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Importance of the route of administration of CCl_4 in the protective effect of promethazine

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RECENTLY, it was shown that 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) decreased carbon tetrachloride (CCl_4) tissue concentration during the first 2 hr after oral administration of the halogenated hydrocarbon in rats.[1] This was interpreted as an inhibition of SKF 525-A on the gastrointestinal absorption of CCl_4.[1,2] Since SKF 525-A protects against the hepatotoxicity of CCl_4, it was proposed that the decrease in CCl_4 concentration in the liver could play a role in the protective effect of SKF 525-A;[3] this hypothesis was not incompatible with the possible inhibition of SKF 525-A on the metabolism of CCl_4 to form an hepatotoxic metabolite.[4,5] It was then of interest to study the effect of promethazine on CCl_4 liver and blood concentrations since the drug, which is chemically related to SKF 525-A, is believed to protect against CCl_4 hepatotoxicity through another mechanism of action, namely its antioxidant properties.[6]

Sprague–Dawley male rats, weighing between 160 and 200 g, were fasted for 18 hr before administration of promethazine hydrochloride, 25 mg/kg, i.p., and CCl_4, 2 ml/kg, administered either i.p or per os. At different times after treatment, rats were sacrificed by decapitation and blood was collected. Serum glutamic pyruvic transaminase activity (SGPT) was determined according to the method of Reitman and Frankel[7] (one Sigma Frankel unit will form $4{\cdot}82 \pm 10^{-4}$ μmoles of glutamate/min at pH 7·5 and 25°). A piece of liver was rapidly taken out and glucose 6-phosphatase activity (G-6-Pase) was measured as previously described.[3] In another series of experiments, the same protocol was followed except that $^{14}CCl_4$, 2 ml (0·03 mCi)/kg, was administered i.p. or per os and determinations of ^{14}C were made as previously described.[1] We made the assumption that toluene-soluble ^{14}C is $^{14}CCl_4$. Significance of the difference between control and treated rats was assessed by the *t*-test and a P value of 0·05 or less was considered significant.

TABLE 1. EFFECT OF PROMETHAZINE, 25 mg/kg, I.P., ON BLOOD AND LIVER CONCENTRATION OF CCl_4, 2·0 ml/kg, ADMINISTERED SIMULTANEOUSLY

Treatment	Time after treatment (hr)	Liver CCl_4 (μg/g ± S. E.)	Blood CCl_4 (μg/ml ± S. E.)
		CCl_4, administered orally	
Saline (6)*	2	445·5 ± 62·1	77·5 ± 12·3
Promethazine (6)		190·7 ± 51·9†	31·9 ± 9·3†
Saline (6)	3	265·7 ± 21·3	84·6 ± 5·5
Promethazine (6)		143·4 ± 35·5†	40·1 ± 10·1†
Saline (6)	6	229·1 ± 17·6	68·2 ± 6·6
Promethazine (6)		212·9 ± 49·9	62·7 ± 14·6
		CCl_4, administered intraperitoneally	
Saline (7)	2	1852·3 ± 191·2	82·1 ± 13·8
Promethazine (7)		1572·9 ± 216·9	124·9 ± 7·8†
Saline (7)	3	1661·2 ± 148·3	75·4 ± 5·9
Promethazine (7)		1548·5 ± 109·0	109·8 ± 9·7†
Saline (6)	6	917·9 ± 102·5	56·5 ± 5·0
Promethazine (6)		672·9 ± 44·3	99·6 ± 8·3†

* Number in parentheses refers to the number of animals in each group.
† $P < 0{\cdot}05$.

TABLE 2. EFFECT OF PROMETHAZINE, 25 mg/kg, I.P., ON THE HEPATOTOXICITY OF CCl_4, 2·0 ml/kg, ADMINISTERED SIMULTANEOUSLY

Treatment	Time after treatment (hr)	SGPT (units ± S. E.)	Comparison of treatments (P)	G-6-Pase (μg/g/20 min ± S. E.)	Comparison of treatments (P)
		CCl_4, administered orally			
Saline + saline (7)*	2	58·7 ± 2·8	1–2 < 0·05	12·0 ± 0·5	1–2 < 0·05
Saline + CCl_4 (7)		72·3 ± 2·6	2–3 < 0·05	5·9 ± 0·2	2–3 < 0·05
Promethazine + CCl_4 (7)		56·7 ± 2·1	1–3 NS†	8·6 ± 0·4	1–3 < 0·05
Saline + saline (10)	3	62·9 ± 3·2	1–2 < 0·05	10·0 ± 0·4	1–2 < 0·05
Saline + CCl_4 (10)		92·0 ± 3·9	2–3 < 0·05	5·1 ± 0·2	2–3 < 0·05
Promethazine + CCl_4 (9)		71·2 ± 1·8	1–3 < 0·05	6·8 ± 0·3	1–3 < 0·05
Saline + saline (6)	6	52·5 ± 3·1	1–2 < 0·05	10·8 ± 0·6	1–2 < 0·05
Saline + CCl_4 (6)		348·3 ± 40·2	2–3 NS	3·6 ± 0·1	2–3 NS
Promethazine + CCl_4 (6)		241·8 ± 40·5	1–3 < 0·05	3·9 ± 0·2	1–3 < 0·05
		CCl_4, administered intraperitoneally			
Saline + saline (7)	2	50·1 ± 2·6	1–2 < 0·05	11·2 ± 1·1	1–2 < 0·05
Saline + CCl_4 (7)		178·5 ± 42·5	2–3 NS	5·1 ± 0·3	2–3 NS
Promethazine + CCl_4 (7)		133·9 ± 16·9	1–3 < 0·05	4·5 ± 0·3	1–3 < 0·05
Saline + saline (7)	3	51·9 ± 3·2	1–2 < 0·05	11·9 ± 0·7	1–2 < 0·05
Saline + CCl_4 (7)		223·9 ± 31·5	2–3 NS	6·0 ± 0·5	2–3 < 0·05
Promethazine + CCl_4 (7)		401·4 ± 95·8	1–3 < 0·05	4·4 ± 0·4	1–3 < 0·05
Saline + saline (7)	6	59·0 ± 3·3	1–2 < 0·05	8·8 ± 0·7	1–2 < 0·05
Saline + CCl_4 (7)		692·1 ± 96·0	2–3 < 0·05	3·4 ± 0·2	2–3 NS
Promethazine + CCl_4 (7)		976·3 ± 34·4	1–3 < 0·05	3·9 ± 0·3	1–3 < 0·05

* Number in parentheses refers to the number of animals in each group.
† NS = not significant.

Because of the close chemical similarity between SKF 525-A and promethazine, it was not surprising to find that the antihistaminic decreased blood and liver CCl_4 concentration when CCl_4 was administered orally, 2 and 3 hr after promethazine treatment (Table 1). As was reported in animals pretreated with SKF 525-A,[1] there was no difference between control and treated rats 6 hr after injection of the antihistaminic. Using SGPT and G-6-Pase as early indexes of hepatotoxicity, promethazine administration gave partial protection against the hepatotoxicity of CCl_4 administered orally, 2 and 3 hr after promethazine treatment (Table 2). By the sixth hr, the protection disappeared (Table 2). Three hr after CCl_4 oral administration, Cignoli and Castro[8] found no difference in G-6-Pase between control and promethazine-treated rats. In experiments not reported here, promethazine alone had no effect on SGPT or G-6-Pase.

In order to assess more precisely the possible role of delayed gastrointestinal absorption of CCl_4 in the protective effect of promethazine, CCl_4 was administered intraperitoneally instead of orally. The same promethazine treatment had little effect on the hepatotoxicity of i.p. CCl_4, 2 hr after injection (Table 2). Three and 6 hr after administration of CCl_4, there were even indications of increase in toxicity in rats treated with promethazine as indicated by SGPT and, to a certain degree, G-6-Pase. Although the G-6-Pase activity of the liver is a sensitive test, it is difficult to establish a good correlation between G-6-Pase activity and the dose of CCl_4.[3,9,10] Parallel to this absence of protection by promethazine, when CCl_4 was administered i.p., CCl_4 liver concentrations were similar in both groups (Table 1). Contrary to what had been found after oral administration of CCl_4, an increase in CCl_4 blood concentration was found in rats treated with promethazine. We have observed an increase in blood and liver drug concentrations after i.v. and i.p. administration of sulfacetamide in rats treated with SKF 525-A.[11]

The close parallelism between the decrease in CCl_4 liver concentration and the protection against CCl_4 hepatotoxicity indicates that CCl_4 liver concentration may play a role in the protective effect of promethazine. However, the increase in the hepatotoxicity of CCl_4 administered intraperitoneally in rats treated with promethazine cannot be explained by an increase in CCl_4 liver concentration although more $^{14}CCl_4$ was found in the blood of animals treated with promethazine. It is surprising that increase in blood CCl_4 concentration is not associated with a parallel augmentation in liver CCl_4 concentration.[1]

Whatever may be the role of CCl_4 liver concentration in the hepatotoxicity of CCl_4, these observations are difficult to reconcile with the hypothesis that the antioxidant properties of promethazine are playing a major role in the protective effect against CCl_4 hepatotoxicity.[6,12,13] It will be interesting to study the effect of promethazine not only on the early[13] but on late[14] changes in liver biochemistry and morphology after intraperitoneal administration of CCl_4.

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