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Biochemical Pharmacology, Vol. 21, pp. 1053–1055. Pergamon Press, 1972. Printed in Great Britain.

Increased plasma enzyme activity induced by 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A)

(*Received* 29 *August* 1971; *accepted* 5 *November* 1971)

Considerable interest has been focused on the effects of 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) as an inhibitor of drug metabolism[1] and thereby as a modifier of chemically induced hepatotoxicity. Pretreatment of rats with SKF 525-A protects against the hepatotoxicity of carbon tetrachloride assessed histologically[2] and by measurement of serum enzyme activity and liver triglycerides.[3] Although this effect has been attributed to the inhibitory action of SKF 525-A on microsomal enzymes, the responsible mechanism has not been established.[3] During the course of studies in this laboratory on the toxicity of halogenated hydrocarbons, treatment with SKF 525-A *per se* was found to produce significant elevations of plasma levels of glutamic oxaloacetic transaminase (GOT), resembling that observed in hepatic cell necrosis. Marchand *et al.*[3] recently noted elevation of serum glutamic pyruvic transaminase (GPT) in rats treated with SKF 525-A. The present report extends these observations and indicates that the pattern of increased plasma enzyme activity induced by SKF 525-A is consistent with altered permeability of muscle and, to a lesser extent, hepatocyte membranes, resulting in leakage of intracellular enzymes.

Female, Sprague–Dawley rats weighing 180–200 g were fasted but allowed access to water for 2–4 hr prior to and after the intraperitoneal (i.p.) injection of isotonic saline or SKF 525-A, dissolved in isotonic saline (pH of injected solutions ranged between 4·4 and 5·6). Doses of SKF 525-A administered are indicated in Table 1. One hr after injection, the rats were sacrificed by bleeding from the abdominal aorta. Plasma was assayed for GOT, GPT[4] and creatine phosphokinase (CPK)[5] by standard spectrophotometric methods. Liver and skeletal and cardiac muscle tissue fixed in buffered formalin was stained with hematoxylin and eosin for light microscopic examination. As shown in Table 1, elevated levels of plasma GOT and CPK were noted in rats treated with as little as 5 mg/kg of SKF 525-A, and higher enzyme levels were found with increasing dosage. Increased GPT activity was observed at the highest dose employed, 100 mg/kg.

In a second series of experiments, SKF 525-A was administered in a fixed dose of either 100 or 40 mg/kg by i.p. injection, and groups of rats were sacrificed at the intervals indicated in Tables 2 and 3 respectively. Peak plasma enzyme activity was observed at 1 hr after the larger dose and at approximately 8 hr after the lower dose. Plasma GPT and CPK levels had returned to normal at 24 hr after the 100 mg/kg dose and at 48 hr after the 40 mg/kg dose. GOT was still moderately elevated at 48 hr after the i.p. injection at each dose level. Light microscopic examination of sections of liver and cardiac and skeletal muscle, obtained in both sets of experiments, failed to show evidence of hepatic or muscle necrosis or inflammation. Liver triglycerides, measured by standard chemical techniques,[6] were similar in control and SKF 525-A-treated animals (100 mg/kg) at 24 and 48 hr after i.p. injection.

TABLE 1. PLASMA ENZYME ACTIVITY 1 hr AFTER INTRAPERITONEAL ADMINISTRATION OF SKF 525-A

Dose SKF 525-A (mg/kg)	GOT* (units/ml)	GPT* (units/ml)	CPK* (units/ml)
Control	40 ± 1	21 ± 1	13 ± 4
5	54 ± 6	8 ± 1	28 ± 7
10	132 ± 52	16 ± 6	102 ± 48
20	180 ± 93	27 ± 14	86 ± 27
50	309 ± 28	26 ± 3	740 ± 97
100	520 ± 147	170 ± 48	804 ± 226

* Each value represents the mean ± S.E. from a group of four rats.

In other groups of rats SKF 525-A, in doses ranging between 5 and 50 mg/kg, was given by intravenous injection. One hr later, plasma was collected for assay of enzyme activity. Although GPT activity rose only minimally in rats receiving 10, 20 or 50 mg/kg, plasma GOT and CPK levels were significantly increased in these animals. These data indicate that the elevation of plasma enzyme activity is not specific to the i.p. route of administration.

TABLE 2. PLASMA ENZYME ACTIVITY AFTER INTRAPERITONEAL ADMINISTRATION OF SKF 525-A (100 mg/kg)

Time (hr)	GOT* (units/ml)	GPT* (units/ml)	CPK* (units/ml)
1·0	590 ± 60	295 ± 35	613 ± 96
4·5	405 ± 46	80 ± 7	513 ± 98
8·0	454 ± 98	64 ± 4	68 ± 25
16·0	249 ± 28	48 ± 8	23 ± 3
24·0	106 ± 26	25 ± 8	14 ± 1
48·0	109 ± 11	22 ± 3	3 ± 2

* Each value represents the mean ± S.E. from a group of four rats.

TABLE 3. PLASMA ENZYME ACTIVITY AFTER INTRAPERITONEAL ADMINISTRATION OF SKF 525-A (40 mg/kg)

Time (hr)	GOT* (units/ml)	GPT* (units/ml)	CPK* (units/ml)
4	248 ± 73	33 ± 8	64 ± 24
8	339 ± 93	61 ± 11	63 ± 11
12	238 ± 105	53 ± 6	37 ± 10
24	162 ± 58	39 ± 17	29 ± 8
48	62 ± 18	17 ± 3	16 ± 1

* Each value represents the mean ± S.E. from a group of four rats.

Since SKF 525-A affects body temperature, rectal temperature was monitored in rats treated with SKF 525-A (50 or 100 mg/kg). Plasma enzymes were measured in rats maintained normothermic with heating pads, and in rats in which no attempt to alter body temperature was made (room temperature 22°). One hr after SKF 525-A, mean body temperature had fallen 2° and plasma enzyme levels

were elevated in animals not receiving external heat. In the rats whose body temperature was maintained at normothermic levels, elevations of enzyme activity were similarly observed. Thus hypothermia induced by SKF 525-A, which is associated with inhibition of bile flow,[7] could not be implicated as a factor in the increased enzyme activity.

Lee *et al.*[8] have shown that SKF 525-A can result in concentration-dependent hemolysis or stabilization of the erythrocyte membrane. At the higher concentrations (10^{-3} M) employed by these workers hemolysis, which increased as temperature decreased, was observed. Hemolysis was not observed in our studies *in vivo* and could not explain the increased level of plasma GPT, but might have been responsible for a minor fraction of the elevated levels of plasma GOT and CPK.

Although the mechanism by which SKF 525-A produces these striking increases in plasma enzyme activity remains to be determined, the pattern noted suggests that muscle is the major organ source of the enzyme leakage, but liver may also be contributing. Future studies of the effects of SKF 525-A on leakage of enzymes from muscle preparations *in vitro* are planned. The recognition of this phenomenon may be important to workers using this agent, and indicates that SKF 525-A has appreciable extramicrosomal actions which have not yet received adequate attention.

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