EFFECT OF D-GALACTOSAMINE ON BLOOD CLEARANCE OF ^{99m}Tc -PHYTATE IN RATS

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Abstract.....The effect of D-galactosamine (GalN) on the blood clearance of ^{99m}Tc -phytate (^{99m}Tc -P) in rats was examined, and the blood clearance test of ^{99m}Tc -P was compared with the cases of serum transaminase and bilirubin test.

Serum transaminase and bilirubin levels in rats increased dose-dependantly after GalN administration. The disappearance rate of ^{99m}Tc -P from blood decreased with the increase in dose of GalN and with the passage of time after the GalN administration. Changes of the blood clearance of ^{99m}Tc -P after GalN treatment in rats may be influenced by the disorder in the hepatocytes and the alteration of the liver reticuloendothelial system phagocytic function. The blood clearance test of ^{99m}Tc -P in rats showed a sensitive reaction for the acute hepatic dysfunction induced by GalN equally to or higher than the serum transaminase and bilirubin test.

Key words: ^{99m}Tc -phytate, blood clearance, D-galactosamine, hepatic dysfunction, reticuloendothelial system.

INTRODUCTION

D-Galactosamine (GalN) is a chemical substance which has been shown to induce biochemical and histological evidence of hepatocellular necrosis and fatty liver that closely resemble human viral hepatitis in laboratory animals (Keppler et al., 1968 ; Medline et al., 1970 ; Koff et al., 1971a, 1971b ; Shinozuka et al., 1973 ; Farber et al., 1973). In the earlier study (Keppler et al., 1968), the lesions were induced by repeated injections of frequent intervals, resulting in a relatively extensive hepatocellular necrosis. It soon became apparent that a single injection is as effective as multiple injections in inducing typical lesions and that the severity of the lesion is dose dependent (Koff et al., 1971a, 1971b ; Shinozuka et al., 1973 ; Farber et al., 1973). Furthermore, it is proposed the reticuloendothelial system is implicated in

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GalN-induced hepatic injury in rats because of a correlation between the phagocytic alteration and the development of hepatocellular necrosis (Grun et al., 1974 ; Al-Tuwaijri et al., 1981).

On the other hand, we have attempted to apply ^{99m}Tc -phytate (^{99m}Tc -P) for the evaluation of hepatic in laboratory animals, and 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 and bilirubin test or the histological test (Shibata and Hara, 1988 ; Shibata et al., 1989).

The purpose of this report is to investigate the effect of GalN on the blood clearance of ^{99m}Tc -P in rats. That is, by using the rats with GalN-induced hepatic injury, the relation between the blood clearance of ^{99m}Tc -P and the severity of hepatic dysfunction was examined, and the blood clearance test of ^{99m}Tc -P was compared with the cases of the serum transaminase and bilirubin determination.

MATERIALS AND METHODS

Male Wistar strain rats weighing 220–260 g 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.

D-Galactosamine (GalN) HCl was dissolved in aqueous solution neutralized with 1N NaOH at the concentration of 50 mg/ml, and it was intraperitoneally administered to the rats 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 the ^{99m}Tc -P concentration in blood after the intravenous injection of ^{99m}Tc -P, the blood clearance rate of ^{99m}Tc -P (K) was calculated from the first 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 ^{99m}Tc -P, and the K-values from the second phase (K_2 -value) were obtained from the values at 30 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 alkali azobilirubin method (Wako Pure Chemical Industries, Ltd.).

RESULTS

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

GalN was given to the rats at doses of 50, 100, 250, 500 and 1000 mg/kg, and the blood clearance of $^{99m}\text{Tc-P}$, serum transaminase activity and serum bilirubin concentration were determined 48 hours after the administration of each dose.

The $^{99m}\text{Tc-P}$ concentration in blood showed a tendency to rise with the increase in dose level of GalN (Fig. 1). The K_1 -value decreased from 0.133 (control : treated with aqueous solution alone) to 0.051 (1000 mg/kg GalN) with the increase of GalN dose level. A significant decrease was observed in the K_1 -value compared with the control at dose of GalN 100 mg/kg ($P < 0.05$). On the other hand, significant changes with the increase in dose of GalN were not observed in the K_2 -value (Table 1).

Serum GOT, GPT and bilirubin values rose with the increase in dose of GalN. Significantly high values were obtained compared with the control at dose of GalN 250 mg/kg (GOT, GPT ; $P < 0.01$) or 500 mg/kg (bilirubin ; $P < 0.01$) (Fig. 2).

Fig. 1. Relation between blood clearance of $^{99m}\text{Tc-P}$ and dose of GalN examined 48 hours after the administration of each dose.

Table 1. Relation between blood clearance of $^{99m}\text{Tc-P}$ and dose of GalN examined 48 hours after the administration of each dose.

GalN(mg/kg)	K_1 -value	K_2 -value
control	0.133 ± 0.012	0.0098 ± 0.0016
50	0.116 ± 0.010	0.0157 ± 0.0020
100	0.108 ± 0.010	0.0119 ± 0.0018
250	0.094 ± 0.008	0.0109 ± 0.0018
500	0.068 ± 0.007	0.0129 ± 0.0018
1000	0.051 ± 0.005	0.0126 ± 0.0018

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

Fig. 2. Relation between serum parameter values and dose of GalN examined 48 hours after the administration of each dose.

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

After the administration of GalN at dose 500 mg/kg, the blood clearance of $^{99m}\text{Tc-P}$, serum transaminase activity and serum bilirubin concentration were determined at hours 2, 4, 8, 16, 24 and 48.

The $^{99m}\text{Tc-P}$ concentration in blood tended to rise with the passage of post-administrative time (Fig. 3). The K_1 -value decreased with the passage of time from 0.133 (control: before the administration) to 0.068 (48 hours after the administration). A significant decrease was observed in the K_1 -value compared with the control 4 hours after the administration of GalN ($P < 0.05$). However, significant changes with the passage of time after the GalN administration were not observed in the K_2 -value (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 4 hours (GOT, GPT ; $P < 0.05$) or 16 hours (bilirubin ; $P < 0.05$) after the administration of GalN (Fig. 4).

Fig. 3. Relation between blood clearance of $^{99m}\text{Tc-P}$ and time following the administration of GalN at dose 500 mg/kg.

Table 2. Relation between blood clearance of $^{99m}\text{Tc-P}$ and time following the administration of GalN at dose 500 mg/kg.

Time(hr)	K_1 -value	K_2 -value
control	0.133 ± 0.012	0.0098 ± 0.0016
2	0.116 ± 0.010	0.0108 ± 0.0016
4	0.102 ± 0.010	0.0144 ± 0.0020
8	0.087 ± 0.008	0.0145 ± 0.0018
16	0.077 ± 0.007	0.0151 ± 0.0020
24	0.068 ± 0.007	0.0135 ± 0.0018
48	0.068 ± 0.007	0.0129 ± 0.0018

Values show mean $\pm$ S.D. of 12 rats. **Fig. 4.** Relation between serum parameter values and time following the administration of GalN at dose 500 mg/kg.

DISCUSSION

It has been reported that administration of GalN induces biochemical evidence of hepatocellular necrosis in laboratory animals and the severity of the lesion is dose dependent (Keppler et al., 1986 ; Koff et al., 1971a, 1971b ; Shinozuka et al., 1973 ; Farber et al., 1973). The data presented in our experiment confirm these studies. That is, serum GOT and GPT activities were elevated gradually from 4 hours after the administration of GalN (500 mg/kg), while serum bilirubin concentration increased from 16 hours, and the elevation of serum transaminase activity was dose dependent.

Though the relation between the hepatocellular disorder and the liver reticuloendothelial system (RES) function has not been clarified, it is proposed that the alteration of RES phagocytic function contributes to the development of GalN-induced hepatocellular necrosis in rats (Grun et al., 1974 ; Al-Tuwaijri et al., 1981). On the other hand, intravenous administration of $^{99m}\text{Tc-P}$ causes immediate chelation of blood calcium, and this makes microcolloidal substance which is taken up into the RES (Subramanian et al., 1973). And, the uptake ratio of $^{99m}\text{Tc-P}$ into the rat liver RES reached approximately 92% 15 minutes after the administration (Shibata and Hara, 1988). In addition, though 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), the liver blood flow and cardiac output in the rat with GalN-induced hepatic injury shows no significant changes (Liehr et al., 1972). Therefore, the blood clearance of $^{99m}\text{Tc-P}$ in the rat with GalN-induced hepatic injury may reflect the alteration of the liver RES phagocytic function.

In this study, the blood clearance of $^{99m}\text{Tc-P}$ was determined from the K_1 -value, since significant changes with the increase in dose of GalN and with the passage of time after GalN administration were not observed in the K_2 -value. The present study has made it clear that the blood clearance of $^{99m}\text{Tc-P}$ in rats changes with the increase of the dose and with the passage of time after the administration of GalN similarly to the activity of serum transaminase and the concentration of serum bilirubin. Furthermore, the minimum dose of GalN for the induction of obvious reaction was 100 mg/kg 48 hours after the administration in the blood clearance of $^{99m}\text{Tc-P}$, while 250 mg/kg in serum transaminase level and 500 mg/kg in serum bilirubin level. However, the earliest time when obvious changes appeared was 4 hours after the administration of GalN (500 mg/kg) in the blood clearance of $^{99m}\text{Tc-P}$ and serum transaminase level, 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 GalN equally to or higher than the serum transaminase and bilirubin test. And, changes of the blood clearance of $^{99m}\text{Tc-P}$ after GalN treatment in rats may be influenced by the disorder in the hepatocytes.

The degree of decrease in K_1 -value with the increase in dose and with the passage

of time after the administration of GalN, compared with the control value were almost similar to the cases of carbon tetrachloride (CCl₄) or α -naphthylisothiocyanate (ANIT) administration (Shibata and Hara, 1988 ; Shibata et al., 1989). Furthermore, the minimum dose for the induction of obvious changes and the earliest time when obvious changes appeared in the blood clearance of ^{99m}Tc-P were almost similar to the cases in serum transaminase level after CCl₄, ANIT or GalN treatment. These are because CCl₄, ANIT and GalN cause the disorder in the hepatocytes, and this disorder may change the blood clearance of ^{99m}Tc-P in rats.

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