Hepatic Lipid Deposition and Plasma Transaminase Activity Influenced by Oral Administration of Carbon Tetrachloride in Growing Chicks

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Carbon tetrachloride (CCl_4) is a hepatotoxin inducing necrosis and fatty infiltration in liver^{1,2)}. Compared with mammals, chicks are less susceptible to liver injury by CCl_4 ^{3,4)}. FRIEDMAN *et al.*⁵⁾ have shown that hepatic lipid accumulation was induced by 10 times oral dose of CCl_4 which produced it in rats. Hepatic lipid accumulation^{5,6)} and increased plasma transaminase activities^{7,8)} by large dose of CCl_4 have been separately reported in chicks. The present study was undertaken to assess the changes of hepatic lipid content and plasma transaminase activities in chicks after single and repeated doses of CCl_4 .

Materials and Methods

White Leghorn male chicks were housed in wire cages under controlled light (14 hours light photoperiod) in air-conditioned room at 22°C and fed *ad libitum* a practical type mash diet containing 20.7% CP and 2.9 kcal of metabolizable energy per gram.

Experiment 1.

Forty 14-day-old chicks were allocated to five groups of 8 chicks each. A mixture of olive oil and CCl_4 was infused daily for 14 days into the crop to allow chicks receiving 0, 2.5, 5, 10 or 15 ml CCl_4 /kg body weight. All chicks were given identical amount of olive oil (15 ml/kg body weight). The birds were killed by heart puncture 24 hours after the last administration of CCl_4 .

Experiment 2.

Either 30 ml CCl_4 /kg body weight, dissolved in the equivalent volume of olive oil or olive oil alone (controls) were infused into the crop of seven 21-day-old chicks at 48 hours before killing.

Experiment 3.

One hundred and twelve 21-day-old chicks were divided into 4 groups. A mixture of CCl_4 and olive oil was infused into the crop of 28 chicks each to allow chicks receiving 10, 20 or 25 ml CCl_4 /kg body weight. All chicks were given identical amount of olive oil (25 ml/kg body weight). Seven chicks in each group were killed before and 1, 2 and 3 days after the administration.

Analytical methods

Blood was collected by cardiac puncture with a heparinized syringe prior to slaughter and centrifuged for 15 minutes at $1,500 \times g$ and the plasma was stored for later analysis. Liver lipid was extracted⁹⁾ and determined gravimetrically. Plasma total lipids were deter-

mined by the method of BRAGDON¹⁰). Triglyceride and phospholipid contents in the liver and plasma were determined colorimetrically^{11,12}). Activities of glutamate-oxalacetate transaminase (GOT, EC 2.6.1.1) and glutamate-pyruvate transaminase (GPT, EC 2.6.1.2) were determined by a modified method of REITMAN and FRANKEL¹³), using a colorimetric measuring kits (Wako Purechemical Industries, Osaka, Japan). The data were subjected to analysis of variance¹⁴).

Results

Experiment 1

Fourteen day administration of CCl₄ at rates of 5 and 10 ml/kg body weight reduced body weight gain and killed one and seven chicks out of eight chicks, respectively (Table 1). Liver weight and total lipid and triglyceride contents in the liver were significantly increased with increasing CCl₄ doses. Plasma triglyceride concentration was elevated by 5 ml CCl₄ though no changes in total lipid concentration in the plasma were observed in chicks administered CCl₄ (Table 2). Plasma GOT and GPT activities were significantly and proportionately elevated with increasing CCl₄ doses.

Table 1. Effect of 14 day administration of CCl₄ on body weight gain, liver weight and liver lipid contents¹⁾ (Experiment 1)

Treatment ²⁾ CCl ₄ (ml/kg BW/day)	Body weight gain (g)	Liver weight (g/100 g BW)	Liver			
			Total lipid (% wet weight)	Total lipid (mg/100 g BW)	Triglyceride (% total lipid)	Triglyceride (mg/100 g BW)
0	139±13 ^{a3)}	3.4±0.3 ^b	5.8±0.9 ^c	202±49 ^c	36.3±6.1 ^b	61±23 ^c
2.5	118±17 ^a	5.5±0.5 ^a	7.5±1.3 ^b	425±104 ^b	61.7±4.8 ^a	212±65 ^b
5.0	83±12 ^b	5.8±1.3 ^a	14.1±3.5 ^a	822±282 ^a	69.9±3.9 ^a	438±225 ^a
10.0	-2	7.5	18.5	1391	72.4	1007

- 1) All chicks were fed for 14 days from 14th day of age. Average initial body weight for all chicks was 125 g.
- 2) CCl₄ was orally administered with tube for 14 days. One and seven out of eight birds died in groups administered CCl₄ at 5 or 10 ml, respectively.
- 3) Mean±SD of 7 or 8 observations. Means with different superscripts in columns are significantly (P<0.05) different.

Table 2. Effect of 14 day administration of CCl₄ on plasma lipid content and plasma transaminase activity (Experiment 1)

Treatment CCl ₄ (ml/kg BW/day)	Plasma			
	Total lipids (mg/100 ml)	Triglyceride (mg/100 ml)	GOT ¹⁾ (Karmen unit)	GPT ¹⁾
0	914±67 ^{a2)}	190±30 ^b	91±8 ^c	7.2±1.8 ^c
2.5	867±55 ^a	206±30 ^{a,b}	180±49 ^b	14.1±4.8 ^b
5.0	728±198 ^a	276±80 ^a	290±130 ^a	25.6±8.8 ^a
10.0	900	301	—	—

- 1) Abbreviations: GOT, glutamate-oxalacetate transaminase; GPT, glutamate-pyruvate transaminase.
- 2) Mean±SD of 7 or 8 observations. Means with different superscripts in columns are significantly (P<0.05) different.

Experiment 2

Single oral administration of CCl₄ at rate of 30 ml/kg body weight increased liver weight, total lipid and triglyceride contents in liver and plasma GOT and GPT activities while it decreased body weight gain, liver phospholipid content and plasma total lipid content (Table 3).

Experiment 3

Changes after single oral administration of CCl₄ of liver weight, total lipid and triglyceride contents in liver, plasma lipids and plasma GOT and GPT activities were illustrated in Fig. 1. Liver weight increased proportionately with increasing CCl₄ after the administration and attained the maximum in each dose at 2 days after the administration, then decreased (Fig. 1A). The maximum total and triglyceride contents in the liver were demonstrated in chicks given CCl₄ in each dose at 2 or 3 days after the administration and those in chicks given 10 and 20 ml CCl₄ were reduced, while those in chicks given 25 ml CCl₄ were maintained, at 3 days after the administration (Fig. 1B and C). Single administration of CCl₄ at rates of 20 and 25 ml lowered plasma total lipid concentration at 1 day, but not 3 days, after the administration (Fig. 1D). The highest plasma GOT activity was demonstrated at 1 day after the administration of CCl₄ and was not significantly different among CCl₄ doses (Fig. 1E). On 3 days after the administration, the increased plasma GOT activity was maintained in chicks given 25 ml CCl₄ while those in chicks given 15 and 20 ml returned to the control values.

Table 3. Effect of single administration of CCl₄ on body weight gain, liver weight, liver lipid contents, plasma lipid contents and plasma transaminase activities¹⁾ (Experiment 2)

	CCl ₄ (ml/kg BW)	
	0	30
Body weight gain (g)	16 ± 7 ³⁾	-31 ± 6* ⁴⁾
Liver weight (g/100 g BW)	3.2 ± 0.2	4.3 ± 0.2*
Liver total lipid		
(% wet weight)	4.4 ± 0.1	12.9 ± 1.6*
(mg/100 g BW)	141 ± 10	555 ± 69*
Liver triglyceride		
(% total lipids)	19.4 ± 2.4	36.6 ± 7.1*
(mg/100 g BW)	27 ± 3	203 ± 27*
Liver phospholipids		
(% total lipids)	74.4 ± 3.0	26.8 ± 4.8*
(mg/100 g BW)	105 ± 5	149 ± 30
Plasma total lipids		
(mg/100 ml)	243 ± 27	102 ± 22*
Plasma triglyceride		
(mg/100 ml)	51 ± 19	43 ± 21
Plasma GOT ³⁾ (Karmen unit)	89 ± 9	293 ± 44*
Plasma GPT ³⁾ (Karmen unit)	15 ± 2	36 ± 12*

1) Twentyone-day-old chicks were administered orally with CCl₄ and killed two days after the administration.

2) Abbreviations: see Table 2.

3) Mean ± SD of 7 observations.

4) Significantly different from corresponding chicks without CCl₄ (P < 0.05)

Fig. 1. Changes of liver weight, total lipid and triglyceride contents in liver, plasma lipids, and plasma transaminase activities after single administration of CCl₄ (Experiment 3).

A: Liver weight (g/100 g BW). B: Liver total lipids (mg/100 g BW). C: Liver triglyceride (mg/100 g BW). D: Plasma lipids (mg/100 ml). E: Plasma GOT activity (Karmen unit). F: Plasma GPT activity (Karmen unit).
 — No CCl₄ (Control) CCl₄ 10 ml/kg BW
 - - - CCl₄ 20 ml/kg BW - · - · CCl₄ 25 ml/kg BW

No significant changes in plasma GPT activity were demonstrated (Fig. 1F).

Discussion

The present data in experiment 1 demonstrated that fourteen day administration of 2.5 ml/kg body weight resulted in neither mortality nor reduced body weight gain. Moreover no mortality was found even when a large dose (15~30 ml/kg body weight) of CCl₄ was acutely introduced, being clearly different from findings in rats that a single oral dose of 2 ml/kg body weight provoked approximately 50% mortality⁵⁾. These results are consistent with findings^{3,4)} that the chicken are resistant to CCl₄ poisoning in comparison with the mammals.

Acute and prolonged administrations of CCl₄ in chicks increased liver weight and hepatic lipid deposition in accord with findings by FRIEDMAN *et al.*⁵⁾ and WHITEHEAD *et al.*⁶⁾. This increase was mainly due to increase of triglyceride, but not to increase of phospholipid, and

was in proportion to CCl₄ doses in the case of prolonged administration.

Fourteen day administration of CCl₄ lowered plasma total lipid concentration, though it increased plasma triglyceride content, in experiment 1, consequently suggesting that plasma phospholipids, a main component in the plasma lipids of normal chicks, was lowered by CCl₄. The reduced phospholipid content in the liver would favor to support the above suggestion and accords with findings in rats¹⁵⁾ that hepatic synthesis of lipoprotein was inhibited by CCl₄.

A significant increase in the activities of GOT and GPT in the plasma accompanied by a decrease in their activities in the liver has been observed in liver dysfunction^{16,17)}. The present study demonstrated that the administration of CCl₄ increased the plasma GOT activity in good accordance with previous findings in chicks by FOWLER⁷⁾ and NARASHIMHAN *et al.*⁸⁾, essentially suggesting that the liver function was impaired by CCl₄ in the present study. Moreover it was proved that the increased plasma GOT activity was accompanied with the increased lipid deposition in the liver. In addition it would be of worth to indicate that as shown in Fig. 1 the maximum increase in the hepatic lipid deposition was given at 2 or 3 days after the administration of CCl₄ whereas the maximum rise in plasma GOT activity was attained at 1 day after the administration. These findings suggest that CCl₄ causes degeneration of hepatic cells, subsequently leading to the lipid deposition in liver of chicks.

Summary

Lipid and triglyceride contents in liver and plasma, and plasma transaminase activities were determined in growing White Leghorn male chicks orally administered with carbon tetrachloride (CCl₄). Fourteen day administration of CCl₄ at rates of 2.5, 5 and 10 ml/kg body weight and the single administration at rate of 15 to 30 ml/kg body weight increased, with increasing CCl₄ doses, liver weight, total lipid and triglyceride contents in the liver, plasma triglyceride concentration and plasma GOT activity but reduced body weight gain and plasma total lipid concentration. Seven out of eight chicks died by 14 day administration of 10 ml CCl₄. The maximum increase of the hepatic lipid deposition was demonstrated at 2 or 3 days after whereas that of plasma GOT activity was observed at 1 day after the single administration. It is suggested that a large dose of CCl₄ in chicks induces degeneration of hepatic cells, subsequently leading to the hepatic lipid deposition.

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鶏の肝臓脂質蓄積と血漿トランスアミナーゼ活性に 対する四塩化炭素経口投与の影響

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鶏は、哺乳動物と比較して、四塩化炭素 (CCl_4) によって引き起される脂肪肝および肝障害に対する抵抗性が大きいことが知られているが、これまで CCl_4 投与後の肝臓脂質量の変化を、肝機能の変化と対応して検討した報告はみられない。本報告では、鶏ヒナの肝臓および血漿脂質、ならびに血漿トランスアミナーゼ (GOT および GPT) 活性に対する CCl_4 の影響を検討する目的で、3 回の実験を行なった。 CCl_4 はオリーブ油と混合して、ヒナの喉のう内に注入した。なおオリーブ油の投与量は、 CCl_4 投与量にかかわらず一定にした。

[実験 1] 14 日齢のヒナに CCl_4 を 1 日体重 1 kg 当り 2.5, 5 および 10 ml の割合で、14 日間投与した。10 ml CCl_4 投与の場合には、8 羽のヒナのうち 7 羽が死亡した。 CCl_4 投与により、肝臓総脂質、肝臓トリグリセリドおよび血漿 GOT 活性は CCl_4 投与量の増加に伴って増加し、脂肪肝と肝障害が発現した。

[実験 2] 21 日齢のヒナに、体重 1 kg 当り 30 ml の CCl_4 を 1 回経口投与し、その 48 時間後に採血と殺した。 CCl_4 投与により肝臓総脂質および血漿 GOT と GPT 活性が著るしく増加した。一方、血漿総脂質は CCl_4 投与により減少した。肝臓脂質画分では、 CCl_4 投与によりトリグ

リセリドが増加し、リン脂質が減少した。

[実験 3] 21 日齢のヒナに、体重 1 kg 当り 10, 20 または 25 ml の CCl_4 を 1 回経口投与し、その 1, 2 および 3 日後に各 7 羽づつを採血と殺した。肝臓重量は投与 1 日後より増加がみられて 2 日後には最高値に達し、3 日後には減少した。 CCl_4 10 または 20 ml 投与区では肝臓総脂質およびトリグリセリドは、 CCl_4 投与 2 日後に最高値に達し、投与 3 日後には肝臓総脂質とトリグリセリドが減少したが、 CCl_4 25 ml 投与区では、3 日後でも増加もしくは維持する傾向にあった。血漿総脂質は、 CCl_4 投与 1 日後に減少する傾向がみられた。血漿 GOT 活性は CCl_4 投与 1 日後に最高値に達し、 CCl_4 投与量にかかわらずその最高値はほぼ一定であった。 CCl_4 投与 3 日後に、10 および 20 ml 投与区の血漿 GOT 活性は対照区と同程度にまで回復したが 25 ml 投与区では 3 日後も高い血漿 GOT 活性が維持された。

以上の事から、 CCl_4 投与は、鶏ヒナの肝細胞の変性を引き起して肝機能を減退させ、それに引き続いて肝臓における脂質 (特にトリグリセリド) の蓄積を促すものと考えられる。

(家禽会誌 20, 277~283, 1983)