EFFECT OF DL-ETHIONINE ON BLOOD CLEARANCE OF ^{99m}Tc -PHYTATE IN DOGS

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Accepted April 20, 1990

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

Serum transaminase and bilirubin levels in dogs increased dose-dependently after the administration of EthN. The disappearance rate of ^{99m}Tc -P from blood in dogs decreased with the increase in dose of EthN and with the passage of time after EthN administration. Changes of the blood clearance of ^{99m}Tc -P after EthN treatment in dogs may be influenced by the disorder in the hepatocytes. The blood clearance test of ^{99m}Tc -P in dogs showed a reaction for the acute hepatic dysfunction induced by EthN equally to the serum transaminase and bilirubin test.

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

INTRODUCTION

Intravenous administration of ^{99m}Tc -phytate (^{99m}Tc -P) causes immediate chelation with blood calcium and this makes microcolloidal substance which is taken up mainly by the liver reticuloendothelial system (RES) (Subramanian et al., 1973 ; Shibata and Hara, 1988 ; Shibata et al., 1988). Then, we have attempted to apply ^{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 reaction for the acute hepatic dysfunction induced by carbon tetrachloride (CCl_4) (Shibata et al., 1988), α -naphthylisothiocyanate (ANIT) (Shibata et al., 1989) or D-galactosamine (GalN) (Shibata and Kohno, 1989) equally to the serum transaminase and bilirubin test. DL-Ethionine (EthN) is well-known to induce inhibition of hepatic protein synthesis and fatty liver in rats (Farber, 1967), but there have been few reports about the RES function in this type of injury. On the other hand, there

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have been some reports about the effect of EthN in dogs (Kroboth, 1954 ; Wang et al., 1958 ; Cobb et al., 1985), but EthN-induced liver injury has not been clarified.

The purposes of this report are twofold. The first is to determine whether hepatocytes degeneration and bilirubinemia are occurred after EthN treatment in dogs by means of examination of changes in serum transaminase and bilirubin. The second purpose is to investigate the effect of EthN on the blood clearance of ^{99m}Tc -P in dogs. That is, by using the dogs with EthN-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

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.

DL-Ethionine (EthN) was dissolved in 0.9% NaCl solution at the concentration of 50 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 changes was observed in the ^{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 (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 direct and total bilirubin concentration by the alkaliazobilirubin method (Wako Pure Chemical Industries, Ltd.).

RESULTS

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

EthN was given to the dogs at dose of 100, 250, 500, 1000 or 2000 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 250 mg/kg were administered, and these parameters began to rise dose-dependently from

the dose of 500 mg/kg, however, at the dose of 2000 mg/kg, all dogs died apparently by peritonitis.

After the administration of EthN at dose of 500 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 24 hours after the administration, and the GOT level reached 5–6 times higher and the GPT level 8–9 times higher than the control level (before the administration) after 3 days, and these activities returned to the control level after 14 days. The concentration of total bilirubin rose to 4–5 times after 3 days, and the control level was recovered after 14 days. However, as the concentration of direct bilirubin rose, the increase in the ratio of direct / total bilirubin was not observed (Fig. 1).

Fig. 1. Changes of serum transaminase activities and bilirubin concentration after the administration of EthN at dose 500 mg/kg.
T-Bil and D-Bil are total and direct bilirubin concentration.
The data represent mean of 6 dogs.

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

EthN was given to the dogs at dose of 100, 250 500 or 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}Tc -P concentration in blood showed a tendency to rise with the increase in dose level of EthN (Fig. 2). The K_1 -value decreased from 0.277 (control : treated with 0.9% NaCl solution alone) to 0.216 (1000 mg/kg EthN) with the increase of EthN dose level. A significant decrease was observed in the K_1 -value compared with the control at dose of EthN 500 mg/kg ($P < 0.05$). On the other hand, significant changes with the increase in dose of EthN were not observed in the K_2 -value (Table 1).

Serum GOT, GPT and bilirubin values rose with the increase in dose of EthN. Significantly high values were obtained compared with the control at dose of EthN 500 mg/kg ($P < 0.01$) (Fig. 3).

Fig. 2. Blood clearance of ^{99m}Tc -P 48 hours after the administration of various doses of EthN. The data represent mean of 6 dogs.

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

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

EthN(mg/kg)	K_1 -value	K_2 -value
control	0.277 ± 0.021	0.0084 ± 0.0014
100	0.274 ± 0.020	0.0064 ± 0.0012
250	0.251 ± 0.020	0.0117 ± 0.0018
500	0.220 ± 0.016	0.0106 ± 0.0016
1000	0.216 ± 0.016	0.0093 ± 0.0016

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

Fig. 3. Serum parameter values 48 hours after the administration of various doses of EthN. Bil is total bilirubin concentration. The data represent mean $\pm$ S. D. of 6 dogs.

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

After the administration of EthN at dose 500 mg/kg, the blood clearance of $^{99m}\text{Tc-P}$, serum transaminase activity and serum bilirubin concentration were determined at hours 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. 4). The K_1 -value decreased with the passage of time from 0.277 (control : before the administration) to 0.220 (48 hours after the administration). A significant decrease was observed in the K_1 -value compared with the control 24 hours after the administration of EthN ($P < 0.05$). However, significant changes with the passage of time after the EthN 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 24 hours after the administration of EthN (GOT, GPT : $P < 0.01$; bilirubin : $P < 0.05$) (Fig. 5).

Fig. 4. Blood clearance of $^{99m}\text{Tc-P}$ after the administration of EthN at dose 500 mg/kg. The data represent mean of 6 dogs.

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

Table 2. Blood clearance of $^{99m}\text{Tc-P}$ after the administration of EthN at dose of 500 mg/kg.

Time(hr)	K_1 -value	K_2 -value
control	0.277 ± 0.021	0.0084 ± 0.0014
4	0.263 ± 0.020	0.0086 ± 0.0014
8	0.251 ± 0.018	0.0118 ± 0.0018
16	0.238 ± 0.018	0.0096 ± 0.0016
24	0.226 ± 0.016	0.0048 ± 0.0012
48	0.220 ± 0.016	0.0106 ± 0.0016

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

Fig. 5. Serum parameter values after the administration of EthN at dose 500 mg/kg. Bil is total bilirubin concentration. The data represent mean $\pm$ S. D. of 6 dogs.

DISCUSSION

Some reports have indicated that there is a sex difference in the response of the liver to EthN using rats (Farber and Corban, 1958 ; Farber, 1967). Therefore, at first, we examined the changes of serum transaminase and bilirubin levels after administration of EthN by using male and female dogs. However, there was no sex difference in these responses (data not shown). Therefore, we used dogs of both sexes.

EthN, the ethyl analogue of methionine, has provided a model for study of the biochemical effects leading to acute liver injury in rats. After its administration (1000mg/kg, ip), hepatic ATP levels are drastically lowered (Villa-Trevino et al., 1963, 1966), resulting in a decreased rate of protein synthesis (Villa-Trevino et al., 1963, 1966) and a decreased rate of RNA synthesis (Villa-Trevino et al., 1966). Fatty liver (Farber, 1967) and morphologic changes in the nucleoli (Goldblatt et al., 1973) are also noted. However, EthN-induced liver injury in dogs has not been clarified. Then, we attempted to determine whether hepatocytes degeneration and bilirubinemia were occurred after EthN treatment in dogs by means of examination of changes in serum transaminase and bilirubin. Our data demonstrated that serum transaminase activity and bilirubin concentration were increased after the administration of EthN, however, the degree of increase in these parameters was much lower than after CCl₄ (Shibata et al., 1988) or GalN (Shibata and Kohno, 1989) administration. Furthermore, the increase in the ratio of direct / total bilirubin was not observed. These results suggest that moderate hepatocytes degeneration may be occurred in dogs after the administration of EthN.

In this study, the blood clearance of ^{99m}Tc-P was determined from the K₁-values, since significant changes with the increase in dose of EthN and with the passage of time after EthN administration were not observed in the K₂-values. The present study has made it clear that the blood clearance of ^{99m}Tc-P in dogs changed with the increase of the dose and with the passage of time after the administration of EthN similarly to the activity of serum transaminase and the concentration of serum bilirubin. Furthermore, the minimum dose of EthN for the induction of obvious reaction in the blood clearance of ^{99m}Tc-P was 500 mg/kg (48 hours after the administration) as same as the cases in serum transaminase and bilirubin levels. And, the earliest time when obvious changes appeared was 24 hours after the administration of EthN (500 mg/kg) both in the blood clearance of ^{99m}Tc-P and in serum transaminase and bilirubin levels. These results suggest that the blood clearance test of ^{99m}Tc-P in dogs may be show a reaction for the acute hepatic dysfunction induced by EthN equally to the serum transaminase and bilirubin test. And, changes of the blood clearance of ^{99m}Tc-P after EthN treatment in dogs may be influenced by the disorder in the hepatocytes.

Our previous studies indicated that the blood clearance test of ^{99m}Tc-P in dogs showed a sensitive reaction for the acute hepatic dysfunction induced by CCl₄

(Shibata et al., 1988), ANIT (Shibata et al., 1989) or GalN (Shibata and Kohno, 1989) equally to the serum transaminase and bilirubin test. And, the present study indicated that this test showed a reaction for the acute hepatic dysfunction induced by EthN equally to the biochemical examination. That is, in the dogs induced hepatic injury with CCl_4 , ANIT, GalN or EthN, the minimum dose for the induction of obvious reaction and the earliest time when obvious changes appeared in the blood clearance of $^{99m}\text{Tc-P}$ were almost similar as compared with the cases in these biochemical parameters. However, the degree of decrease in the blood clearance rate of $^{99m}\text{Tc-P}$ with the increase in the dose and with the passage of time after the administration was much higher in the cases of CCl_4 , ANIT or GalN treatment than in EthN treatment.

The relation between the hepatocellular disorder and liver RES function has not been clarified. A clear correlation between the alteration of RES activity and the degree of hepatic injury in both CCl_4 and EthN treated mice was not noted (Okazaki et al., 1985, 1987), while the development of GalN-induced hepatocellular necrosis in rats contributed to altered RES function (Al-Tuwaijri et al., 1981 ; Grun et al., 1974). In addition, the blood clearance rate of $^{99m}\text{Tc-P}$ may be affected not only by the RES function but by intravascular coagulation and fibrinolysis, platelet aggregation and disaggregation and blood flow through the RES (Donald, 1980 ; Takahashi and Matsuoka, 1980). Therefore, the blood clearance of $^{99m}\text{Tc-P}$ may reflect the hepatic function in a broad sense, including the RES function.

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