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**BLOOD CLEARANCES OF ^{99m}Tc -PHYTATE
AND INDOCYANINE GREEN IN CARBON
TETRACHLORIDE TREATED DOGS**

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Abstract.....The comparative study of the blood clearances of ^{99m}Tc -phytate (^{99m}Tc -P) and indocyanine green (ICG) was carried out in dogs with hepatic injury induced by carbon tetrachloride (CCl_4), and the blood clearance of ^{99m}Tc -P or ICG was compared with the levels of serum transaminase and bilirubin.

The blood clearances of ^{99m}Tc -P and ICG in dogs decreased with increase in doses of CCl_4 and with elapsed time after CCl_4 administration. The decreases were correspondent to the increase in serum transaminase activity and bilirubin level. The blood clearance test of ^{99m}Tc -P in dogs showed a equal degree of sensitivity and a higher degree of accuracy for the acute hepatic dysfunction induced by CCl_4 as compared with the blood clearance test of ICG.

Key words : ^{99m}Tc -phytate, indocyanine green, carbon tetrachloride, dog.

INTRODUCTION

Indocyanine green (ICG) is widely used to assess hepatic function in laboratory animals by measuring the plasma or serum retention of this dye after intravenous injection (Krarup and Larsen, 1976 ; Iga et al., 1980 ; Tsao et al., 1983 ; Kawasaki et al., 1984). On the other hand, we have attempted to apply ^{99m}Tc -phytate (^{99m}Tc -P) for the evaluation of hepatic dysfunction in laboratory animals and have reported that the blood clearance test of ^{99m}Tc -P in dogs shows a sensitive procedure for

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detecting the acute hepatic dysfunction induced by carbon tetrachloride (CCl_4) (Shibata et al., 1988), α -naphthylisothiocyanate (Shibata et al., 1989), D-galactosamine (Shibata and Kohno, 1989) and DL-ethionine (Shibata and Kohno, 1990), as the serum transaminase and bilirubin levels. The blood clearance tests of $^{99\text{m}}\text{Tc-P}$ and ICG are of value because a time course of the alteration of hepatic function can be grasped dynamically.

The purpose of this study is to compare the blood clearance of $^{99\text{m}}\text{Tc-P}$ with the blood clearance of ICG in dogs with CCl_4 -induced hepatic injury, and to compare the blood clearance of $^{99\text{m}}\text{Tc-P}$ or ICG with elevating of serum transaminase and bilirubin.

MATERIALS AND METHODS

Mongrel dogs of both sexes weighing 6–10 kg were used at the age of 5–10 months, and were allocated to each group consisting of 6 heads of littermate.

Carbon tetrachloride (CCl_4) solution in 50 % liquid paraffin was intraperitoneally administered to the dogs.

Methods to prepare $^{99\text{m}}\text{Tc-P}$ and to measure the radioactivity of $^{99\text{m}}\text{Tc-P}$ in blood sample were described in the previous paper (Shibata and Hara, 1988).

ICG was purchased from the Daiichi Pure Chemical Company Ltd.. After the ICG was administered at dose of 0.5 mg/kg via a femoral vein, blood samples were obtained at 5, 10, 15, 20, 30, 45 and 60 minutes in heparinized polyethylene centrifuge tubes. For ICG analysis in the blood sample, 1.0 ml of serum was diluted with 2.0 ml of 0.9 % NaCl solution and immediately measured at 805 nm in a Hitachi 100–20 spectrophotometer.

Since two-phase changes was observed in the $^{99\text{m}}\text{Tc-P}$ or ICG concentration in blood after intravenous injection of these markers, the blood clearance rate (K) of $^{99\text{m}}\text{Tc-P}$ or ICG was calculated from the first phase and second phase of the clearance curve, respectively. The K-values from the first phase (K_1 -value) were obtained from the values of blood concentration at 5 and 10 minutes after the injection of marker, and the K-values from the second phase (K_2 -value) were obtained from the values at 30 and 60 minutes. The K-values were calculated from the following formula :

$$K = 2.303 \times (\log C_1 - \log C_2) / (t_1 - t_2)$$

where C_1 and C_2 are the concentration of marker at times t_1 and t_2 , respectively.

Serum GOT and GPT activities were determined by the pyruvate oxidase method (Wako Pure Chemical Industries, Ltd.), while serum total bilirubin concentration by the alkali azobilirubin method (Wako Pure Chemical Industries, Ltd.).

RESULTS

1. The Relation between Dose of CCl_4 and Blood Clearance of $^{99m}\text{Tc-P}$ or ICG, Serum Transaminase Activity, Serum Bilirubin Concentration

CCl_4 was given to the dogs at dose of 0.1, 0.25, 0.5 and 1.0 ml/kg, and the blood clearance of $^{99m}\text{Tc-P}$ or ICG, serum transaminase activity and serum bilirubin concentration were determined 48 hours after the administration of each dose.

Both $^{99m}\text{Tc-P}$ and ICG concentrations in blood showed a tendency to rise with the increase in dose level of CCl_4 (Figs. 1, 2). K_1 -value in the blood clearance test of $^{99m}\text{Tc-P}$ decreased from 0.277 (control : treated with liquid paraffin alone) to 0.128 (1.0 ml/kg CCl_4) with increase of CCl_4 dose level, while K_1 -value in the blood clearance test of ICG decreased from 0.260 (control) to 0.057 (1.0 ml/kg CCl_4). A significant decrease in the K_1 -value compared with the control was observed at dose of CCl_4 0.1 ml/kg in the $^{99m}\text{Tc-P}$ test ($P < 0.05$) or 0.25 ml/kg in the ICG test ($P < 0.01$) (Table 1, Fig. 3). On the other hand, significant changes with the increase in dose of CCl_4 were not observed in the K_2 -value of both $^{99m}\text{Tc-P}$ and ICG clearance (Table 1).

Serum GOT, GPT and bilirubin values rose with the increase in dose of CCl_4 . Significant increase, were obtained at dose of CCl_4 0.25 ml/kg ($P < 0.01$) (Fig.4).

Fig. 1. Blood clearance of $^{99m}\text{Tc-P}$ 48 hours after the administration of various doses of CCl_4 . The data represent mean of 6 dogs.

Fig. 2. Blood clearance of ICG 48 hours after the administration of various doses of CCl_4 . The data represent mean of 6 dogs.

Fig. 3. Blood clearance rate of $^{99\text{m}}\text{Tc-P}$ or ICG 48 hours after the administration of various doses of CCl_4 . The data represent mean $\pm$ S. D. of 6 dogs.

Blood clearances of $^{99m}\text{Tc-P}$ and ICG in CCl_4 -injured dogs

Table 1. Blood clearance of $^{99m}\text{Tc-P}$ or ICG 48 hours after the administration of various doses of CCl_4 .

CCl_4 (ml/kg)	$^{99m}\text{Tc-P}$		ICG	
	K_1 -value	K_2 -value	K_1 -value	K_2 -value
control	0.277 ± 0.021	0.009 ± 0.002	0.260 ± 0.024	0.095 ± 0.014
0.1	0.217 ± 0.016	0.008 ± 0.002	0.249 ± 0.022	0.080 ± 0.011
0.25	0.210 ± 0.016	0.008 ± 0.001	0.144 ± 0.010	0.104 ± 0.015
0.5	0.154 ± 0.012	0.009 ± 0.003	0.098 ± 0.010	0.081 ± 0.010
1.0	0.128 ± 0.012	0.010 ± 0.003	0.057 ± 0.010	0.031 ± 0.008

Values show mean $\pm$ S. D. of 6 dogs.

Fig. 4. Serum parameter values 48 hours after the administration of various doses of CCl_4 . Bil is total bilirubin concentration. The data represent mean $\pm$ S. D. of 6 dogs.

2. The Relation between Time Following the Administration of CCl_4 and Blood Clearance of $^{99\text{m}}\text{Tc-P}$ or ICG, Serum Transaminase Activity, Serum Bilirubin Concentration

After the administration of CCl_4 at dose of 0.5 ml/kg the blood clearance of $^{99\text{m}}\text{Tc-P}$ or ICG, serum transaminase activity and serum bilirubin concentration were determined at hours 2, 4, 8, 16, 24, and 48.

Both $^{99\text{m}}\text{Tc-P}$ and ICG concentrations in blood tended to rise with the passage of post-administrative time (Figs. 5, 6). K_1 -value in the blood clearance of $^{99\text{m}}\text{Tc-P}$ decreased with time from 0.277 (control : before the administration) to 0.154 (48 hours after the administration), while K_1 -value in the blood clearance of ICG decreased from 0.260 (control) to 0.098–0.099 (16–48 hours after the administration). A significant decrease was observed in the K_1 -value at 4 hours in the $^{99\text{m}}\text{Tc-P}$ test ($P < 0.01$) and at 2 hours in the ICG test ($P < 0.05$) after the administration of CCl_4 , respectively (Table 2, Fig. 7). However, no significant changes were observed in the K_2 -value of both $^{99\text{m}}\text{Tc-P}$ and ICG clearance (Table 2).

Serum GOT, GPT and bilirubin values increased also with the time after administration of CCl_4 . A significant increase was observed at 4 hours after the administration ($P < 0.05$) (Fig. 8).

Fig. 5. Blood clearance of $^{99\text{m}}\text{Tc-P}$ after the administration of CCl_4 at dose of 0.5 ml/kg. The data represent mean of 6 dogs.

Fig. 6. Blood clearance of ICG after the administration of CCl_4 at dose of 0.5 ml/kg. The data represent mean of 6 dogs.

Fig. 7. Blood clearance rate of $^{99m}\text{Tc-P}$ or ICG after the administration of CCl_4 at dose of 0.5 ml/kg. The data represent mean $\pm$ S. D. of 6 dogs.

Table 2. Blood clearance of $^{99m}\text{Tc-P}$ or ICG after the administration of CCl_4 at dose of 0.5 ml/kg.

Time(hr)	$^{99m}\text{Tc-P}$		ICG	
	K_1 -value	K_2 -value	K_1 -value	K_2 -value
control	0.277 ± 0.021	0.009 ± 0.002	0.260 ± 0.024	0.095 ± 0.014
2	0.267 ± 0.020	0.008 ± 0.002	0.188 ± 0.020	0.083 ± 0.012
4	0.231 ± 0.018	0.006 ± 0.001	0.164 ± 0.018	0.077 ± 0.010
8	0.217 ± 0.016	0.005 ± 0.001	0.118 ± 0.016	0.065 ± 0.010
16	0.210 ± 0.016	0.008 ± 0.002	0.099 ± 0.016	0.079 ± 0.014
24	0.204 ± 0.014	0.010 ± 0.003	0.096 ± 0.012	0.066 ± 0.010
48	0.154 ± 0.012	0.009 ± 0.003	0.098 ± 0.010	0.081 ± 0.010

Values show mean $\pm$ S. D. of 6 dogs.

Fig. 8. Serum parameter values after the administration of CCl_4 at dose of 0.5 ml/kg. Bil is total bilirubin concentration. The data represent mean $\pm$ S. D. of 6 dogs.

DISCUSSION

The present study showed that the blood clearances of ^{99m}Tc -P and ICG decreased in dogs with liver injury induced by CCl_4 . The slowed clearance of the markers was dependent on the dose of CCl_4 and with the elapsed time after its administration. The changes in clearance of the markers were in accordance with increases in serum transaminase activity and serum bilirubin concentration, indicating that the decrease in clearance reflected the extent of liver injury. However, the minimum dose of CCl_4 for the induction of obvious change was 0.1 ml/kg (48 hours after the administration) in the blood clearance of ^{99m}Tc -P, while 0.25 ml/kg in the blood clearance of ICG and serum transaminase and bilirubin levels. Furthermore, the earliest time when obvious changes appeared was 2 hours after the administration of CCl_4 (0.5 ml/kg) in the blood clearance of ICG, while 4 hours in the blood clearance of ^{99m}Tc -P and serum transaminase and bilirubin levels. In addition, K_1 -value in the ^{99m}Tc -P test continued to decrease from 4 to 48 hours, while K_1 -value in the ICG test decreased from 2 to 16 hours but did not change from 16 to 48 hours. These results suggest that ^{99m}Tc -P test in dogs may show a equal sensitivity to ICG test but a higher accuracy for detecting the acute hepatic dysfunction induced by CCl_4 .

It seemed that CCl_4 caused cholestasis and hyperbilirubinemia in this study of dogs. It is reported that the excretion of ICG into bile is decreased and ICG concentration in serum is consequently increased by cholestasis (Namiyama and Yamashiro, 1987). Therefore, it was estimated that a similar result might be obtained by CCl_4 -induced cholestasis in this study of ICG test. On this subject, since the biliary excretion of ^{99m}Tc -P is not recognized, the consideration of ^{99m}Tc -P concentration in blood was not necessary in ^{99m}Tc -P test.

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