EFFECT OF α -NAPHTHYLISOTHIOCYANATE ON BLOOD CLEARANCE OF ^{99m}Tc -PHYTATE IN RATS

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Abstract.....The effect of α -naphthylisothiocyanate (ANIT) on blood clearance of ^{99m}Tc -phytate (^{99m}Tc -P) in rats was examined, and blood clearance test of ^{99m}Tc -P was compared with the cases of serum transaminase test, serum bilirubin test or histological test of the liver.

The disappearance rate of ^{99m}Tc -P from blood 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 rats 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 rats showed a sensitive reaction for the acute hepatic dysfunction induced by ANIT equally to the serum transaminase test, the serum bilirubin test or the histological test of the liver.

Key words : ^{99m}Tc -phytate, blood clearance, α -naphthylisothiocyanate, hepatic dysfunction, rat.

INTRODUCTION

α -Naphthylisothiocyanate (ANIT) is a chemical substance which has been shown to disturb functionally and histologically both the bile ductules and bile ducts and to produce hyperbilirubinemia and cholestasis in experimental animals (Plaa and Priestly, 1976). Therefore, the cholestasis induced by this agent has been used as one experimental model. The acute administration of ANIT also causes elevation of plasma transaminase activity (Fukumoto et al., 1980; Aoki et al., 1986) and bromosulfophthalein retention (Indacochea-Redmond et al., 1974) and inhibition of hepatic drug metabolizing enzymes (El-Hawari and Plaa, 1979). Since some of these alterations have been detected before the onset of cholestasis, the acute response to ANIT are thought to be due to its effects on the hepatocytes, rather than on the bile ductule cells.

On the other hand, we have attempted to apply ^{99m}Tc -phytate (^{99m}Tc -P) for the

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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 effect of ANIT on blood clearance of ^{99m}Tc -P in rats. That is, by using the rats 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 or histological examination of the liver.

MATERIALS AND METHODS

Male Wistar strain rats weighing 220–260g were used at the age of 7–8 weeks, and were allocated to each group consisting of 12 heads. During the blood clearance experiment, they were anesthetized with sodium pentobarbital given intraperitoneally at a dose of 40 mg/kg.

ANIT was dissolved in olive oil at the concentration of 25 mg/ml, and it was intraperitoneally administered to the rats at each dose.

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

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

The liver was prepared in tissue sections by the conventional procedure and they were stained with hematoxylin and eosin for histological examination.

RESULTS

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

ANIT was given to the rats at doses of 10, 25, 50, 100 and 200 mg/kg, and blood 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. Both C_0 and $T_{1/2}$ grew from 0.44 %/ml (control : treated with olive oil alone) to 1.00 %/ml (200 mg/kg ANIT), and from 5.2 minutes (control) to 11.5 minutes (200 mg/kg ANIT) as the dose level of ANIT was increased. Therefore, the K-value decreased to about a half, from 0.133 (control) to 0.060 (200 mg/kg ANIT) with the increase of ANIT dose level. A significant decrease was observed in the K-value compared with the control at such a small dose of ANIT as

25 mg/kg ($P < 0.05$) (Fig. 1, Table 1).

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 50 mg/kg ($P < 0.01$) (Fig. 2).

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

After the administration of ANIT at dose 100mg/kg, blood clearance of $^{99m}\text{Tc-P}$, serum transaminase activity and serum bilirubin concentration were determined at

Fig. 1. Relation between blood clearance of $^{99m}\text{Tc-P}$ and dose of ANIT.

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

ANIT(mg/kg)	C_0 (%/ml)	C_{10} (%/ml)	$T_{1/2}$ (min)	K
control	0.44	0.123	5.2	0.133 ± 0.012
10	0.56	0.170	5.7	0.122 ± 0.010
25	0.64	0.250	6.6	0.105 ± 0.010
50	0.82	0.320	7.4	0.094 ± 0.008
100	0.98	0.460	9.2	0.075 ± 0.006
200	1.00	0.540	11.5	0.060 ± 0.005

Values show mean of 12 rats or mean $\pm$ S.D. of 12 rats.

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

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

Fig. 2. Relation between serum parameter values and dose of ANIT.

hours 2, 4, 8, 16, 24 and 48.

^{99m}Tc -P concentration in blood tended to rise with the passage of post-administrative time. Co and T $_{1/2}$ grew as the time went by from 0.44 %/ml (control : before the administration) to 0.98 %/ml (48 hours after the administration), and 5.2 minutes (control) to 9.2 minutes (48 hours after the administration), respectively. Therefore, the K-value decreased with the passage of time from 0.133 (control) to 0.075 (48 hours after the administration). A significant decrease was observed compared with the control 8 hours after the administration of ANIT ($P < 0.05$) (Fig. 3, 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 8 hours (GOT, GPT : $P < 0.05$) or 16 hours (bilirubin : $P < 0.01$) after the administration of ANIT (Fig. 4).

3. Changes in Histological Appearance of the Liver :

The liver was histological examined 48 hours after the administration of ANIT at the dose of 10–200 mg/kg. While at the dose of 25 mg/kg, there were no significant changes, slight degeneration of hepatocells and bile duct epithelium were first observed at the dose of 50 mg/kg. More prominent alterations from vacuolar

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

Fig. 3. Relation between blood clearance of $^{99m}\text{Tc-P}$ and time following the administration of ANIT.

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

Time(hr)	C_0 (%/ml)	C_{10} (%/ml)	$T_{1/2}$ (min)	K
control	0.44	0.123	5.2	0.133 ± 0.012
2	0.51	0.145	5.6	0.124 ± 0.011
4	0.55	0.170	5.7	0.122 ± 0.010
8	0.61	0.212	6.5	0.107 ± 0.009
16	0.76	0.300	7.4	0.094 ± 0.008
24	0.86	0.372	8.3	0.083 ± 0.008
48	0.98	0.460	9.2	0.075 ± 0.006

Values show mean of 12 rats or mean $\pm$ S. D. of 12 rats.

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

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

degeneration and necrosis of hepatocells to degeneration and necrosis of bile duct epithelium, were observed at the dose of 100 mg/kg or more (Table 3, Photograph 1).

The appearance of histological changes was examined from 2 to 48 hours after the administration of ANIT at the dose of 100 mg/kg. Any significant changes was not observed 4 hours after administration. Slight degeneration of hepatocells was first observed at 8 hours. Thereafter, the damage got more severe, including degeneration and necrosis of bile duct epithelium (Table 3, Photograph 2). However, changes of Kuppfer cells were not observed 48 hours after administration.

Fig. 4. Relation between serum parameter values and time following the administration of ANIT.

Table 3. Changes in histological appearance of the liver after administration of ANIT.

Dose of ANIT (48 hours after administration)			Time following the administration of ANIT (100mg/kg)		
	H	D		H	D
control	—	—	control	—	—
10 mg/kg	—	—	4 hours	—	—
25 mg/kg	—	—	8 hours	+	—
50 mg/kg	+	+	16 hours	+	+
100 mg/kg	+	#	24 hours	+	#
200 mg/kg	+	#	48 hours	+	#

H : changes of hepatocells.

D : changes of bile ductule cells.

— : no change, + : slight, # : moderate.

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

Photo. 1. Photomicrograph of the rat liver 48 hours after the administration of ANIT at the dose of 50 mg/kg (H-E, $\times 200$).

Photo. 2. Photomicrograph of the rat liver 48 hours after the administration of ANIT at the dose of 100 mg/kg (H-E, $\times 200$).

DISCUSSION

ANIT was administered to various kinds of experimental animals and rats were found to be quite sensitive to ANIT intoxication (Capizzo and Roberts, 1971 ; Indacochea-Redmond and Plaa, 1971). In our experiment, serum GOT and GPT activities and serum bilirubin concentration began to increase dose-dependantly from the dose 50 mg/kg of ANIT (48 hours after the administration). Furthermore, it has been reported that ANIT produces toxic effects which have an early onset such as elevation of plasma transaminase (Fukumoto et al., 1980 ; Aoki et al., 1986) and bromosulfophthalein retention (Indacochea-Redmond et al., 1974) and effects with a late onset such as hyperbilirubinemia and cholestasis (Indacochea-Redmond and Plaa, 1971 ; El-Hawari and Plaa, 1979) in rats. The data presented in this report confirm these studies. That is, serum GOT and GPT activities were elevated gradually from 8 hours after the administration of ANIT (100 mg/kg), while serum bilirubin concentration was elevated from 16 hours. And, the histological appearance of the liver examined in this study supported these data.

The blood clearance test of $^{99m}\text{Tc-P}$ may be of significant value to grasp dynamically the hepatic dysfunction in rats (Shibata and Hara, 1988). Then, we applied this test for the rats with acute hepatic dysfunction experimentally induced by ANIT administration. Intravenous administration of $^{99m}\text{Tc-P}$ causes immediate chelation of blood calcium, and this makes microcolloidal substance which is taken up into the reticuloendothelial system (RES) (Subramanian et al., 1973). The spleen, an organ of RES, is an important one besides liver. However, since the uptake ratio of $^{99m}\text{Tc-P}$ into the liver (activity in liver / total activity in body) reached approximately 92% 15 minutes after the administration (Shibata and Hara, 1988) and the accumulation of ^{14}C in the liver is much higher than that in the spleen after ANIT- ^{14}C administration in rats (Capizzo and Roberts, 1970), the blood clearance of $^{99m}\text{Tc-P}$ may reflect hepatic function.

The acute administration of ANIT causes not only marked hyperbilirubinemia and cholestasis but also hepatocellular disorder in rats (Plaa and Priestly, 1976 ; El-Hawari and Plaa, 1979 ; Fukumoto et al., 1980 ; Aoki et al., 1986). And, though histological changes of liver Kuppfer cells were not observed after the administration of ANIT in our experiment, the RES function in the liver may be disturbed by the treatment of rats with ANIT. 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 $^{99m}\text{Tc-P}$ from blood may be influenced by the liver blood flow and the whole circulating blood volume (Takahashi and Matsuoka, 1980a, 1980b). Therefore, the blood clearance of $^{99m}\text{Tc-P}$ as represented by K-value reflects hepatic function in a broad sense.

The present study has made it clear that the blood clearance of $^{99m}\text{Tc-P}$ in rats changes with the passage of time after the administration of ANIT. Furthermore, the decrease of blood clearance of $^{99m}\text{Tc-P}$ after the administration of ANIT was

observed at the same time as the increase of serum transaminase level, but earlier than the increase of serum bilirubin level. Therefore, changes of the blood clearance of $^{99m}\text{Tc-P}$ after ANIT treatment in rats may be influenced by the disorder in the hepatocytes rather than in the bile ductule cells.

The minimum dose of ANIT for the induction of obvious reaction was 25 mg/kg 48 hours after administration in the blood clearance of $^{99m}\text{Tc-P}$, while 50 mg/kg in serum transaminase and bilirubin levels and histological changes of the liver. Furthermore, the earliest time when obvious changes appeared was 8 hours after the administration of ANIT (100 mg/kg) in the blood clearance of $^{99m}\text{Tc-P}$, serum transaminase level and histological appearance of the liver, while 16 hours in serum bilirubin level. These results suggest that the blood clearance test of $^{99m}\text{Tc-P}$ in rats may show a sensitive reaction for the acute hepatic dysfunction induced by ANIT equally to the serum transaminase test, the serum bilirubin test or the histological test of the liver.

As compared with the cases of CCl_4 administration (Shibata and Hara, 1988), the degree of K-value decrease with the increase in dose and with the passage of time after the administration were almost similar. Furthermore, the minimum dose for the induction of obvious changes and the earliest time when obvious changes appeared in the blood clearance of $^{99m}\text{Tc-P}$ were almost similar to the cases in serum transaminase level after both ANIT and CCl_4 treatment. These are because both ANIT and CCl_4 cause the disorder in the hepatocytes, and this disorder may change the blood clearance of $^{99m}\text{Tc-P}$ in rats.

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