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PROTECTION BY 2-DIETHYLAMINOETHYL-2,2-DIPHENYLVALERATE HYDROCHLORIDE AGAINST CARBON TETRACHLORIDE HEPATOTOXICITY

A POSSIBLE MECHANISM OF ACTION

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Abstract—Recently, it was found that pretreatment with SKF 525-A decreases the concentrations of CCl_4 in the liver and other tissues after oral administration of the halogenated hydrocarbon. This was interpreted as a possible explanation for the protective effect of SKF 525-A on the hepatotoxicity of CCl_4. To test this hypothesis, the relationship between the liver concentration of CCl_4 and its hepatotoxicity has been studied. Rats were given an oral dose of $^{14}CCl_4$, which produced liver concentrations similar to those found in rats given twice the dose of $^{14}CCl_4$ but pretreated with SKF 525-A. A comparative study of the toxicity of CCl_4 revealed a good correlation between CCl_4 liver concentration and both serum glutamic pyruvic transaminase activity and hepatic triglycerides. There seemed to be no correlation with liver glucose 6-phosphatase activity. It is suggested that the protective effect of SKF 525-A on CCl_4 hepatotoxicity can be explained by a decrease in liver CCl_4 concentration.

RECENTLY, Marchand *et al.*[1] showed that pretreatment of rats with 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) 40 min before oral administration of carbon tetrachloride (CCl_4) decreased the concentrations of CCl_4 in the blood, liver, fat, muscle and brain during the first 2 hr after gavage. Furthermore, rats pretreated with SKF 525-A expired less CCl_4 than control animals. Since almost all of a dose of CCl_4 is excreted through the lungs[2] and there was no shift in organ distribution, these observations were interpreted as being due to an inhibitory effect of SKF 525-A on the absorption of CCl_4 from the gastrointestinal tract.

Slater *et al.*[3] have reported that pretreatment with SKF 525-A protects against the hepatotoxicity of CCl_4. This observation was confirmed by Castro *et al.*,[4] who attributed the protective effect of SKF 525-A to the inhibition of microsomal enzymes responsible for the metabolism of CCl_4 to a proposed active metabolite.[5,6] However, the present study indicates that the decrease in CCl_4 concentration in the liver of rats pretreated with SKF 525-A could well explain the protective effect of this last compound.

METHODS

Male Sprague–Dawley rats, fasted for 18 hr and weighing between 190 and 250 g, were pretreated with SKF 525-A, i.p., 40 mg/kg; control animals received saline. Forty min later, animals were gavaged with undiluted CCl_4. After 6 hr, the rats were

killed by decapitation and the blood was collected. Serum glutamic pyruvic transaminase (SGPT) activity was determined according to the method of Reitman and Frankel[7] (1 Sigma Frankel unit will form $4{\cdot}82 \times 10^{-4}$ μmoles of glutamate/min at pH 7·5 and 25°). Two aliquots of liver were rapidly removed; one was frozen for later determination of triglyceride according to the method of Butler *et al.*,[8] while the other aliquot was immediately used for the determination of glucose 6-phosphatase activity (G-6-Pase). The activity of this enzyme was determined by homogenizing 100–200 mg tissue in 0·1 M tris–maleate buffer (pH 6·2). Final conditions for the enzyme assay were as follows: 20 μmoles tris–maleate buffer (pH 6·2); 13·4 μmoles glucose 6-phosphate (pH 6·2); 0·2 ml of liver homogenate (20 mg/ml). The final volume of 1·1 ml was incubated for 20 min at 37° in a Dubnoff metabolic shaker in a gas phase of air. The reaction was terminated by the addition of 5·0 ml of 10% trichloroacetic acid. Inorganic phosphorus was determined in 2·0-ml aliquots of the supernatant by the method of Fiske and Subbarow,[9] as modified by Gomori.[10]

In another series of experiments, 40 min after pretreatment with SKF 525-A or saline, as above, the rats were gavaged with $^{14}CCl_4$, 2 ml/kg (0·1 mc/kg) or 1 ml/kg (0·05 mc/kg; New England Nuclear, Boston, Mass.). Control rats were given water orally. One hr later, the rats were killed by decapitation and a piece of liver and both kidneys were removed, weighed and put into 10 ml toluene and kept at 4°, with occasional shaking, for 3 days before measuring the radioactivity. Taking aliquots of toluene at different times showed the radioactivity extracted from the tissues to be maximal at 3 days.[1] Using tissue homogenates, Dingell and Heimberg[11] reported values of the same order. We made the assumption that toluene-soluble ^{14}C is $^{14}CCl_4$.

RESULTS

Pretreatment with SKF 525-A caused a significant decrease in liver CCl_4 concentration 1 hr after administration of the halogenated hydrocarbon (Table 1). This effect was apparent whether 2 or 1 ml/kg of CCl_4 was administered. It is of interest that the CCl_4 concentration in the kidney was much lower than that in the liver. The effect of SKF 525-A on the CCl_4 concentration in the kidneys was comparable to that

TABLE 1. EFFECT OF PRETREATMENT WITH SKF 525-A ON TISSUE LEVELS OF $^{14}CCl_4$ 1 hr AFTER ORAL ADMINISTRATION

Dose $^{14}CCl_4$ (ml/kg)	Pretreatment†	$^{14}CCl_4$ (μg/g tissue ± S.E.)*	
		Liver	Kidney
2	Saline	519·4 ± 30·8	187·4 ± 5·6
2	SKF 525-A	213·3 ± 53·4‡	81·0 ± 18·7‡
1	Saline	314·3 ± 18·2‡	123·6 ± 8·8‡
1	SKF 525-A	122·4 ± 29·3‡§	43·1 ± 10·4‡§

* Each value is the mean from seven rats.
† SKF 525-A, 40 mg/kg, i.p., was given 40 min before $^{14}CCl_4$.
‡ $P < 0{\cdot}001$ with respect to control group given $^{14}CCl_4$, 2·0 ml/kg.
§ $P < 0{\cdot}001$ with respect to control group given $^{14}CCl_4$, 1·0 ml/kg.

found in the liver. It has previously been shown that the CCl_4 concentrations in the blood and in a given tissue are proportional to one another.[1]

As expected, treatment with CCl_4 caused a marked increase in SGPT activity (Fig. 1). There was a good correlation between the dose of CCl_4 (1·0 and 2·0 ml/kg) and the rise in SGPT activity. In a separate experiment, it was possible to show that a good correlation existed between various doses of CCl_4 and the elevation in SGPT activity at 6 hr (Table 2). Pretreatment with SKF 525-A was associated with a marked drop in SGPT activity in rats gavaged with CCl_4 (Fig. 1). The SGPT activity measured in the SKF 525-A-treated animals given 2·0 ml/kg of CCl_4 was found to be similar to that in rats receiving 1·0 ml/kg without pretreatment with SKF 525-A. It must be pointed out here that SKF 525-A alone caused a small but significant increase in the enzyme activity. This observation is consistent with the morphological and biochemical changes observed in rats receiving SKF 525-A.[4,12]

SKF 525-A is a well known inhibitor of drug-metabolizing enzyme activity. The possibility arose that its protection against CCl_4-induced SGPT elevation might be due to a direct inhibition of serum enzyme activity by the SKF 525-A or its metabolites remaining in the serum at 6 hr. We mixed plasma obtained from rats treated with only SKF 525-A with plasma from rats treated with only CCl_4. The plasma from the SKF

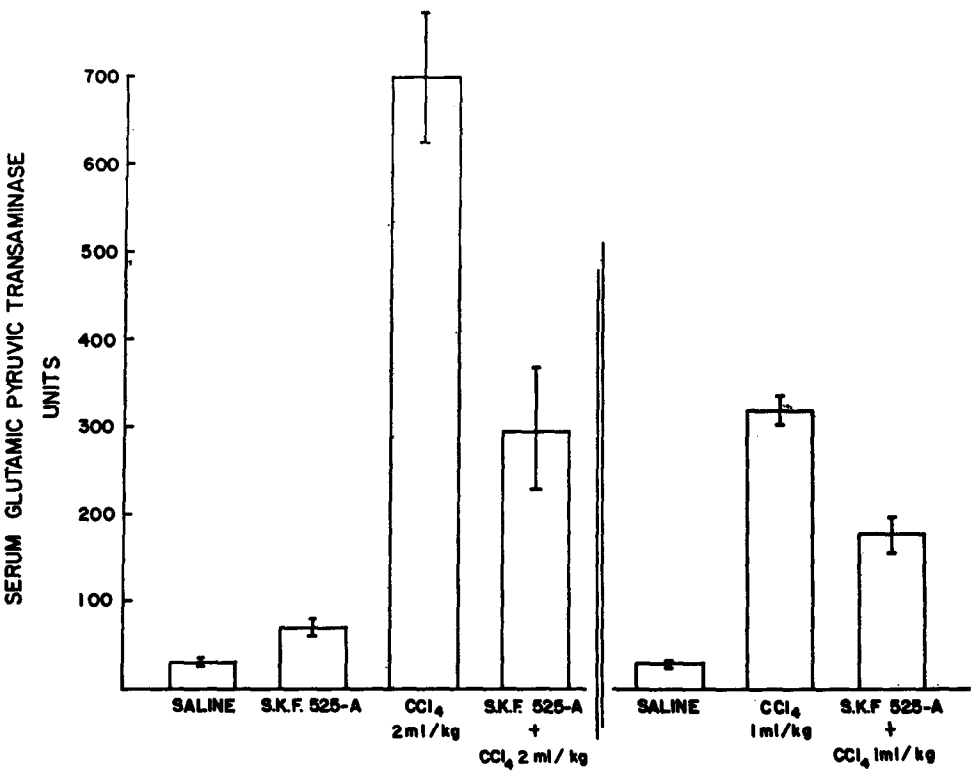

FIG. 1. Effect of SKF 525-A, 40 min before administration of CCl_4, on serum glutamic pyruvic transaminase activity in rats sacrificed 6 hr after oral administration of CCl_4. Each value represents the mean of six rats. Vertical bars depict the standard errors. All means are significantly different from one another ($P < 0·05$) in a given experiment.

TABLE 2. SGPT ACTIVITY 6 hr AFTER THE ORAL ADMINISTRATION OF CCl_4

Dose CCl_4 (ml/kg)	SGPT activity* (units/ml)
Control	29 ± 2
0·5	328 ± 75
1·0	486 ± 47
2·0	568 ± 84
4·0	883 ± 110

* Each value represents the mean ± S.E. from a group of five animals.

525-A-treated rats did not inhibit the SGPT activity of the CCl_4-treated animals. Thus, the amount of SKF 525-A or metabolites (or both) present in the plasma at 6 hr exerted no appreciable inhibition of SGPT activity in our experiments.

Treatment with CCl_4, 2 ml/kg, caused the expected rise in liver triglycerides[13] and pretreatment with SKF 525-A significantly decreased liver triglycerides of animals given CCl_4 (Fig. 2). Although the amount of triglycerides observed in the liver after administration of 1 ml/kg of CCl_4 was less than that observed after 2 ml/kg, the relationship between the dose of CCl_4 and the amount of triglycerides was not as good as with SGPT. On a percentage basis, CCl_4 at a dose of 2 ml/kg caused triglycerides to increase to a value which was 630 per cent of the control, whereas pretreatment with SKF 525-A reduced this augmentation to 312 per cent. Rats given CCl_4, 1 ml/kg,

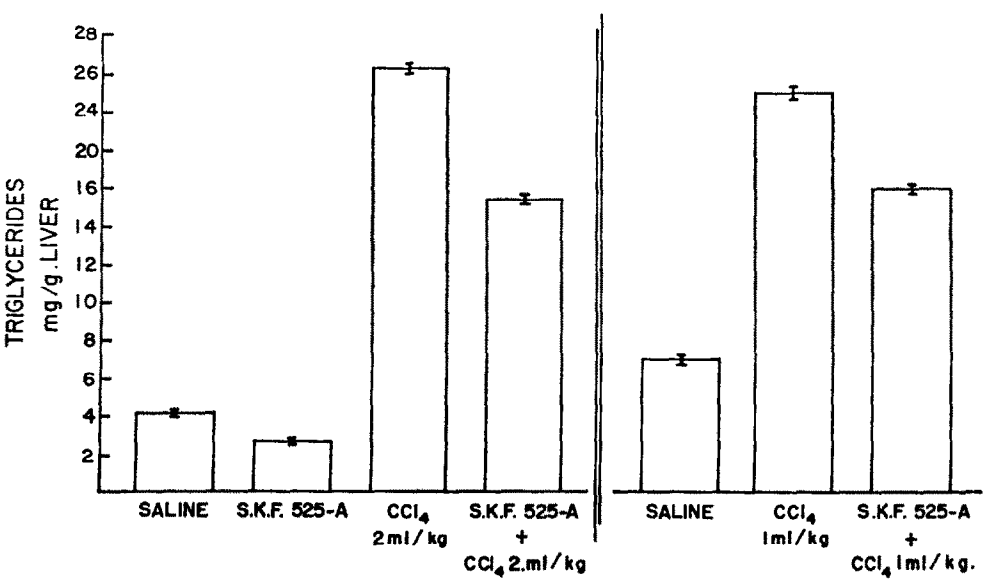

FIG. 2. Effect of SKF 525-A, 40 min before administration of CCl_4, on liver triglycerides in rats sacrificed 6 hr after oral administration of CCl_4. Each value represents the mean of six rats. Vertical bars depict the standard errors. All means are significantly different from one another ($P < 0·05$) in a given experiment.

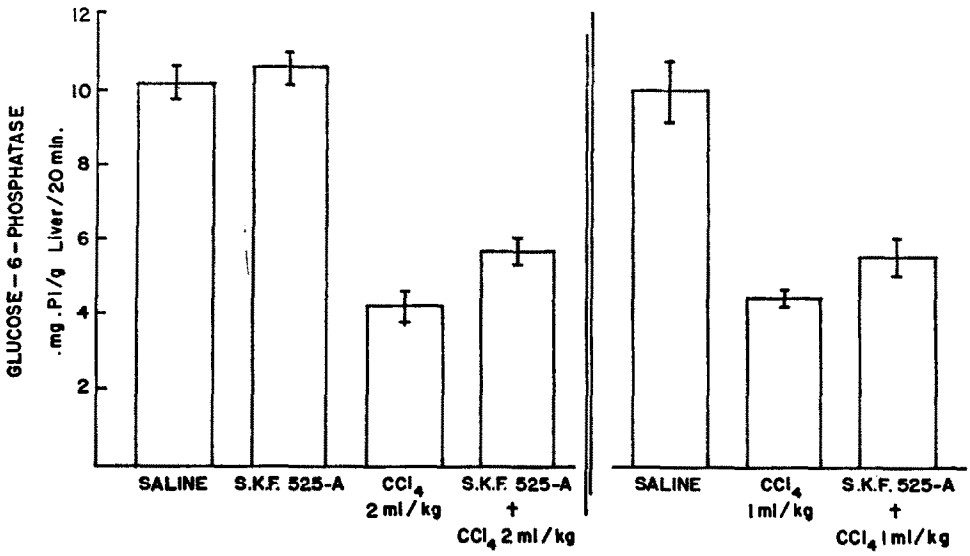

FIG. 3. Effect of SKF 525-A, 40 min before administration of CCl_4, on liver glucose 6-phosphatase activity in rats sacrificed 6 hr after oral administration of CCl_4. Each value represents the mean of six rats. Vertical bars depict the standard errors. Gavage with CCl_4 caused a significant ($P < 0{\cdot}05$) decrease in G-6-Pase liver activity. In rats pretreated with SKF 525-A, and gavaged with CCl_4, 2 ml/kg, the G-6-Pase liver activity was significantly higher ($P < 0{\cdot}05$) than that of rats pretreated with saline.

showed a triglyceride level that was 350 per cent of the control value and SKF 525-A pretreatment reduced it to 225 per cent.

When liver G-6-Pase activity was used as an index of toxicity, we found that CCl_4, 1 and 2 ml/kg, caused a significant decrease in liver G-6-Pase activity (Fig. 3). Pretreatment with SKF 525-A significantly raised the level of liver G-6-Pase activity in rats given 2 ml/kg of CCl_4, but there was little difference when the CCl_4 dose was 1 ml/kg. Moreover, there was a poor correlation between the dose of CCl_4 and the hepatic G-6-Pase activity, since CCl_4 had similar effects at 2 and 1 ml/kg.

DISCUSSION

We are unaware of studies establishing a direct relationship between the hepatotoxicity of CCl_4 and the liver concentration of the halogenated hydrocarbon. However, correlations have been described between the doses of CCl_4 and certain hepatotoxic effects. Balazs *et al.*[14] and Zimmerman *et al.*[15] have shown a relationship between SGPT activity and the dose of CCl_4. Klaassen and Plaa[16] have reported that, within a certain dosage range, the quantity of liver triglyceride is proportional to the dose of CCl_4. Although a sensitive test, the G-6-Pase activity in the liver and the serum seems to be less dependent on the dose of CCl_4.[15–17]

The present study establishes a parallel between the SGPT activity and the CCl_4 concentration in the liver. There is a marked difference in both CCl_4 liver concentration and SGPT activity between controls and rats pretreated with SKF 525-A. On the other hand, no significant difference was found in either liver concentration or SGPT activity between rats that received CCl_4, 1 ml/kg, and those that were given

2 ml/kg but pretreated with SKF 525-A. A similar correlation seemed to exist when the liver triglycerides were used as an index of liver injury.

It is believed by some that a metabolite is responsible for the hepatotoxicity of CCl_4.[5,6] Our data are not incompatible with this hypothesis. However, the protective effect of SKF 525-A on the hepatotoxicity of CCl_4 has been interpreted as an inhibition of microsomal enzymes responsible for the metabolism of CCl_4. Although our data do not eliminate such a possibility, the protective effect of SKF 525-A can be explained by the decrease in CCl_4 concentration in the liver. If SKF 525-A were exerting its protective effect against CCl_4 in the liver, then for equal liver concentrations of CCl_4, one would expect less damage in rats treated with SKF 525-A than in those without; this was not found. Rats given SKF 525-A and CCl_4, 2 ml/kg, had a liver CCl_4 concentration similar to that of rats given saline and CCl_4, 1 ml/kg, and both the SGPT activity and hepatic triglycerides were similar in both groups. Castro *et al.*[4] showed an almost complete blocking effect of SKF 525-A on the impairment of liver microsomal activity observed 3 hr after CCl_4 administration, whereas the morphological changes in the liver of the same animals were quite extensive 24 hr after CCl_4 administration. Our previous study[1] showed that during the first 2 hr after CCl_4 administration there was less CCl_4 in the blood and liver of rats pretreated with SKF 525-A, but at 8 and 12 hr after CCl_4 administration the opposite was observed. The observations of Castro *et al.*[4] may then easily be explained on the basis of changes in CCl_4 liver concentration.

It has been shown that the CCl_4 hepatotoxicity is potentiated by pretreatment with phenobarbital or DDT, while rats given a protein-free diet become resistant;[12,18] there was no commensurate change in CCl_4 liver concentration measured 1 and 3 hr after gavage. It was confirmed by Marchand *et al.*[1] that pretreatment with phenobarbital did not alter the concentration of CCl_4 2 hr after administration of the halogenated hydrocarbon. Garner and McLean[12] concluded that CCl_4 hepatotoxicity was not related to its liver concentration but to its conversion to a toxic metabolite. Such a conclusion appears premature, since the toxicity studies were carried out 24 hr after administration of CCl_4 without a full time-study of CCl_4 tissue concentration. However, it is conceivable that factors other than liver concentration of CCl_4 are involved in the potentiating and inhibitory effects of these pretreatments.

The observation that CCl_4 concentrations in the kidneys were much lower than in the liver is not new[19] and it may be of significance in the light of the hypothesis raised by the present work. It is known that, in several species, the kidney seems to be less responsive to doses of CCl_4 than the liver.[20–22] It may be that the relative absence of nephrotoxic effect after CCl_4 administration is simply due to a lower CCl_4 concentration in that organ. More data are needed to support such a hypothesis.

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