[15] However, TMCA has never been studied in liver diseases.

On the basis of these considerations, the aim of the present work was to study some side-effects of colchicine and compare them with those of TMCA, as well as to compare the beneficial activities of both compounds on liver damage induced by bile duct ligation (BDL). In fact, we observed that TMCA is less toxic than colchicine and that it is capable of preventing liver damage induced by BDL.



COLCHICINE TRIMETHYLCOLCHICINIC ACID

Figure 1. Chemical structures of colchicine and trimethylcolchicinic acid.

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EXPERIMENTAL

Materials

Colchicine and trimethylcolchicinic acid were obtained from Sigma Chemical Co. (St. Louis, MO). The rest of the reagents were of the best quality commercially available.

Animal treatments and biliary obstruction

Male Wistar rats weighing around 200 g were used. Animals had free access to food (Standard Purina chow diet, St. Louis, MO) and water. Obstructive jaundice was induced by double ligation and division of the common bile duct. Control rats were sham operated. Colchicine and trimethylcolchicinic acid (TMCA) were dissolved in water and administered at different doses (as specified in the Results section) through an intragastric tube, daily after surgery. The animals were sacrificed after 2 months of biliary obstruction or sham operation under light ether anaesthesia; blood was collected by heart puncture and the liver was rapidly removed.

Small liver sections fixed in Bowin's solution were used for trichromic staining for histological examination under light microscopy.

Serum enzyme activities and bilirubins

Serum was obtained for the following determinations: the activities of alkaline phosphatase[16] and γ-glutamyl transpeptidase,[17] and for bilirubin content (Merck, Mexico).

Determination of hepatic lipid peroxidation and glycogen

Liver pieces were separated for glycogen quantification with the anthrone reagent.[18] Malondialdehyde (MDA) was determined in liver homogenates using the TBA method according to Ohkawa *et al.*[19]

Isolation of hepatocyte and erythrocyte plasma membranes

Hepatocyte plasma membranes were isolated according to the method described by Pohl and Birnbaumer.[20] The membranes were frozen in liquid nitrogen and for further use were resuspended in 20 mM imidazole (pH 7.8). Protein determinations were performed according to the method described by Bradford.[21] Preparation of erythrocyte membranes was performed according to the procedure described by Rose and Oklander.[22]

Quantification of ATPase enzyme activities

The activities of hepatocyte Na^+/K^+- and high affinity Ca^{2+}-ATPases were determined in liver plasma membranes according to the methods described by Ames[23] and Lotersztajn and Pecker,[24] respectively. The activities of erythrocyte Na^+/K^+ and Ca^{2+}-ATPases were determined in plasma membranes according to Lee and Au[25] and Ames.[23]

Extraction of lipids

The erythrocyte and hepatocyte plasma membrane lipids were extracted according to Folch *et al.*[26] The pure lipid solutions were evaporated under a stream of nitrogen and then were dissolved in a known volume of benzene. Cholesterol and the total lipid phosphorus content of phospholipids were determined in each extract using the methods described by Ames[23] and Abell *et al.*,[27] respectively.

For statistical analysis, ANOVA with the Tukey test modified by Spjotvoll and Stoline[28] was used in order to compare the groups. In the case of percentage mortality, a chi-squared test was performed. In all cases, a difference was considered significant when $P < 0.05$.

RESULTS

Administration of colchicine 10 μg rat^{-1} day^{-1} for 2 months to normal rats did not produce diarrhoea, but 50 μg rat^{-1} day^{-1} produced diarrhoea in all animals treated. Colchicine at a dose of 200 μg rat^{-1} day^{-1} produced, in addition to a severe diarrhoea, a 40% mortality after 2 months of treatment. Thus, in the following experiments, colchicine was administered only at a dose of 10 μg rat^{-1} day^{-1}.

Trimethylcolchicinic acid (TMCA) was administered to normal rats for 2 months at different doses: 10, 50 and 100 μg and 1 mg rat^{-1} day^{-1}; no diarrhoea or mortality was observed after 2 months of treatment at any dose used. Because of this, the effect of TMCA on liver damage induced by 2 months of biliary obstruction was evaluated at different doses until a full beneficial effect was observed in most markers of liver damage.

Liver damage induced by 4 weeks of biliary obstruction (BDL) was accompanied by an increase in collagen content around the central vein. Treatment of BDL rats with 10 μg of colchicine resulted in a partial improvement of histology. Trimethylcolchicinic acid preserved parenchymal architecture in a dose-dependent manner; the BDL group receiving 100 μg of TMCA showed less collagen content and resembled more the control group (not shown).

Table 1 shows mortality after BDL and its prevention by colchicine and TMCA. Rats that died in the first 24 h after surgery were not considered, because they probably died as a consequence of anaesthesia or surgery manipulation. As can be seen in Table 1, both drugs prevented mortality but the best effect was observed in the group receiving 50 μg of TMCA per rat per day; 100 μg did not further increase survival significantly.

Biliary obstruction increased total bilirubins about 50-fold over sham-operated rats. A similar effect was observed in unconjugated and conjugated bilirubins. Colchicine (10 μg) decreased the elevation of conjugated and unconjugated bilirubins by about 60%. Trimethylcolchicinic acid showed a dose-dependent response on bilirubins. Unconjugated bilirubins remained within the sham-operated group levels in the BDL group receiving 100 μg of TMCA (Table 2).

Table 1. Effect of colchicine (COLCH) and trimethylcolchicinic acid (TMCA) on mortality induced by 2 months of bile duct ligation (BDL)

Group	$(n_i)^a$	Mortality (%)	$(n_s)^b$
Sham-operated	15	6.7	14
BDL	15	66.7[c]	5
BDL + 10 μg COLCH	15	46.7[c]	8
BDL + 10 μg TMCA	15	33.3[c,d]	10
BDL + 50 μg TMCA	15	26.7[d]	11
BDL + 100 μg TMCA	15	20.0[d]	12

[a]n_i: initial number of rats.
[b]n_s: survived rats after 2 months of BDL.
[c]Means different from the Sham group, $P < 0.05$.
[d]Means different from the BDL group, $P < 0.05$.

Serum alkaline phosphatase and γ-glutamyl transpeptidase (γ-GT) activities are shown in Table 2. Alkaline phosphatase serum activity increased twofold while γ-GT increased sevenfold by biliary obstruction. Colchicine was not capable of preventing the increase in alkaline phosphatase but had a partial (although no significant) effect on γ-GT activity. Trimethylcolchicinic acid partially prevented both enzyme activities, but not significantly, when given at a 10 μg dose; however, total protection was observed at 50 or 100 μg doses ($P < 0.05$).

Hepatic lipid peroxidation (Fig. 2A) increased more than tenfold in BDL rats. Colchicine (10 μg) administration completely prevented this effect, while 10 μg of TMCA prevented it only partially. Fifty micrograms of TMCA is enough to prevent it completely. Hepatic glycogen content (Fig. 2B) decreased about fourfold in the BDL group. Colchicine did not prevent this effect significantly, whereas 10 μg of TMCA prevented glycogen depletion partially (but not significantly) and 50 or 100 μg preserved normal hepatic glycogen content.

Figure 3 depicts Na^+/K^+-ATPase activity in plasma membranes derived from erythrocytes (panel A) or hepatocytes (panel B). In both cell types, Na^+/K^+-ATPase decreased significantly by bile duct obstruction. Erythrocyte membrane Na^+/K^+-ATPase activity was preserved partially (but not significantly) by 10 μg of

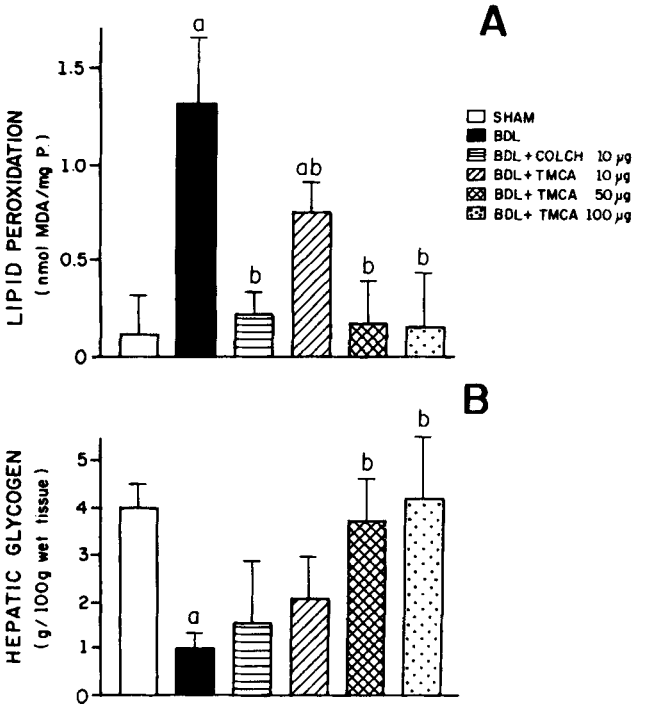

Figure 2. Lipoperoxidation (A) and glycogen content (B) determined in livers from sham-operated rats (SHAM), bile duct ligated rats (BDL) and BDL rats treated with colchicine (COLCH) or trimethylcolchicinic acid (TMCA). Each bar represents the mean ± SD in experiments performed in duplicate. [a]Means different from the SHAM group, $P < 0.05$. [b]Means different from the BDL group ($P < 0.05$).

colchicine or TMCA; however, 50 or 100 μg of TMCA preserved it completely. Colchicine (10 μg) and TMCA (10 μg) did not prevent the fall in hepatocyte Na^+/K^+-ATPase activity, but 50 μg of TMCA preserved it partially ($P < 0.05$) and 100 μg preserved it totally.

Calcium ATPase activity in plasma membranes derived from erythrocytes was decreased by about 50% by biliary obstruction (Fig. 4A). Colchicine failed to improve its activity. Administration of 100 μg of TMCA preserved Ca^{2+}-ATPase activity completely. Membrane hepatocyte Ca^{2+}-ATPase (Fig. 4B) decreased significantly in the BDL group too; again, colchicine was not capable of preserving it, and 100

Table 2. Bilirubins and enzyme activities of alkaline phosphatase (AP) and γ-glutamyl transpeptidase (γ-GT) determined in serum[a]

	Bilirubins (μmol/l)		Serum enzyme activities (μmol l^{-1} min^{-1})	
Group	Conjugated	Unconjugated	AP	γ-GT
SHAM	0.18 ± 0.07	0.32 ± 0.21	72.7 ± 16.0	4.2 ± 0.76
BDL	24.0 ± 8.1[b]	71.1 ± 19.7[b]	166.1 ± 31.4[b]	28.5 ± 5.1[b]
BDL + 10 μg COLCH	9.9 ± 4.2[b]	27.8 ± 12.0[b]	150.0 ± 40.2[b]	15.7 ± 7.8
BDL + 10 μg TMCA	11.6 ± 6.1[b]	5.5 ± 3.7[c]	98.9 ± 34.1	17.7 ± 9.3
BDL + 50 μg TMCA	4.2 ± 1.8[b,c]	2.4 ± 1.1[c]	56.4 ± 12.0[c]	8.1 ± 1.2[c]
BDL + 100 μg TMCA	2.7 ± 1.1[c]	1.48 ± 0.6[c]	72.0 ± 9.0[c]	7.0 ± 1.4[c]

[a]Each value represents the mean ± SD in experiments performed in duplicate; SHAM: sham-operated rats; BDL: bile duct ligated rats; TMCA: trimethylcolchicinic acid.
[b]Means different from the SHAM group, $P < 0.05$.
[c]Means different from the BDL group, $P < 0.05$.

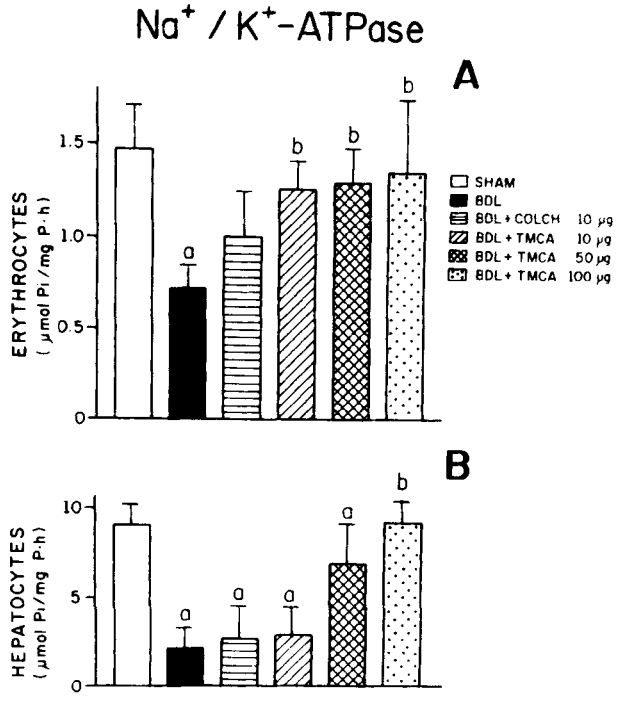

Figure 3. The Na^+/K^+-ATPase activity determined in erythrocyte (A) and hepatocyte (B) plasma membranes derived from sham-operated rats (SHAM), bile duct ligated rats (BDL) and BDL rats treated with colchicine (COLCH) or trimethylcolchicinic acid (TMCA). Each bar represents the mean ± SD in experiments performed in duplicate. [a]Means different from the SHAM group, $P < 0.05$. [b]Means different from the BDL group, $P < 0.05$.

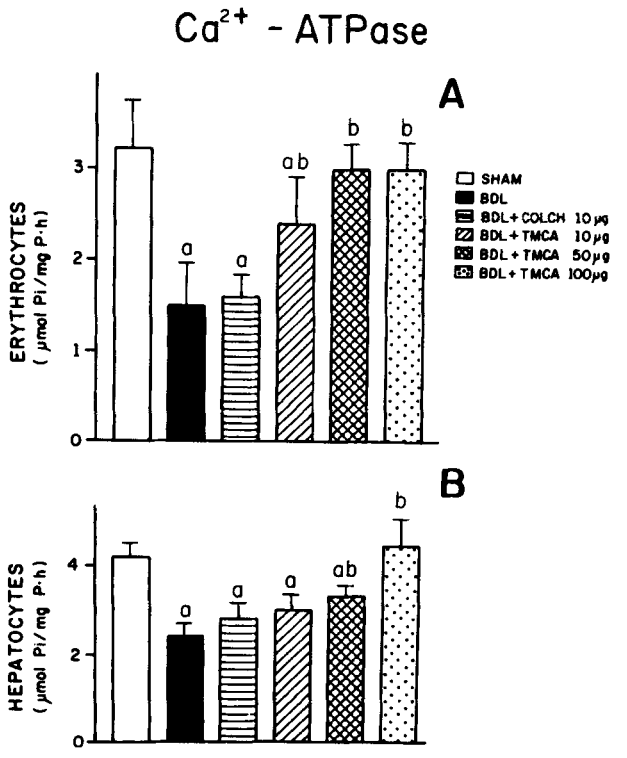

Figure 4. The Ca^{2+}-ATPase activity determined in erythrocyte (A) and hepatocyte (B) plasma membranes derived from sham-operated rats (SHAM), bile duct ligated rats (BDL) and BDL rats treated with colchicine (COLCH) or trimethylcolchicinic acid (TMCA). Each bar represents the mean ± SD in experiments performed in duplicate. [a]Means different from the SHAM group, $P < 0.05$. [b]Means different from the BDL group, $P < 0.05$.

µg of TMCA was needed to preserve completely its normal activity.

Table 3 shows erythrocyte plasma membrane cholesterol, total phospholipids and the cholesterol/ phospholipid (CH/PL) ratio. As can be seen, BDL increased cholesterol content and thus the CH/PL ratio ($P < 0.05$). Treatment with colchicine (10 µg) or TMCA (10 µg) preserved the normal cholesterol content and CH/PL ratio.

Biliary obstruction produced a decrease in cholesterol content and, as a result, a decrease in CH/PL ratio in plasma membranes derived from liver (Table 4); colchicine failed to preserve the cholesterol content. Fifty or 100 µg of TMCA maintained the normal cholesterol content and the CH/PL ratio.

Colchicine (10 µg) and TMCA (10, 50 and 100 µg and 1 mg rat^{-1} day^{-1}) administered daily to normal or sham-operated rats for 2 months did not modify significantly any of the determined parameters (data not shown).

DISCUSSION

Our results show that trimethylcolchicinic acid is less toxic than colchicine and thus better at preventing liver damage induced by bile duct ligation.

Colchicine, the major alkaloid in *Colchicum autumnale*[29] is best known for its antimitotic effects[13,14] and is medically used for the treatment of gout.[30] Besides its antitumour activity,[31,32] colchicine also has anti-inflammatory properties[32] and is useful in the treatment of familial Mediterranean fever.[33] In addition, colchicine protects the liver of experimental animals against several hepatotoxins[5–8] and shows some beneficial effects in humans.[9,10]

In light of the many pharmacological actions of colchicine, it seemed worthwhile studying whether these activities, in particular its beneficial effects on liver damage, were related to colchicine's affinity for tubulin, or possibly whether they were separable by chemical modifications of the molecule. This is particularly important because colchicine toxicity has been associated with its ability to bind microtubule protein.[13,14]

The chemistry of colchicine and tropolonic analogues has been summarized elsewhere.[29,34] Trimethylcolchicinic acid is an interesting compound because it did not displace [^{3}H]colchicine from rat brain microtuble protein.[15] In addition, historically it is important to note that TMCA was selected for clinical trials more than 20 years ago,[34] mainly on the basis of its lower toxicity (LD_{50} = 15 mg kg^{-1} in mice) in comparison to colchicine (LD_{50} = 0.095 mg kg^{-1} in mice).[35] However, until now, TMCA had never been proved as an agent to prevent liver damage. It is very important to distinguish TMCA from trimethylcolchicinic acid methyl ether (also abbreviated to TMCA), which has been demonstrated to be useful in various malignancies[36] but is very toxic[37] and displaces [^{3}H]colchicine from microtubule protein.[15]

Table 3. Cholesterol, phospholipids and cholesterol/phospholipid ratio determined in erythrocyte plasma membranes[a]

Group	Cholesterol (μmol mg^{-1} P)	Phospholipids (μmol mg^{-1} P)	Cholesterol/ phospholipids
SHAM	0.33 ± 0.024	0.65 ± 0.051	0.51 ± 0.034
BDL	0.42 ± 0.018[b]	0.67 ± 0.106	0.66 ± 0.039[b]
BDL + 10 μg COLCH	0.34 ± 0.034[c]	0.66 ± 0.091	0.52 ± 0.042[c]
BDL + 10 μg TMCA	0.34 ± 0.027[c]	0.65 ± 0.064	0.52 ± 0.029[c]
BDL + 50 μg TMCA	0.32 ± 0.029[c]	0.63 ± 0.088	0.51 ± 0.044[c]
BDL + 100 μg TMCA	0.33 ± 0.027[c]	0.66 ± 0.044	0.50 ± 0.029[c]

[a]Each value represents the mean ± SD in experiments performed in duplicate; SHAM: sham-operated rats; BDL: bile duct ligated rats; COLCH: colchicine; TMCA: trimethylcolchicinic acid.
[b]Means different from the SHAM group, $P < 0.05$.
[c]Means different from the BDL group, $P < 0.05$.

Table 4. Cholesterol, phospholipids and cholesterol/phospholipid ratio determined in hepatocyte plasma membranes[a]

Group	Cholesterol (μmol mg^{-1} P)	Phospholipids (μmol mg^{-1} P)	Cholesterol/ phospholipids
SHAM	0.31 ± 0.029	0.67 ± 0.049	0.46 ± 0.049
BDL	0.20 ± 0.040[b]	0.69 ± 0.056	0.29 ± 0.027[b]
BDL + 10 μg COLCH	0.21 ± 0.032[b]	0.68 ± 0.071	0.31 ± 0.019[b]
BDL + 10 μg TMCA	0.23 ± 0.032[b]	0.67 ± 0.058	0.34 ± 0.046[b]
BDL + 50 μg TMCA	0.30 ± 0.019[c]	0.66 ± 0.066	0.45 ± 0.044[c]
BDL + 100 μg TMCA	0.31 ± 0.022[c]	0.66 ± 0.064	0.47 ± 0.039[c]

[a]Each value represents the mean ± SD in experiments performed in duplicate; SHAM: sham-operated rats; BDL: bile duct ligated rats; COLCH: colchicine; TMCA: trimethylcolchicinic acid.
[b]Means different from the SHAM group, $P < 0.05$.
[c]Means different from the BDL group, $P < 0.05$.

In this work we used TMCA at doses ranging from 10 μg rat^{-1} day^{-1} to 1 mg rat^{-1} day^{-1} for 2 months without noticing any side-effects. Moreover, no more than 100 μg rat^{-1} day^{-1} was necessary to prevent completely the liver damage induced by BDL in the rat, thus showing that TMCA is safe at therapeutic doses. On the other hand, colchicine showed diarrhoea in all animals treated with 50 μg rat^{-1} day^{-1}; when 200 μg rat^{-1} day^{-1} was used a 40% mortality was observed. Owing to the high toxicity of colchicine, only the dose of 10 μg rat^{-1} day^{-1} was used in BDL rats. Colchicine at this dose showed partial action according to the majority of the liver damage markers employed, however its toxicity did not permit the dose to be increased in order to observe a better therapeutic effect. It was found that at the same dose (10 μg) both colchicinoids show quite the same effects in BDL rats.

It has been reported that the protective effects of some colchicinoids (at least colchicine) on liver damage induced by CCl_4 intoxication are due in part to cytochrome P-450 inhibition (and thus interfering with CCl_4 activation to the free radical $CCl_3\cdot$).[38] There is also indirect evidence that colchicine could be a good free-radical scavenger.[8] In order to avoid interfering with the mechanism of action of an external hepatotoxin (i.e. CCl_4), in this work we decided to use the model of liver damage induced by BDL, because it is not caused by lipid peroxidation processes[39] and it is independent of cytochrome P-450 activity.

It is important to emphasize that both colchicine and TMCA improved histology by reducing necrosis and collagen accumulation.

In addition to liver glycogen content, lipid peroxidation, serum enzyme activities and bilirubins, erythrocyte and hepatocyte Na^+/K^+- and Ca^{2+}-ATPase activities were also determined, due to their important roles in liver and erythrocyte functions: ATPases located in the plasma membrane of all animal cells maintain the characteristic intracellular concentration of ions and determine the transmembrane electrochemical gradient.[40] It is generally accepted that the electrochemical sodium gradient in the hepatocyte provides the driving force for Na^+-coupled transport of a variety of solutes,[41] including certain amino acids[42–44] and bile acids.[44,45] In addition, intracellular Ca^{2+} is essential in the regulation of a large number of liver enzymatic activities such as those participating in gluconeogenesis and glycogenolysis.[46] It is also well known that red cells are able to extrude Ca^{2+} ions across the plasma membrane against a large gradient by an ATP-fuelled pumping mechanism. It has been demonstrated that the mechanical and permeability properties of erythrocyte membrane depend upon the intracellular level of Ca^{2+} and ATP.[47] In addition, Na^+/K^+-ATPase is an integral part of the red cell membrane.[48] This pump is able to mediate a number of ouabain-sensitive transport processes, each with characteristic cation and substrate requirements.[49] Moreover, our previous studies[50] showed that erythrocytes are good indicators of hepatocyte function; the present results confirm these findings because erythrocyte alterations produced by BDL resembled their counterpart on hepatocytes.

It is worth noting that 100 μg rat^{-1} day^{-1} of TMCA completely preserves erythrocyte and hepatocyte plasma membrane Na^+/K^+- and Ca^{2+}-ATPases.

The beneficial effects of TMCA on ATPase activities could be explained by alterations in the regulatory mechanisms of each enzyme; however, it is also possible that the normalization of membrane lipid composition and fluidity is responsible for that improvement.[49] Several factors such as the lipid/protein ratio, amount of cholesterol, CH/PL ratio and degree of saturation of fatty acyl chains, among others, can modify the fluidity of the membrane.[51] On the other hand, all the observed beneficial effects could be explained by 'stabilization' of canalicular membranes by an unknown mechanism.

It is also interesting that 50 or 100 μg rat^{-1} day^{-1} of TMCA preserved liver glycogen. Previously we have reported that glycogen is exhausted in CCl_4-cirrhotic animals[52] and in BDL rats.[8] An association between cAMP and liver glycogen content has also been observed.[53] It is well known that an increase in cAMP activates phosphorylase and glycogen breakdown; thus, it seems likely that bile salts acting on hepatocyte plasma membrane could disturb adenylate cyclase activity, as CCl_4 does,[53] increasing cAMP levels and glycogen breakdown. Trimethylcolchicinic acid could be acting by preserving normal adenylate cyclase activity, as it does with ATPases; however, this point requires further investigation.

Colchicine (10 μg) was capable of preventing the increase in lipid peroxidation but it only partially preserved some of the markers of liver damage, indicating that lipid peroxidation by itself cannot fully explain liver damage induced by BDL. These results are in agreement with our previous findings that lipid peroxidation is a consequence rather than the cause of liver damage induced by BDL in the rat.[39] Sokol *et al.*[54] have shown an increase in lipid peroxidation 17 days after BDL, but we have observed that liver damage occurred earlier than lipid peroxidation and that vitamin E (400 IU kg^{-1} day^{-1}, p.o.) prevents lipid peroxidation but not liver injury.[39]

The mechanism by which colchicine or TMCA prevents liver damage remains to be investigated. However, it is known that colchicine inhibits collagen synthesis and favours the removal of scar tissue.[6,9,55,56] Colchicine is currently in use for the treatment of liver cirrhosis and has been shown to produce an improvement in liver function in most patients and to ameliorate fibrosis in some cirrhotics.[9,55,56]

Several actions reported for colchicine[56] may be due to its capacity to lower the concentration of plasma cholesterol and to revert the elevated cholesterol/phospholipid ratio (CH/PL) in liver plasma membranes of CCl_4-cirrhotic animals; accordingly, the decrease in ATPase activity in the CCl_4-cirrhotic animal is not due to a lack of enzyme protein; the enzyme could be present in a cryptic form due to the alteration in the CH/PL ratio.[57] However, this work shows that in BDL-cirrhotic animals, cholesterol is elevated in erythrocyte plasma membranes but is decreased in hepatocytes. An explanation for these results cannot be given with the present data; however, it is possible that bile salts accumulated in the liver solubilize membrane cholesterol, decreasing it in liver membranes but increasing it in serum and, in turn, increasing erythrocyte membrane cholesterol.

It has been reported that alterations in the CH/PL ratio are responsible for ATPase inactivation.[57] However, as can be seen in the results, in some groups (BDL + 10 μg of colchicine; BDL + 10 and 50 μg of TMCA) the CH/PL ratios were normal while ATPase activity remained below control values; this means that the CH/PL ratio by itself cannot completely explain ATPase activity. On the other hand, administration of 100 μg of TMCA to BDL rats completely preserved ATPase activity, showing that TMCA is not only acting by preserving CH/PL, but other effects must be considered. It is likely that TMCA possess some of the beneficial effects of colchicine (as their chemical formulae are quite similar; Fig. 1), such as inhibition of collagen synthesis and removal of scar tissue,[6,9,55,56] with the advantage that TMCA is less toxic and thus more suitable for use in humans. However, the beneficial mechanism of action of TMCA is not known at present, and further work is needed to understand it. The low toxicity of TMCA as compared with colchicine allows higher and more effective doses to be used without the common side-effects observed with colchicine treatment.[11–14] Nevertheless, much more work is needed in the fields of toxicology and pharmacology before recommending TMCA for human use.

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