EFFECT OF $\alpha$-NAPHTHYLISOTHIOCYANATE ON BLOOD CLEARANCE OF $^{99m}$Tc-PHYTATE IN DOGS

Hiroshi SHIBATA, Masahiro KOHNO and Hirotake KATOH
Department of Veterinary Pharmacology, Faculty of Agriculture,
Yamaguchi University, 1677-1, Yoshida, Yamaguchi 753, Japan
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Abstract······The effect of $\alpha$-naphthylisothiocyanate (ANIT) on blood clearance of $^{99m}$Tc-phytate ($^{99m}$Tc-P) in dogs was examined, and blood clearance test of $^{99m}$Tc-P was compared with the cases of serum transaminase test or serum bilirubin test.

Serum transaminase and bilirubin levels in dogs increased after ANIT administration, however, the degree of increase in these parameters was much lower than the cases in rats. The disappearance rate of $^{99m}$Tc-P from blood in dogs decreased with the increase in dose of ANIT and with the passage of time after the ANIT administration. Changes of the blood clearance of $^{99m}$Tc-P after ANIT treatment in dogs may be influenced by the disorder in the hepatocytes rather than in the bile ductule cells. The blood clearance test of $^{99m}$Tc-P in dogs showed a sensitive reaction for the acute hepatic dysfunction induced by ANIT equally to the serum transaminase test or the serum bilirubin test.

Key words: $^{99m}$Tc-phytate, blood clearance, $\alpha$-naphthylisothiocyanate, hepatic dysfunction, dog.

INTRODUCTION

The acute administration of $\alpha$-naphthylisothiocyanate (ANIT) causes not only marked hyperbilirubinemia and cholestasis but also hepatocellular disorder in mice and rats (Plaa and Priestly, 1976; El-Hawari and Plaa, 1979; Fukumoto et al., 1980; Aoki et al., 1986). However, it is reported that no hyperbilirubinemia is produced even when high doses of ANIT are administered in dogs, although bilirubinuria

Correspondence: Dr. Hiroshi SHIBATA at the above address.
appears to be present and serum GPT activity is elevated (Indacochea-Redmond and Plaa, 1971). These reports suggest that dogs respond to ANIT in a different manner as compared with rats, but hepatocytes degeneration occurs in dogs similarly to the cases in rats.

On the other hand, we reported that the blood clearance test of $^{99m}$Tc-phytate ($^{99m}$Tc-P) was one of more sensitive methods for evaluation of hepatic dysfunction in dogs, equally to the serum transaminase test (Shibata et al., 1988), and changes of the blood clearance of $^{99m}$Tc-P after ANIT treatment in rats might be influenced by the disorder in the hepatocytes rather than in the bile ductule cells (Shibata et al., 1989).

The purposes of this report are twofold. The first is determine whether hepatocytes degeneration and bilirubinemia are occurred after ANIT treatment in dogs by means of examination of changes in serum transaminase and bilirubin. The second purpose is to investigate the effect of ANIT on blood clearance of $^{99m}$Tc-P in dogs. That is, by using the dogs with ANIT-induced hepatic injury, the relation between the blood clearance of $^{99m}$Tc-P and the severity of hepatic dysfunction was examined, and blood clearance test of $^{99m}$Tc-P was compared with the cases of the serum transaminase and bilirubin 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.

ANIT was dissolved in olive oil at the concentration of 25 mg/ml, and it 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 the previous paper (Shibata and Hara, 1988). Since two-phase change was observed in $^{99m}$Tc-P concentration in blood after intravenous injection of $^{99m}$Tc-P, the blood clearance rate of $^{99m}$Tc-P (K) was calculated from the first phase and second phase of the clearance curve, respectively. The K-values from the first phase were obtained from the values of blood concentration at 5 and 10 minutes after the injection of $^{99m}$Tc-P, and the K-values from the second phase were obtained from the values at 30, 45 and 60 minutes. The K-value was 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 $^{99m}$Tc-P 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 bilirubin concentration by the alkaiazobilirubin method (Wako Pure Chemical Industries, Ltd.).
Effect of ANIT on blood clearance of $^{99m}$Tc-P in dogs

RESULTS

1. Changes of Serum Transaminase Activity and Bilirubin Concentration after Administration of ANIT:

   ANIT was given to the dogs at doses of 25, 50, 70, 100, 140 and 200 mg/kg, and the dose-dependent changes of serum GOT and GPT activities and bilirubin concentration were examined 48 hours after the administration of each dose. No elevation of serum GOT, GPT and bilirubin levels could be detected when doses as high as 50 mg/kg were administered, and these parameters began to rise dose-dependently from the dose of 70 mg/kg, however, at the dose of 200 mg/kg, all dogs died apparently by peritonitis.

   After the administration of ANIT at dose of 100 mg/kg, time-dependent changes of serum GOT and GPT activities and total and direct bilirubin concentration were examined. The activities of GOT and GPT began to increase within 16 hours after the administration, and reached the level 5–6 times higher than control (before the administration) level after 2 days, and returned to the control level after 10–14 days. The concentration of total bilirubin rose to 3–4 times after 2 days, and the control level was recovered after 8–10 days, however, as direct bilirubin also rose, increase of direct bilirubin/total bilirubin was not observed (Fig. 1).

2. The Relation between Dose of ANIT and Blood Clearance of $^{99m}$Tc-P, Serum Transaminase Activity, Serum Bilirubin Concentration:

   ANIT was given to the dogs at doses of 50, 70, 100 and 140 mg/kg, and blood

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Fig. 1. Changes of serum transaminase activities and bilirubin concentration after administration of ANIT.

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clearance of $^{99m}$Tc-P, serum transaminase activity and serum bilirubin concentration were 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 ANIT (Fig. 2). The K-value from the first phase decreased from 0.277 (control: treated with olive oil alone) to 0.154 (140 mg/kg ANIT) with the increase of ANIT dose level. A significant decrease was observed in the K-value compared with the control at dose of ANIT 70 mg/kg ($P<0.05$). On the other hand, significant changes with the increase in dose of ANIT were not observed in the K-value from the second phase (Table 1).

![Graph showing the relation between blood clearance of $^{99m}$Tc-P and dose of ANIT.](image)

**Fig. 2.** Relation between blood clearance of $^{99m}$Tc-P and dose of ANIT.

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

<table>
<thead>
<tr>
<th>ANIT (mg/kg)</th>
<th>K (first phase)</th>
<th>K (second phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.277±0.021</td>
<td>0.0084±0.0014</td>
</tr>
<tr>
<td>50</td>
<td>0.257±0.020</td>
<td>0.0116±0.0016</td>
</tr>
<tr>
<td>70</td>
<td>0.231±0.017</td>
<td>0.0105±0.0016</td>
</tr>
<tr>
<td>100</td>
<td>0.198±0.017</td>
<td>0.0137±0.0018</td>
</tr>
<tr>
<td>140</td>
<td>0.154±0.012</td>
<td>0.0124±0.0018</td>
</tr>
</tbody>
</table>

Values show mean ± S.D. of 6 dogs.
Effect of ANIT on blood clearance of $^{99m}$Tc-P in dogs

Serum GOT, GPT and bilirubin values rose with the increase in dose of ANIT. Significantly high values were obtained compared with the control at dose of ANIT 70 mg/kg (P<0.05) (Fig. 3).

3. The Relation between Time Following the Administration of ANIT and Blood Clearance of $^{99m}$Tc-P, Serum Transaminase Activity, Serum Bilirubin Concentration:

After the administration of ANIT at dose 100 mg/kg, blood clearance of $^{99m}$Tc-P, serum transaminase activity and serum bilirubin concentration were determined at hours 4, 8, 16, 24 and 48.

$^{99m}$Tc-P concentration in blood tended to rise with the passage of post-administrative time (Fig. 4). The K-value from the first phase decreased with the passage of time from 0.277 (control: before the administration) to 0.198 (48 hours after the administration). A significant decrease was observed compared with the control 16 hours after the administration of ANIT (P<0.05). However, significant changes with the passage of time after the ANIT administration were not observed in the K-value from the second phase (Table 2).

Serum GOT, GPT and bilirubin values increased with the passage of post-administrative time. A significant increase was observed compared with the control 16 hours (GOT, GPT; P<0.05) or 24 hours (bilirubin; P<0.05) after the administration of ANIT (Fig. 5).

![Graph](image-url)  
*Fig. 3. Relation between serum parameter values and dose of ANIT.*
Fig. 4. Relation between blood clearance of $^{99m}$Tc-P and time following the administration of ANIT.

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

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>K (first phase)</th>
<th>K (second phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.277±0.021</td>
<td>0.0084±0.0014</td>
</tr>
<tr>
<td>4</td>
<td>0.248±0.021</td>
<td>0.0065±0.0012</td>
</tr>
<tr>
<td>8</td>
<td>0.239±0.020</td>
<td>0.0066±0.0012</td>
</tr>
<tr>
<td>16</td>
<td>0.231±0.018</td>
<td>0.0092±0.0014</td>
</tr>
<tr>
<td>24</td>
<td>0.231±0.017</td>
<td>0.0086±0.0014</td>
</tr>
<tr>
<td>48</td>
<td>0.198±0.017</td>
<td>0.0156±0.0016</td>
</tr>
</tbody>
</table>

Values show mean ± S.D. of 6 dogs.

DISCUSSION

In this paper, changes of blood clearance of $^{99m}$Tc-phytate ($^{99m}$Tc-P), serum transaminase activity and serum bilirubin concentration after a single administration of $\alpha$-naphthylisothiocyanate (ANIT) in dogs were examined.

It is reported that rats, mice and guinea pigs are sensitive to ANIT intoxication, while hamsters, rabbits and dogs are more resistant (Capizzo and Roberts, 1971; Indacochea-Redmond and Plaa, 1971), and, particularly in dogs, no hyperbilir-
Effect of ANIT on blood clearance of $^{99m}$Tc-P in dogs

![Graph showing the effect of ANIT on blood clearance of $^{99m}$Tc-P in dogs]

Fig. 5. Relation between serum parameter values and time following the administration of ANIT.

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minimum dose of ANIT for the induction of obvious reaction in the blood clearance of $^{99m}$Tc-P was 70 mg/kg (48 hours after administration) as same as the cases in serum transaminase and bilirubin levels. However, the earliest time when obvious changes appeared was 16 hours after the administration of ANIT (100 mg/kg) in the blood clearance of $^{99m}$Tc-P and serum transaminase level, while 24 hours in serum bilirubin level. These results suggest that the blood clearance test of $^{99m}$Tc-P in dogs may show a sensitive reaction for the acute hepatic dysfunction induced by ANIT equally to the serum transaminase test or the serum bilirubin test. And, changes of the blood clearance of $^{99m}$Tc-P after ANIT treatment in dogs may be influenced by the disorder in the hepatocytes rather than in the bile ductule cells similarly to the cases in rats (Shibata et al., 1989).

As compared with the cases in rats (Shibata et al., 1989), the degree of K-value (from the first phase) decrease with the increase in the dose of ANIT was almost similar, however, that with the passage of time after the ANIT administration was lower. Furthermore, the minimum dose of ANIT for the induction of obvious reaction in the K-value was much higher, and the earliest time when obvious changes appeared was later. These facts suggest that the dog may be insensitive to ANIT intoxication not only in the serum transaminase test or the serum bilirubin test but also in the blood clearance test of $^{99m}$Tc-P.

REFERENCES


Effect of ANIT on blood clearance of $^{99m}$Tc--P in dogs