

Change of Serum Glutamic Oxaloacetic Transaminase Activities after Administration of Carbon Tetrachloride to Mice

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Carbon tetrachloride (CCl₄) was orally administered to female C57BL mice (0.02, 0.1, 0.5, 1.0 or 1.5 ml/kg). Activities of serum glutamic oxaloacetic transaminase (SGOT) were recorded at 15, 24, 39, 48, 63, 72 and 87 hr after administration. SGOT levels rose more slowly but reached higher maximum levels later in mice given higher doses of CCl₄. Therefore, an inverse dose response was observed at earlier times after drug administration. This phenomenon was observed also in female C3H mice and male and adrenalectomized female C57BL mice.

Keywords—carbon tetrachloride; SGOT activity; mouse blood; time course; dose response; phenobarbital induction

Glutamic oxaloacetic transaminase (GOT) is thought to be released from injured cells into circulation²⁾ and is used as a marker to diagnose diseases³⁾ such as hepatitis and myocardial infarct. Administration of carbon tetrachloride (CCl₄) to animals leads to centrilobular necrosis in the liver and causes a marked change of the activity of enzymes including GOT in serum (SGOT).⁴⁾ However, wide variances are observed in SGOT activities of mice given the same doses of CCl₄, making estimation of the extent of liver injury from the enzyme activities difficult. These variances result from the different responses of animals to the drug which can be easily altered by a variety of factors.⁵⁾ As CCl₄ administration has been reported to make rats resistant to the toxic effects of CCl₄ itself,⁶⁾ response to the drug may be altered by repeated administration.

The present experiment was designed to investigate the time course of the change in SGOT activities after a single administration of the drug in order to find the best time to estimate the extent of liver injuries on the enzyme level and to accumulate control data for comparison with values obtained after repeated administration of the drug.

Materials and Methods

Animals—C57BL/6J and C3H/He mice derived from lines maintained by brother-sister mating at Aburahi Laboratories, Shionogi and Co., Ltd. were used. They were housed five in each transparent plastic cage with wooden bedding and exposed to a daily schedule of 12 hr light and 12 hr darkness (light on at 8 a.m. and turned off at 8 p.m.). The room was maintained at 24 ± 1° with relative humidity between 50 and 60%. Diet (CLEA CA-1 pellet) and water were provided *ad libitum*. CCl₄ was diluted with liquid paraffin and given through a stomach tube (0.1 ml/10 g body weight). Control animals received an equal volume of the vehicle.

Chemicals—Commercial guaranteed reagents and phenobarbital (sodium salt, J.P. VIII) were used without further purification. CCl₄ (guaranteed) and liquid paraffin (extra pure) were obtained from Wako Pure Chemical Industries, Ltd. α -Ketoglutaric acid (monosodium salt) was obtained from Sigma Chemical Co., U.S.A.

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Enzyme Assay—Blood (20 μ l) was drawn from orbital veins of mice with a heparinized micropipette at scheduled intervals, blown into physiological saline (0.28, 0.48 or 0.98 ml), and centrifuged. The supernatants were frozen at -20° and stored in an electric freezer until assay. GOT was measured by the method proposed by Nippon Shôkakibyô Gakkai⁷⁾ using plasma diluted with saline (0.1 ml). The absorbances of the reaction mixtures were translated into Karmen units using a standard curve.

Experiment 1—Sixty female C57BL mice (60–67 days old at the time of CCl_4 administration) were divided into six equal groups. Animals of groups 1, 2, 3, 4, and 5 received 0.02, 0.1, 0.5, 1.0 and 1.5 ml/kg body weight of CCl_4 , respectively, at 6 p.m. Animals of group 6 were given an aqueous solution of phenobarbital (0.5 g/l) *ad lib.* as drinking water from ten days prior to CCl_4 administration until the end of the experiment and administered CCl_4 (0.5 ml/kg) at 6 p.m. Blood was taken just before (0 hr) and at 15, 24, 39, 48, 63, 72 and 87 hr after CCl_4 administration.

Experiment 2—Twenty female C3H mice (70–77 days old) were divided into two equal groups and given CCl_4 at 0.02 and 0.5 ml/kg, respectively, at 6 p.m. Blood was taken as in Exp. 1.

Experiment 3—Twenty-four male C57BL mice (62–67 days old) were divided into three equal groups. Animals of groups 1, 2 and 3 received CCl_4 at 0.02, 0.1 and 0.5 ml/kg, respectively, at 6 p.m. Blood was taken as in Exp. 1.

Experiment 4—Fifty-eight female C57BL mice (66–82 days old) were divided into seven groups (six mice each in groups 1, 2, 3 and 6, and ten mice each in groups 4 and 7). Mice of groups 2, 5 and 7 were adrenalectomized under hexobarbital narcosis (sodium hexobarbiturate 100 mg/kg *i.p.* at ten days before CCl_4 administration), then received a physiological saline solution as drinking water *ad lib.* until the end of the experiment. Mice of groups 1, 4 and 6 also received a saline solution *ad lib.* Animals other than those of groups 1 and 2 were given CCl_4 at 0.5 ml/kg in groups 3, 4 and 5 and 0.02 ml/kg in groups 6 and 7 at 6 p.m. Blood was taken as in Exp. 1.

Experiment 5—Forty female C57BL mice (81–84 days old) were divided into four equal groups and given CCl_4 at 0.02 ml/kg in groups 1 and 2 and 0.5 ml/kg in groups 3 and 4 at 9 a.m. Blood was taken at 0.5 and 3 hr after administration for groups 1 and 3 and at 1 and 5 hr for groups 2 and 4.

Results

Experiment 1

In group 1 (0.02 ml/kg), SGOT activities peaked at 15 hr after administration in six of ten mice but at 24 hr in the other four, then decreased rapidly and returned to physiological

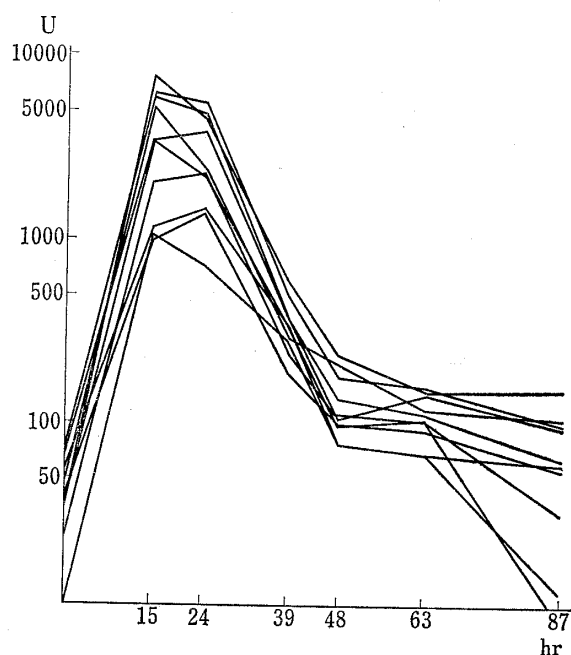


Fig. 1. Change in SGOT Activities of Female C57BL Mice after Administration of CCl_4 (0.02 ml/kg)

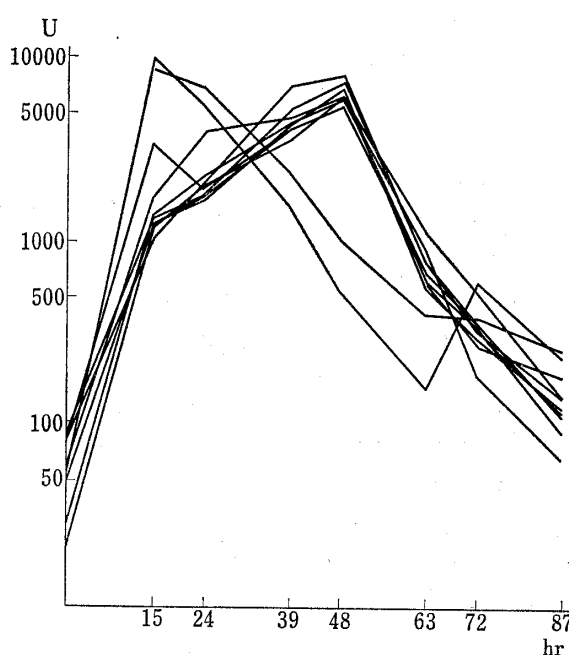


Fig. 2. Change in SGOT Activities of Female C57BL Mice after CCl_4 Administration (1.0 ml/kg)

7) Nippon Shôkakibyô Gakkai, Kankinô Kenkyûhan, *Nippon Shôkakibyô Gakkai Kaishi*, 61, 621 (1961).

levels at 48 or 63 hr (Fig. 1). Although the time courses of SGOT activities of two mice in group 4 differed significantly from those of the other (Fig. 2), such deviations were not observed in any other group. Maximal averages of SGOT activities in each group increased in a dose-related fashion, but appeared later in groups given more CCl_4 (Table I). In mice pretreated with phenobarbital, SGOT activities were elevated much higher and high activities were maintained longer than in mice of other groups given equal or higher doses of CCl_4 without phenobarbital.

TABLE I. Changes of SGOT Levels in Female C57BL Mice after Oral Administration of CCl_4

Dose ml/kg	Number of animals	Hours after CCl_4 administration							
		0	15	24	39	48	63	72	87
0.02	10	43.6 ±21.3	3675.9 ±2376.7	2907.2 ±1631.1	347.6 ±118.5	132.8 ±57.3	113.2 ±33.7	—	70 ±47.2
0.1	10	54.8 ±27.8	3819 ±1537.0	5238.3 ±1775.1	3290.1 ±1411.6	1822.7 ±1398.9	248.7 ±110.1	144 ±32.4	107.6 ±26.0
0.5	10	68.8 ±47.3	1818.8 ±924.0	2680 ±961.2	5406.6 ±524.5	4449 ±1501.4	646.2 ±215.3	376 ±116.3	216 ±46.8
1.0	8	59 ±26.2	1552.5 ±735.0	2170.5 ±739.5	4623.6 ±1011.8	6390 ±886.5	737 ±177.5	336.8 ±116.7	125.1 ±36.6
1.5	10	56 ±31.6	955.4 ±250.3	1726.8 ±584.6	5146 ±1654.4	7807.8 ±2158.6	1542 ±812.8	443.1 ±220.4	152.6 ±51.1
0.5 ^{a)}	10	65.2 ±23.6	2250.4 ±1121.3	6248 ±3321.5	7944.9 ±2132.9	9313.6 ±1606.3	4138.2 ±1108.5	1428.4 ±595.5	356.5 ±122.4

Values are in Karmen units (mean ± S.D.).

a) Phenobarbital solution (0.5 g/L) was given as drinking water.

Experiment 2

SGOT activities increased more rapidly and reached higher levels in C3H mice than in C57BL mice given corresponding doses of CCl_4 . However, the difference between the time courses of the two groups (0.02 and 0.5 ml/kg) was not as distinctly observed in C3H as in C57BL mice (Table II).

TABLE II. Changes of SGOT Levels in Female C3H Mice after CCl_4 Administration

Dose ml/kg	Number of animals	Hours after CCl_4 administration						
		0	15	24	39	48	63	87
0.02	10	63.2 ±15.5	8217.2 ±3149.1	8587.4 ±1441.8	681.6 ±126.2	362.8 ±40.2	250.7 ±39.4	79.3 ±27.9
0.5	10	64.8 ±21.0	3466.7 ±1303.3	11515.5 ±1653.5	2925.0 ±1734.7	718.0 ±214.0	328.0 ±46.7	120.0 ±63.0

Experiment 3

Male mice of each dosage group responded more weakly but more variously to CCl_4 than female mice of the corresponding group. In group 1, the maximal SGOT levels of four mice were lower than 250 U, which made the standard deviations of the enzyme activities of the group larger than the mean values at 15 (821.0±912.9 U, mean±S.D.) and 24 hr (1522.8±1781.4). However, increased dosage delayed the appearance of the peaks of the enzyme activities, although not as apparently as in Exp. 1. The enzyme levels of all animals of group 1 peaked at 24 hr. The peaks for groups 2 and 3 were at 24 (3686.6±1089.5) and 48 hr (2571.1±684.9), respectively, but the enzyme activities of two individual animals of group 3 peaked at 24 hr and two of group 2 and one of group 3 mice at 39 hr.

Experiment 4

Neither administration of saline solution (group 1) nor adrenalectomy (group 2) elevated SGOT activities except for a mouse which had 284 U at 24 hr after operation. Both treatments did not largely affect the time course of the enzyme levels after CCl_4 administration, although the activities rose a little more slowly in adrenalectomized and/or saline-administered mice than in intact ones (Fig. 3).

Experiment 5

Levels of SGOT began to rise 3 hr after administration and much faster in the 0.02 ml/kg group, although a significant difference ($p < 0.01$) between values of the two groups was observed first at 5 hr (269.3 ± 42.9 in group 1 and 159.9 ± 40.3 in group 2).

Comparison of SGOT Activities in Blood from Orbital and Jugular Veins of Mice

Blood was taken from orbital and jugular veins of ten female C57BL mice (70–75 days) at 24 hr after oral CCl_4 (0.5 ml/kg) administration. After measurement of SGOT activities as described above, the activities in blood taken from orbital veins were divided by those in plasma separated from jugular vein blood of the same mice. The ratio was 0.633 ± 0.062 (mean \pm S.D.).

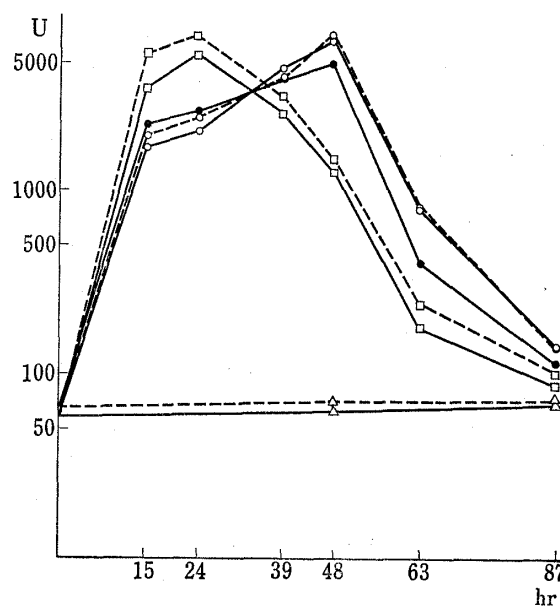


Fig. 3. Effect of Adrenalectomy on the Time Course of SGOT Activities after CCl_4 Administration.

- : saline solution as drinking water, adrenalectomized, CCl_4 at 0.1 ml/kg.
- : saline solution as drinking water, not adrenalectomized, CCl_4 at 0.1 ml/kg.
- : saline solution as drinking water, adrenalectomized, CCl_4 at 0.5 ml/kg.
- : saline solution as drinking water, not adrenalectomized, CCl_4 at 0.5 ml/kg.
- : CCl_4 at 0.5 ml/kg only.
- △—△: saline solution as drinking water, adrenalectomized, CCl_4 not administered.
- △—△: saline solution as drinking water, not adrenalectomized, CCl_4 not administered.

Discussion

The response of animals to CCl_4 is greatly influenced by many factors.⁵⁾ However, in the present work, SGOT activities changed uniformly within mice given the same doses of CCl_4 , except for two in group 4 of experiment 1. The deviation of the response of these two mice from others may have been caused by modifying factors including the absorption rate of the drug from the intestinal tract. The data for the two mice were excluded from later discussion.

SGOT activities increased rapidly, reached maximum and then decreased rapidly in all groups, as reported in literature.^{4,8)} However, this increase was retarded by increasing the CCl_4 dose in female C57BL mice. This was also observed in female C3H and male C57BL mice, although response patterns differed slightly, that is, activities increased more rapidly in C3H mice and more slowly and weakly in male C57BL mice than in female C57BL mice. The serum level of an enzyme is generally regulated by its influx from synthesizing cells and elimination by inactivation, excretion, etc. As the rapid removal of lactic⁹⁾ and malic⁸⁾ dehydrogenase from the serum suggests that other enzymes are also rapidly removed, the rise of SGOT activities after CCl_4 administration can be considered as being chiefly caused by the enhanced release of the mitochondrial and cytoplasmic enzyme of hepatocytes into

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blood, although the rise might also be partly caused by disturbance of the removal functions. On the other hand, the release of a large quantity of hepatocyte enzymes into blood results from an increase in the permeability of hepatocyte membranes in addition to the disruption of cell organelles. CCl_4 has been reported¹⁰⁾ to appear immediately in the liver of prefasted rats and reach maximal concentration at 1.5 hr after oral administration. Metabolic disturbances were detected early^{5,11)} and even necrosis of hepatocytes was observed as early as 6 hr after poisoning.¹²⁾ A protective effect of a small dose of CCl_4 against a second administration, which was observed within 10 min of the first administration to mice,¹³⁾ suggests that metabolic disturbances due to CCl_4 like in rats also occur in mice immediately after administration. The rise of SGOT activities in the present study which started at 3 hr after administration agrees with the report by Kuchii¹⁴⁾ and suggests that the increase in the permeability of the hepatocyte membrane starts a little later.

Dinman *et al.*⁴⁾ reported that the area and severity of liver damage in rabbits exposed to 200 ppm of CCl_4 vapor for 6 hr were less than those of animals dosed with 500 ppm throughout the experiment (from 8 to 72 hr after exposure). Similar correlation between the extent of liver damage and the CCl_4 dose was also observed in rats by other investigators.¹⁵⁾ Therefore, the slower elevation of SGOT activities in mice given more CCl_4 suggests that hepatocyte membranes are more permeable to the enzyme or the elevation of membrane permeability develops faster in mice poisoned with lesser amounts of CCl_4 . The effect of pretreatment of the animals with phenobarbital suggests that the drug not only potentiates the toxic effect of CCl_4 to hepatocyte in agreement with reports by several investigators¹⁶⁾ but also affects the membrane permeability.

The results obtained for male and adrenalectomized female mice suggest that the retardation of the release of GOT from the injured hepatocytes was caused not indirectly through alteration of the hepatocyte membrane permeability by the decrease of estrogens or adrenal hormones due to damage of their synthesizing organs, but directly by CCl_4 itself or its active metabolites. Although we cannot satisfactorily explain the phenomenon at present, it seems unreasonable to estimate the extent of hepatocyte injury by comparing SGOT levels recorded at any one time. Observation of the time course of the enzyme levels is necessary. The same result as ours has already been reported by Dinman *et al.*⁴⁾ However, they regarded it of little significance, thinking that it was due to extreme variation of the responses inherent to the experimental animals, because they had compared the time courses of enzyme levels with exposure to only two doses of CCl_4 .

The result obtained from C3H mice suggests that this phenomenon is not limited only to C57BL mice but occurs generally in mice. As the report Dinman *et al.*⁴⁾ suggests, the inverse dose response should be widely observed in serum enzyme levels of experimental animals administrated CCl_4 .

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