Protective Activity of Silipide on Liver Damage in Rodents

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ABSTRACT—The activity of silipide, a silybin-phosphatidylcholine complex (IdB 1016), was tested in different models of liver damage in rodents. After oral administration, silipide exhibited a significant and dose-related protective effect against the hepatotoxicity induced by CCl₄, praseodymium, ethanol and galactosamine. The ED₅₀ values for inhibition of the rise in ASAT and ALAT levels caused by CCl₄ and praseodymium and for antagonism of the increase in liver triglycerides caused by ethanol ranged from 93 to 156 mg/kg (as silybin). At a dose of 400 mg/kg (as silybin), silipide was also active in protecting against paracetamol-induced hepatotoxicity. Silybin and phosphatidylcholine at doses equivalent to those contained in the active doses of silipide failed to show any significant protective activity in these models. The liver protective effect of silipide is probably related to its antioxidant activities and to a stimulating effect on the hepatic synthesis of RNA and proteins.

Keywords: Silybin, Silipide (IdB 1016), Hepatic intoxication, Flavonoids, Liver protective activity

Silybin is the main active flavonolignan constituent of silymarin, a purified extract from the seeds of *Silybum marianum* widely used for the treatment of hepatic disorders (1).

The pharmacological activity of silybin has been established in vitro in cultured hepatocytes and in vivo after parenteral administration (2–6). Silybin has also been reported to exhibit a scavenging and antiperoxidative activity as well as a stimulating effect on RNA polymerase I, with consequent enhancement of liver protein biosynthesis (7, 8). These properties can explain its protective effects in some experimental models of liver damage such as the hepatotoxicity induced by CCl₄, paracetamol and praseodymium.

When given by the oral route, silybin exhibits a low bioavailability (9, 10). Its complexation with phosphatidylcholine in a 1:1 molar ratio gives rise to silipide* (laboratory code IdB 1016, average molecular weight: 1260), a compound endowed with higher lipophilicity (11). Morazzoni et al. (12) demonstrated that after oral administration of silipide to rats, the plasmatic and the biliary levels of silybin were significantly higher than those obtained after administration of uncomplexed silybin.

The aim of the present study was to assess the effect

of silipide against liver damage induced by different agents such as CCl₄, praseodymium, ethanol, galactosamine and paracetamol, in comparison with the components of the complex, silybin and phosphatidylcholine.

MATERIALS AND METHODS

Animals

Male and female Sprague-Dawley rats, male Wistar rats and female Swiss mice were purchased from Nossan S.r.l. (Correzzana, Italy) and housed in air conditioned rooms at 21 ± 1°C, with free access to food (TR-M, Nossan) and water.

The animals were deprived of food but allowed free access to water for 16–24 hr before sacrifice.

Blood was withdrawn under ether light anesthesia. The animals were killed by an overdose of ether.

Test compounds

Silipide (purity grade >97%) and silybin were from Inverni della Beffa Research and Development Laboratories. Phosphatidylcholine was purchased from Sigma. The test compounds were suspended in water and given by gastric gavage at a volume of 10 ml/kg to rats and 20 ml/kg to mice.

*proposed INN

Materials

Carbon tetrachloride (CCl₄, Rudi Pont), ethanol (Rudi Pont), praseodymium nitrate pentahydrate (Pr, Fluka), paracetamol (Sigma), D-galactosamine hydrochloride (GalN, Merck), D-glucose, sodium chloride, methanol, chloroform (Merck), mineral oil (Sigma), ethyl ether (C. Erba) were used.

Biochemical analyses were performed by standard methods (Boehringer).

Experimental proceedings

Preliminary studies have been performed for each method in order to choose the best experimental conditions and the most suitable range of silipide doses. In the present paper, only the final experiments are reported.

Carbon tetrachloride-induced damage in rats

CCl₄ (3 ml/kg of a 20% solution in mineral oil) was given by gastric gavage to male Sprague-Dawley rats weighing 200–220 g. The dose of CCl₄ (0.6 ml/kg) was in the range of the doses reported by Torielli and Ugazio (13).

Test compounds or water were given by gastric gavage to groups of 20 rats once daily for three consecutive days, the first dose being administered 24 hr before CCl₄. A group of water-treated rats, used as non-intoxicated controls, received 3 ml/kg, p.o. of mineral oil instead of CCl₄.

Liver damage was evaluated 30 hr after CCl₄ administration by determining serum aspartate and alanine amino transferase (ASAT and ALAT) activities.

Praseodymium-induced liver damage in rats

Pr (10 mg/kg dissolved in 0.9% NaCl solution) was injected intravenously to male Wistar rats weighing 320–370 g, according to Strubelt et al. (5).

Test compounds or water were given by gastric gavage at the same time as Pr to groups of 12 rats. A group of water-treated rats received 1 ml/kg (i.v.) of saline instead of Pr.

Liver damage was evaluated 48 hr later by determining serum ASAT and ALAT activities, liver weight and hepatic triglycerides after Folch extraction (14).

Ethanol-induced liver damage in rats

Ethanol (6 g/kg as a 40% aqueous solution w/v) was given by gastric gavage to female Sprague-Dawley rats weighing 180–200 g, according to Baraona and Lieber (15).

Test compounds or water were given by gastric gavage to groups of 10 rats, twice daily for two days and once on the third day, the last dose being given 1 hr be-

fore ethanol administration. Two additional groups of rats received, instead of ethanol or water, 15 ml/kg of a solution of glucose isocaloric with ethanol.

Liver damage was evaluated 24 hr after ethanol administration by determining serum triglycerides, liver weight and hepatic triglycerides.

Galactosamine-induced liver damage in rats

GalN (500 mg/kg dissolved in saline) was injected intraperitoneally to female Sprague-Dawley rats weighing 220–240 g, according to Pickering et al. (16).

Test compounds or water were given by gastric gavage 4 hr before and 4 hr after GalN to groups of 12 rats. A group of water-treated rats received 2 ml/kg (i.p.) of saline instead of GalN.

Liver damage was evaluated 24 hr after GalN by determining serum ASAT and ALAT activities.

Paracetamol-induced liver damage in mice

Paracetamol (400 mg/kg suspended in saline) was injected intraperitoneally to female mice weighing 20–24 g, according to Hjelle et al. (17). The mice were chosen since they are more susceptible than rats to hepatic damage induced by paracetamol (18).

Test compounds or water (20 ml/kg) were given by gastric gavage to groups of 20 mice 1 hr before paracetamol injection. A group of water-treated mice received 10 ml/kg, i.p. of saline instead of paracetamol.

Liver damage was evaluated 48 hr after paracetamol by determining serum ASAT and ALAT activities.

Statistical analyses

Data were expressed as the mean $\pm$ S.E. When a homogeneity of variances was found in Bartlett's test, the statistical significance of differences was evaluated by ANOVA, and differences between the control and treated groups were evaluated by Dunnett's *t*-test or Duncan's multiple range test.

When data showed heterogeneity of variances, Kruskal-Wallis non parametric ANOVA and Dunn's *t*-test were used.

ED₅₀ values were calculated by interpolation when significant log dose-effect relationships were found in the least square or weighed least square regression analysis.

RESULTS

Effect against CCl₄-induced liver damage in rats

Oral administration of CCl₄ in rats induced a significant increase in serum ASAT and ALAT activities in comparison with the controls.

Silipide given by gastric gavage at doses of 25, 82.5

and 250 mg/kg (as silybin) once daily for three consecutive days showed a dose-related protective activity (Table 1). ED₅₀ values (expressed as silybin) were 105 (56–142) mg/kg for ASAT and 132 (66–185) mg/kg for ALAT.

Silybin (25 and 250 mg/kg) and phosphatidylcholine (400 mg/kg, which is equivalent to the amount present in a dose of the complex containing 250 mg of silybin) did not show any significant protective activity.

Effect against praseodymium-induced liver damage in rats

Intravenous injection of Pr induced severe liver damage, as indicated by significant increases in ASAT,

ALAT and hepatic triglycerides.

Single doses of silipide (100 to 800 mg/kg, as silybin), given by gastric gavage at the same time as Pr, significantly reduced serum transaminase activities in a dose-related manner (Table 2). ED₅₀ values (expressed as silybin) were 93 (27–166) mg/kg for ASAT and 156 (52–240) mg/kg for ALAT. At doses of 400 and 800 mg/kg, silipide also reduced significantly the accumulation of triglycerides in the liver by about 60%.

Silybin (100 or 800 mg/kg) did not show any protective activity against Pr-induced liver damage.

Phosphatidylcholine, up to a dose of 320 mg/kg (which is higher than that contained in the ED₅₀ of the complex), was also devoid of activity in the same model.

Table 1. Effects of silipide, silybin and phosphatidylcholine against CCl₄-induced liver damage in rats

Treatment	Dose mg/kg × 3 p.o.	Serum enzymes			
		U/l	ASAT ED ₅₀ mg/kg	U/l	ALAT ED ₅₀ mg/kg
Controls	—	87.2 ± 3.9**		22.0 ± 1.2**	
CCl ₄	—	335.1 ± 38.3		123.9 ± 19.8	
Silipide (as silybin) + CCl ₄	25	300.0 ± 55.0		127.3 ± 30.9	
	82.5	238.0 ± 24.3	105	89.7 ± 13.0	132
	250	145.1 ± 10.8**	(56–142)	51.5 ± 6.4*	(66–185)
Silybin + CCl ₄	25	261.9 ± 31.1		97.3 ± 16.3	
	250	249.8 ± 34.1		107.3 ± 21.4	
Phosphatidylcholine + CCl ₄	400	233.5 ± 29.5		94.6 ± 14.7	

Test compounds were given by gastric gavage to rats once daily for 3 consecutive days starting 24 hr before administration of CCl₄ (3 ml/kg of a 20% solution in mineral oil, p.o.). Liver damage was evaluated 30 hr after CCl₄. Each value represents the mean ± S.E. for 20 rats. *P < 0.05, **P < 0.01 vs. CCl₄ group, Dunnett's *t*-test. The doses producing a 50% reduction (ED₅₀ values) of the rise in ASAT and ALAT were interpolated from log dose-effect plots calculated by weighed least squares regression. Numbers in brackets indicate 95% confidence limits.

Table 2. Effects of silipide, silybin and phosphatidylcholine against praseodymium (Pr) induced liver damage in rats

Treatment	Dose mg/kg p.o.	Serum enzymes			
		U/l	ASAT ED ₅₀ mg/kg	U/l	ALAT ED ₅₀ mg/kg
Controls	—	114.0 ± 5.9**		28.2 ± 1.1**	
Pr	—	2070.0 ± 273.6		1842 ± 380.0	
Silipide (as silybin) + Pr	100	1470.7 ± 375.3		1428.5 ± 430.7	
	200	649.2 ± 118.3		725.0 ± 163.1	
	400	463.3 ± 147.7**	93	447.0 ± 190.4**	156
	800	186.1 ± 16.2**	(27–166)	77.3 ± 8.2**	(52–240)
Silybin + Pr	100	3728.3 ± 606.6		3410.0 ± 669.1	
	800	2912.4 ± 740.2		1901.8 ± 433.9	
Phosphatidylcholine + Pr	160	2036.7 ± 435.2		2296.7 ± 565.9	
	320	1441.7 ± 260.5		1498.3 ± 235.6	

Test compounds were given by gastric gavage at the same time as praseodymium (10 mg/kg, i.v.). Liver damage was evaluated 48 hr after dosing. Each value represents the mean ± S.E. for 12 rats. **P < 0.01 vs. praseodymium group, Dunn's *t*-test. The doses producing a 50% reduction (ED₅₀ values) of the rise in ASAT and ALAT were interpolated from log dose-effect plots calculated by weighed least squares regression. Numbers in brackets indicate 95% confidence limits.

Effect against ethanol-induced damage in rats

A single oral dose of ethanol (6 g/kg) induced a significant increase in liver triglycerides, whereas liver weight and serum triglycerides did not differ significantly from those observed in non-intoxicated rats. Isocaloric treatment with glucose did not modify hepatic triglycerides.

Silipide, given at doses ranging from 100 to 400 mg/kg (as silybin) for five repeated administrations, antagonized dose-dependently the accumulation of triglycerides in the hepatic tissue (Table 3). The calculated ED₅₀ value was 148 (81–273) mg/kg (as silybin).

Silybin (200 or 400 mg/kg) and phosphatidylcholine (320 and 640 mg/kg, which correspond to the amounts contained in the active doses of the complex) did not

show any protective activity.

Effect against galactosamine-induced liver damage

A single dose of GalN (500 mg/kg) by the intraperitoneal route induced a significant increase in ASAT and ALAT activities.

Silipide, given by gastric gavage at doses of 400 and 800 mg/kg (as silybin) 4 hr before and 4 hr after GalN, antagonized the GalN-induced increases in ASAT and ALAT activities by about 50 and 70%, respectively. The reduction obtained with the higher dose was statistically significant (Table 4).

Silybin (400 and 800 mg/kg) and phosphatidylcholine (640 and 1280 mg/kg) did not show any significant protective activity against GalN-induced liver damage.

Table 3. Effects of silipide, silybin and phosphatidylcholine against ethanol-induced liver damage in rats

Treatment	Dose mg/kg × 5 p.o.	Serum Triglycerides mg/100 ml	Liver		
			Weight g	Triglycerides mg/100 g	ED ₅₀ mg/kg
Controls + Water	—	82.5 ± 6.4	5.37 ± 0.19	2370 ± 175	
Controls + Glucose	—	89.6 ± 5.9	5.23 ± 0.14	2377 ± 408	
Controls + Ethanol	—	115.1 ± 11.6	5.81 ± 0.12	3802 ± 439 ^a	
Silipide (as silybin) + Ethanol	100	118.6 ± 7.7	6.05 ± 0.19	3447 ± 472	
	200	106.0 ± 9.7	5.71 ± 0.08	2745 ± 329	148
	400	100.8 ± 6.8	6.04 ± 0.11	2370 ± 243 ^b	(81–273)
Silybin + Ethanol	200	106.1 ± 5.3	5.91 ± 0.14	3572 ± 425	
	400	185.8 ± 46.6	6.21 ± 0.13	4341 ± 473	
Phosphatidylcholine + Ethanol	320	110.1 ± 6.7	5.68 ± 0.21	4890 ± 945	
	640	113.9 ± 9.8	6.10 ± 0.22	3967 ± 741	

Test compounds were given by gastric gavage twice daily for two days and once on the third day, the last dose being given 1 hr before the oral administration of ethanol (6 g/kg). Liver damage was evaluated 24 hr after ethanol. Each value represents the mean ± S.E. for 10 rats. ^a = $P < 0.05$ vs. water controls, ^b = $P < 0.05$ vs. ethanol treated group. Dunnett's *t*-test. The dose producing a 50% reduction (ED₅₀ value) of the rise in liver triglycerides was interpolated from log dose-effect plots calculated by least squares regression. Numbers in brackets indicate 95% confidence limits.

Table 4. Effects of silipide, silybin and phosphatidylcholine against galactosamine (GalN)-induced liver damage in rats

Treatment	Dose mg/kg × 2 p.o.	Serum enzymes	
		ASAT U/l	ALAT U/l
Controls	—	109 ± 16 ^{**}	26 ± 2 ^{**}
GalN	—	4304 ± 602	2953 ± 433
Silipide (as silybin) + GalN	400	1908 ± 261	1438 ± 215
	800	1180 ± 258 ^{**}	849 ± 189 ^{**}
Silybin + GalN	400	2478 ± 381	1713 ± 293
	800	2602 ± 763	1618 ± 508
Phosphatidylcholine + GalN	640	2500 ± 452	1537 ± 410
	1280	2747 ± 397	2078 ± 392

The compounds were given by gastric gavage at 4 hr before and 4 hr after GalN (500 mg/kg, i.p.). Liver damage was evaluated 24 hr after GalN. Each value represents the mean ± S.E. for 12 animals. ^{**} $P < 0.01$ vs. GalN group. Dunn's *t*-test.

Fig. 1. Effect of silipide, silybin and phosphatidylcholine against paracetamol-induced liver damage in mice. Test compounds were given by gastric gavage 1 hr before paracetamol (400 mg/kg, i.p.). Liver damage was evaluated 48 hr after paracetamol. Each bar represents the mean $\pm$ S.E. for 20 mice. Values of serum enzymes (U/l) in non-intoxicated mice were: 90.5 ± 7.9 (ASAT) and 48.8 ± 6.9 (ALAT). * $P < 0.05$ vs. paracetamol group, Duncan's multiple range test. $\square$ ASAT, ▨ ALAT.

Effect against paracetamol-induced liver damage in mice

A single dose of paracetamol (400 mg/kg) by the intraperitoneal route induced a significant increase in ASAT and ALAT activities.

A single dose of silipide (400 mg/kg, as silybin) given by gastric gavage antagonized significantly the increase in both ASAT and ALAT activities (Fig. 1).

On the contrary, silybin (400 mg/kg) and phosphatidylcholine (640 mg/kg) were devoid of any protective activity in this model.

DISCUSSION

Silipide, given by the oral route to rats at doses ranging from 25 to 800 mg/kg (as silybin), showed a significant and dose-related protective activity against liver damage induced by toxic agents with different mechanisms of action such as CCl_4 , praseodymium, ethanol and galactosamine. The range of doses varied according to the severity of the experimental damage and the sensitivity to the protective action of silipide. ED_{50} values for the inhibition of the rise in serum enzymes caused by CCl_4 and praseodymium and for the antagonism of the increased hepatic triglyceride content caused by ethanol ranged from 93 to 156 mg/kg (as silybin). Silipide was also active in protecting against paracetamol-induced liver damage in mice.

Under the same experimental conditions, uncom-

plexed silybin and phosphatidylcholine were devoid of any significant activity at doses equivalent to those contained in the active doses of silipide.

The protective activity of silipide against the liver damage induced by CCl_4 , ethanol and paracetamol could be related to its antioxidant property and inhibiting effect on membrane lipid peroxidation, believed to be involved in the damaging effect of the three xenobiotics used (6, 19–21). Comoglio et al. (22) recently reported that silipide given by the oral route to rats antagonized the lipid peroxidation induced in liver microsomes by NADPH, CCl_4 and cumene hydroperoxide and acted as an effective free radical scavenger towards different types of radical species. Also the activity against liver injury induced by galactosamine, which produces a damage similar to that observed in human hepatitis (23, 24), partly can be ascribed to the same mechanism of action. Some authors indeed reported that the damage induced by galactosamine/endotoxin can be inhibited by a pretreatment with the free radical scavengers (25).

A stimulating effect on the hepatic synthesis of RNA and proteins could be also involved in the silipide protective action against the decrease in liver RNA in protein synthesis caused by galactosamine (26). This stimulating effect could be also the mechanism of action underlying the protective activity of silipide against praseodymium-induced damage as reported for silybin

(5).

The significant dose-related activities of silipide confirm that this compound is well-absorbed after oral administration in rodents, giving rise to significant plasmatic and biliary levels of silybin (12). These findings are in agreement with those obtained by Comoglio et al. (22), who documented that the complex after oral administration to rats is able to produce an active concentration of silybin at intracellular sites of the liver. The oral efficacy of silipide and the lack of activity of the uncomplexed silybin indicate that silybin reaches active concentrations at the site of action only when administered as a complex, in agreement with the results of pharmacokinetic studies in animals and in humans (12, 27). Acute, sub-acute and chronic toxicity studies in rats, dogs and monkeys revealed that complexation with phosphatidylcholine does not modify the excellent tolerability of orally administered silybin (M.J. Magistretti, an unpublished study).

Favorable results of preliminary clinical trials (28, 29) indicate that silipide can be considered as a promising new therapeutic agent for the treatment of hepatic disorders.

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