BLOOD CLEARANCE OF ^{99m}Tc -PHYTATE FOR EVALUATION OF HEPATIC DYSFUNCTION IN DOGS

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Abstract.....Organ distribution pattern, and blood clearance were examined by giving ^{99m}Tc -phytate (^{99m}Tc -P) to the normal dogs. At the same time, the relation between the severity of hepatic function and blood clearance of ^{99m}Tc -P was studied by using the dogs with acute hepatic dysfunction experimentally induced by carbon tetrachloride (CCl_4) administration. Furthermore, a comparative discussion on blood clearance test of ^{99m}Tc -P was made with serum transaminase test.

It appeared appropriate to administer ^{99m}Tc -P at the dose of 100 $\mu\text{g}/\text{kg}$ in order to obtain an effective blood clearance curve. A major part of ^{99m}Tc -P intravenously administered was taken up into the liver, while the remainder of small amount into the spleen, kidneys, lung, and so on. Little was recognized in the thyroid. The disappearance rate of ^{99m}Tc -P from blood decreased with the increase in dose of CCl_4 and with the passage of time after the CCl_4 administration. The blood clearance test of ^{99m}Tc -P in dogs showed a sensitive reaction for the acute hepatic dysfunction induced by CCl_4 equally to the serum transaminase test.

Key words : ^{99m}Tc -phytate, blood clearance, hepatic dysfunction, carbon tetrachloride, dog.

INTRODUCTION

^{99m}Tc -phytate (^{99m}Tc -P) is a radioactive substance which has been widely used in nuclear medical field (Abe et al., 1975 ; Miyamae, 1974 ; Yamagishi et al., 1975) since it was developed in 1973 (Subramanian et al., 1973). In addition, since ^{99m}Tc has an appropriately short physical half-life of about 6 hours and emits single γ ray at 140 KeV but not β ray, ^{99m}Tc -P can be treated in safety and makes it easy to handle the

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radioisotope-treated animals. Then, we have attempted to apply ^{99m}Tc -P for the evaluation of hepatic dysfunction in laboratory animals. In the previous study (Shibata and Hara, 1988), we reported that the blood clearance test of ^{99m}Tc -P was one of more sensitive methods for evaluation of hepatic dysfunction in rats, equally to or rather than the serum transaminase test or the histological test.

The purpose of this report is to investigate the availability of ^{99m}Tc -P for evaluation of hepatic dysfunction in dogs. First of all, the organ distribution pattern and blood clearance rate of ^{99m}Tc -P were examined in the normal dogs. Then, by using the dogs with carbon tetrachloride-induced hepatic injury, the relation between the blood clearance of ^{99m}Tc -P and the severity of hepatic dysfunction was examined, and was compared with the cases of the serum transaminase determination.

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 4–6 heads of littermate. During the blood clearance experiment, they were not anesthetized.

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

Methods to prepare ^{99m}Tc -P and to measure the radioactivity of ^{99m}Tc -P in blood sample were described in a previous paper (Shibata and Hara, 1988). The initial concentration (Co) of ^{99m}Tc -P was obtained by extrapolation from the values of blood concentration at 5 and 10 minutes after the administration of ^{99m}Tc -P to time 0, and the half-life ($\text{T}_{1/2}$) was calculated. The blood clearance rate of ^{99m}Tc -P (K) was calculated from the following formula : $\text{K} = 0.693 / \text{T}_{1/2}$.

Every four dogs were sacrificed at 1, 2, 5, 10, and 24 hours after intravenous injection of ^{99m}Tc -P to measure the radioactivity of the organ samples. Organ distribution of ^{99m}Tc -P was expressed as an amount of ^{99m}Tc -P per organ.

Serum GOT and GPT values were determined by the pyruvate oxidase method (Wako Pure Chemical Industries, Ltd.).

RESULTS

1. Dose Selection of ^{99m}Tc -phytate :

In order to select the dose of ^{99m}Tc -P for the blood clearance test, the doses of 25, 50 and 100 $\mu\text{g}/\text{kg}$ of ^{99m}Tc -P were intravenously given to the dogs. Although there was a tendency to rise in concentration of ^{99m}Tc -P remaining in blood with the increase of ^{99m}Tc -P dose, the disappearance rates from blood were almost the same among each dose (Fig. 1). Therefore, the dose 100 $\mu\text{g}/\text{kg}$ was chosen as the dosage for the following clearance test, since this dose gives an effective clearance curve.

2. Organ Distribution Patterns of ^{99m}Tc -phytate :

A major part of ^{99m}Tc -P administered intravenously was taken up into the liver

Fig. 1. Blood clearance curve of ^{99m}Tc -P after single intravenous injection of various doses.

while the remainder of small amount into the spleen, kidneys, and lung. Though the uptake ratios into the liver, spleen, and lung decreased with the passage of time, that into the kidneys showed a tendency to increase (Fig. 2). Little uptake of $^{99m}\text{TcO}_4^-$ was recognized into the thyroid gland.

3. The Relation between Dose of CCl_4 and Blood Clearance of ^{99m}Tc -phytate, Activity of Serum Transaminase :

CCl_4 was given to the dogs at doses of 0.05, 0.1, 0.25, 0.5 and 1.0 ml/kg, and blood clearance of ^{99m}Tc -P was determined 48 hours after the administration of each dose.

The ^{99m}Tc -P concentration in blood showed a tendency to rise with the increase in dose level of CCl_4 . Both Co and T1/2 grew from 0.38 %/ml (control : treated with liquid paraffin alone) to 0.92 %/ml (1.0 ml/kg CCl_4), and from 2.5 minutes (control) to 5.4 minutes (1.0 ml/kg CCl_4) as the dose level of CCl_4 was increased. Therefore, the K-value decreased to about a half, from 0.277 (control) to 0.128 (1.0 ml/kg CCl_4) with the increase of CCl_4 dose level. A significant decrease was observed in the K-value compared with the control at dose of CCl_4 0.1 ml/kg ($P < 0.05$) (Fig. 3, Table 1).

Both serum GOT and GPT values rose with the increase in dose of CCl_4 . Significantly high values were obtained compared with the control at dose of CCl_4 0.1 ml/kg ($P < 0.05$) (Fig. 4).

4. The Relation between Time Following the Administration of CCl_4 and Blood Clearance of ^{99m}Tc -phytate, Activity of Serum Transaminase :

Fig. 2. Organ distribution of radioactivity after intravenous injection of $^{99m}\text{Tc-P}$.

Fig. 3. Relation between blood clearance of $^{99m}\text{Tc-P}$ and dose of CCl_4 .

Blood clearance of ^{99m}Tc -phytate in dogs

Table 1. Relation between blood clearance of ^{99m}Tc -P and dose of CCl_4 .

$\text{CCl}_4(\text{ml/kg})$	$C_0(\%/\text{ml})$	$C_{10}(\%/\text{ml})$	$T_{1/2}(\text{min})$	K
control	0.38	0.027	2.5	0.277 ± 0.021
0.05	0.38	0.036	2.8	0.248 ± 0.020
0.1	0.39	0.042	3.2	0.217 ± 0.016
0.25	0.42	0.048	3.3	0.210 ± 0.016
0.5	0.64	0.138	4.5	0.154 ± 0.012
1.0	0.92	0.199	5.4	0.128 ± 0.012

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

C_0 means the initial concentration of ^{99m}Tc -P.

C_{10} means the concentration of ^{99m}Tc -P at time 10 minutes.

Fig. 4. Relation between activity of serum transaminase and dose of CCl_4 .

After the administration of CCl_4 at dose 0.5 ml/kg, blood clearance of $^{99\text{m}}\text{Tc-P}$ was determined at hours 2, 4, 8, 24, and 48.

$^{99\text{m}}\text{Tc-P}$ concentration in blood tended to rise with the passage of post-administrative time. C_0 and $T_{1/2}$ grew as the time went by from 0.38 %/ml (control : before the administration) to 0.64 %/ml (48 hours after the administration), and from 2.5 minutes (control) to 4.5 minutes (48 hours after the administra-

Fig. 5. Relation between blood clearance of $^{99\text{m}}\text{Tc-P}$ and time following the administration of CCl_4 .

Table 2. Relation between blood clearance of $^{99\text{m}}\text{Tc-P}$ and time following the administration of CCl_4 .

Time(hr)	C_0 (%/ml)	C_{10} (%/ml)	$T_{1/2}$ (min)	K
control	0.38	0.027	2.5	0.277 ± 0.021
2	0.38	0.030	2.6	0.267 ± 0.020
4	0.38	0.036	3.0	0.231 ± 0.018
8	0.48	0.051	3.2	0.217 ± 0.016
24	0.52	0.067	3.4	0.204 ± 0.014
48	0.64	0.138	4.5	0.154 ± 0.012

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

C_0 means the initial concentration of $^{99\text{m}}\text{Tc-P}$.

C_{10} means the concentration of $^{99\text{m}}\text{Tc-P}$ at time 10 minutes.

tion), respectively. Therefore, the K-value decreased with the passage of time from 0.277 (control) to 0.154 (48 hours after the administration). A significant decrease was observed compared with the control 4 hours after the administration of CCl_4 ($P < 0.05$) (Fig. 5, Table 2).

Both of serum GOT and GPT values increased with the passage of post-administrative time. A significant increase was observed compared with the control 4 hours after the administration ($P < 0.05$) (Fig. 6).

Fig. 6. Relation between activity of serum transaminase and time following the administration of CCl_4 .

DISCUSSION

To grasp dynamically the hepatic dysfunction in dogs, the dye clearance test with bromosulphophthalein (Baker and Cuschieri, 1979) or indocyanine green (Kawasaki et al., 1984) has been in use, while the blood clearance test of ^{99m}Tc -P is also of significant value. Then, in the present study, we have pursued some fundamental examinations in view of availability of ^{99m}Tc -P for the evaluation of hepatic function in dogs.

In this study of dogs, the administrations at each dose of 25, 50 and 100 $\mu\text{g/kg}$ of $^{99\text{m}}\text{Tc-P}$ gave similar blood clearance to each other, so it seemed that there would be no difference in hepatic uptake ratio by the doses within this range. Therefore, we chose a dose 100 $\mu\text{g/kg}$ as the dosage for the blood clearance test of $^{99\text{m}}\text{Tc-P}$, since this dose gives an effective clearance curve.

The organ distribution of $^{99\text{m}}\text{Tc-P}$ in dogs was similarly to the cases in rats (Shibata and Hara, 1988), that is, $^{99\text{m}}\text{Tc-P}$ was taken up into the reticuloendothelial system (RES) organs like the liver and spleen, and little uptake of $^{99\text{m}}\text{TcO}_4^-$ was recognized into the thyroid gland.

In this study, two-phase change was observed in the blood concentration of $^{99\text{m}}\text{Tc-P}$, and the K-values were determined from the first phase of the clearance curve. These were similarly to the cases in rats (Shibata and Hara, 1988).

In acute hepatitis, increased RES function has been reported (Cooksley et al., 1973 ; Van Vliet et al., 1981), and in cirrhosis, alcoholics and other liver diseases, decreased RES function has been admitted (Chritie et al., 1967; Kurahori et al., 1979; Liu, 1979). However, the relation between hepatocellular disorder and the RES function including the activity and the number of RES cells has not been clarified. In addition, the disappearance rate of $^{99\text{m}}\text{Tc-P}$ from blood may be influenced by the liver blood flow and the whole circulating blood volume (Takahashi and Matsuoka, 1980a ; Takahashi and Matsuoka, 1980b). Therefore, as reported previously (Shibata and Hara, 1988), the blood clearance of $^{99\text{m}}\text{Tc-P}$ as represented by K-value reflects hepatic function in a broad sense.

The present study has made it clear that the blood clearance of $^{99\text{m}}\text{Tc-P}$ in dogs changes with the increase of the dose and with the passage of time after the administration of CCl_4 similarly to the activity of serum transaminase. And, decrease of K-value with the increase in dose of CCl_4 was almost similar, however, decrease of K-value with the passage of time after the CCl_4 administration was lower as compared with the cases in rats (Shibata and Hara, 1988). In addition, the minimum dose of CCl_4 for the induction of obvious reaction was 0.1 ml/kg in two tests, while the earliest time when obvious changes appeared was 4 hours after the administration of CCl_4 . However, in rats, the blood clearance pattern of $^{99\text{m}}\text{Tc-P}$ was altered at a smaller dose of 0.05 ml/kg, as early as 2 hours after the administration of CCl_4 (Shibata and Hara, 1988). These facts suggest that the careful consideration of the species of the animals are required for the studies on the movement, metabolism and toxicity of the foreign particulate substances in the living body (Takahashi and Matsuoka, 1980b).

From these findings, it would be concluded that the blood clearance test of $^{99\text{m}}\text{Tc-P}$ is one of more sensitive methods for evaluation of hepatic dysfunction in dogs, equally to the serum transaminase test.

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