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The Rate of Elimination and Distribution Volume of Rabbit Muscle Creatine Phosphokinase[1)]

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An *in vivo* study on the elimination of rabbit muscle creatine phosphokinase (CPK) following intravenous administration has been carried out with nine rabbits selected for low endogeneous CPK fluctuation character. A two-compartment open model is proposed for the disposition of CPK. The equation $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ was employed to fit the profile of CPK activity in the plasma. The pharmacokinetic parameters of CPK in rabbits were determined at low, medium and high dose levels, and the factors which affect the estimation of pharmacokinetic parameters are discussed. The elimination of CPK appeared to be independent of the injected dose, but the intersubject variability was significant. The pharmacokinetic parameters α and β at high injected dose (860 U/kg body weight, $n=9$) were estimated to be 0.432 ± 0.274 h^{-1} and 0.099 ± 0.031 h^{-1}. This value for β *in vivo* is significantly larger than the inactivation rate constant of CPK *in vitro*. Hence, there must be some other mechanism involved *in vivo* in addition to simple inactivation in the circulation by the body temperature. The distribution volume of injected CPK in the steady state was estimated to be approximately $4.3 \pm 1.1\%$ of body weight. The result suggests that injected CPK is distributed into a major compartment (plasma) and a minor one (assumed to be interstitium).

Keywords——rabbit muscle creatine phosphokinase; intravenous administration; pharmacokinetic parameter; endogeneous fluctuation

One of the most promising approaches to the quantification of muscular lesions due to intramuscular injection of drug solutions is to determine the muscle creatine phosphokinase (CPK) activity which is released into the circulation from the injured cells. Since CPK is known to be a muscle-localized enzyme, CPK activity elevation in the plasma may serve as an indicator of musculoirritancy or muscular lesions. However, the mechanism of the disappearance of CPK from the circulation is still obscure, and this approach remains unsatisfactory. In the preceding report,[2)] the inactivation profiles of CPK in the biological fluids were demonstrated. *In vitro* study showed that CPK was inactivated in a first order kinetic fashion in heparinized whole blood at pH 7.40, 39 °C, and the inactivation rate constant was estimated to be 0.054 h^{-1} whereas it was 0.058 h^{-1} in 50 mM Tris–acetate buffer solution under the same conditions.[3)] This implies that CPK *in vitro* is inactivated primarily by heat rather than by the proteolytic enzymes in the blood. The apparent half-life of CPK in rabbit plasma was reported by Qureshi and Wilkinson[4)] after administration of a low dose (125 U/5 mg/head rabbit, $n=3$), but they used a rather insensitive activity determination method. The actual rate of elimination of CPK in the rabbit circulation in relation to the dose administered, and other important pharmacokinetic parameters are still uncertain.

This report presents the results of an *in vivo* study on the elimination of CPK after the intravenous administration of low, medium and high doses. The factors which affect the estimation of the pharmacokinetic parameters are also discussed.

Materials and Methods

Materials——Rabbit muscle creatine phosphokinase (CPK: 67.6±1.5 U/mg protein), rabbit serum

albumin (RSA) and sodium heparin were obtained from Sigma Co., U.S.A. Sodium chloride was supplied by E. Merck Co., West Germany, and diazepam solution (5 mg/ml, Cercine injection, Takeda Co., Japan) was used.

Rabbits———Male white rabbits weighing 2.50—3.39 kg were used. Since uric acid level affects the stability of CPK during incubation,[5] rabbits with normal uric acid level (2.0—3.5 mg/dl)[6] were selected for the experiments (uric acid determination kit No. 292-UV, Sigma Co., U.S.A.).[7] Rabbits were handled carefully and were accustomed to the experimental conditions for at least three days. They were fasted for twelve hours before experiments, but allowed drinking water. Rabbits were lightly fixed at the neck position, with attempts to minimize their anxiety. Experiments were carried out in a room with controlled temperature (23±2°C) and humidity (R.H. 70—80%), with moderate illumination and without loud noises. Rabbits were allowed to recover for more than one week after a blank test (an examination of the fluctuation of endogenous CPK during 12—32 h fixation with successive blood sample collections) and then the experiments involving intravenous administration of CPK were carried out. There were only nine rabbits among a group of twenty with a fluctuation of endogenous CPK of less than 500 U/l during a 12 h blank test. Two of the nine rabbits were used for the low dose experiment and three for the medium dose expriment. After a one-month washout time, the five rabbits were used for the high dose experiment together with the four untreated rabbits (n=9). Three rabbits which showed elevation of plasma CPK activity to above 1000 U/l in the blank test were given 100—290 U/kg body weight of CPK intravenously in a separate experiment.

Diazepam Treatment———Oral administration of diazepam solution (5 mg/ml) at 0.1 ml per two hours during successive blood sample collections was carried out with two untreated rabbits which showed the elevation of plasma CPK activity above 1000 U/l in the blank test to examine the effect of diazepam treatment on the fluctuation of endogenous CPK.

Intravenous Administration of CPK———CPK was dissolved in 2% RSA solution and filtered through a 0.22 micrometer Millipore filter (Millipore Corp., U.S.A.). The activity of the filtrate was determined. A suitable dose in a volume of 0.8 ml was injected into the right marginal ear vein of rabbits within 20 s. The injected doses were 100 to 860 U/kg body weight, as given in the legends to figures and tables.

Blood Samples———Venous blood samples were serially collected in heparinized tubes (containing dried sodium heparin to form 0.75% sodium heparin in blood) from the left marginal ear vein. The collected blood was centrifuged at 4°C, 3500 rpm for 3 min within 10 min after collection. The plasma was pipetted into small glass tubes with tight-fitting polyethylene caps and stored at 4°C. The activity determinations were carried out within 24 h after blood collection.

Activity Determinations———If necessary, the plasma was diluted with 5% RSA solution and isotonic saline solution. The activity of CPK was determined at 25°C with a Merckotest kit (E. Merck Co., West Germany) which is based on the optimized Oliver-Rosalki method[8] using a spectrophotometer (Hitachi model 220, Japan). Triplicate determinations were performed on each sample and the coefficient of variation was less than 6% in this study.

Results

Fluctuation of Endogenous CPK

Figure 1 shows the fluctuation of endogenous CPK in twenty healthy untreated rabbits in the blank test. It is clear that plasma CPK was elevated during successive blood sample collections. Some rabbits showed elevation of CPK activity to above 1000 U/l during the experiment, whereas the others showed less than 500 U/l. Figure 2 depicts the effect of diazepam treatment on two rabbits which showed elevation of plasma CPK to above 1000 U/l in the blank test. Diazepam treatment induced sleeping for 20 to 25 min and facilitated blood sample collections after each administration. Unfortunately, it failed to decrease the amplitude of CPK elevation.

Eliminaion Profiles of CPK in the Circulation

Figure 3 depicts the corrected elimination profiles of plasma CPK after intravenous administration of high, medium and low doses of CPK. The fluctuation of endogenous CPK during the experiment was subtracted from the observed plasma activity for each rabbit. Figure 4 shows the fluctuation of endogenous CPK in nine rabbits during the experimental periods. The profiles in Fig. 3 show a biphasic pattern, in which the rapid initial phase (α) is followed by a slower phase (β), during which the enzyme appears to undergo inactivation. The profiles can be fitted to a two-compartment open model as illustrated in Fig. 3. Each curve can be expressed as a biexponential equation by the least-squares method

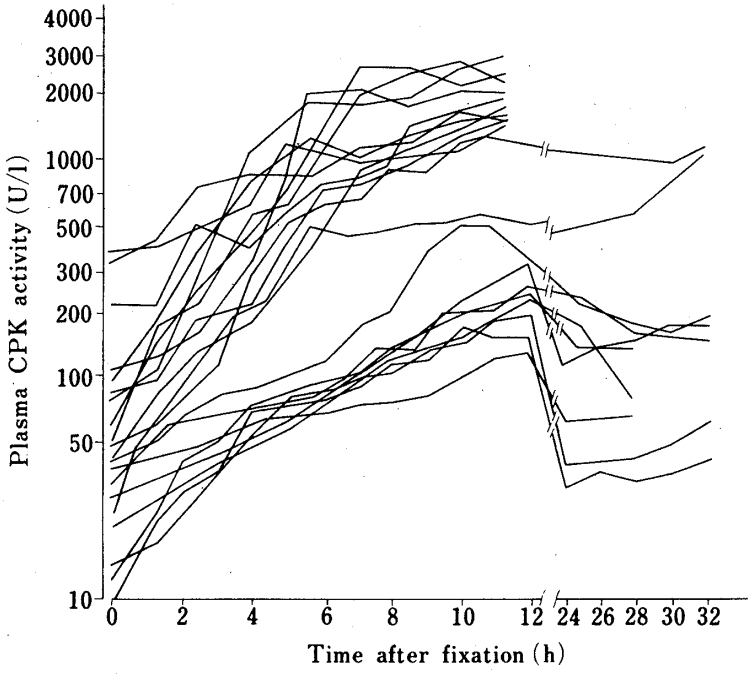

Fig. 1. Fluctuation of Endogenous CPK in Twenty Healthy Untreated Rabbits (Blank Test)

Each letter represents an individual rabbit. Triplicate determinations were made for each point, and the coefficient of variation was less than 5%.

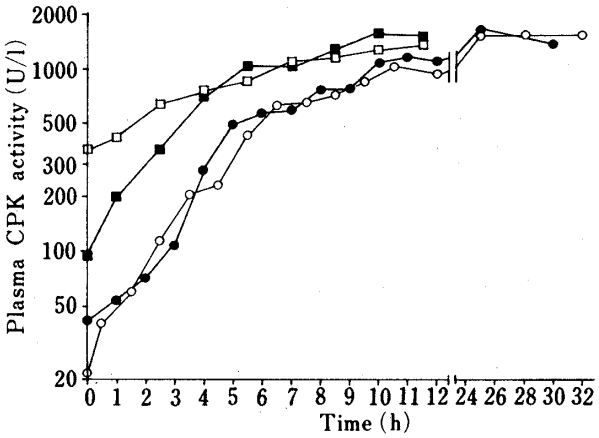

Fig. 2. Effect of Diazepam Treatment on Two Rabbits having High Endogenous CPK Fluctuation Character

(○, □), without diazepam; (●, ■), with diazepam. Triplicate determinations were made for each point, and the coefficient of variation was less than 5%.

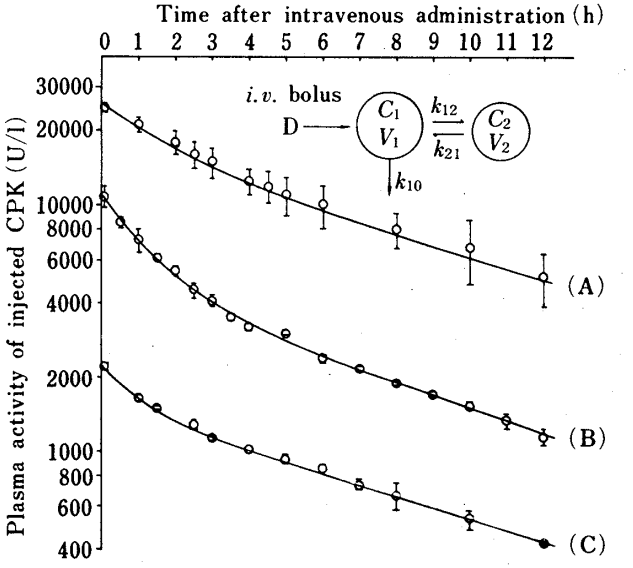

Fig. 3. The Biphasic Elimination of Injected CPK in Rabbits having Low Endogenous CPK Fluctuation Character after Intravenous Administrarion

(A), 860 U/kg dose. $n=9$, mean±S.D.; (B), 380 U/kg dose, $n=3$, mean ±S.D.; (C), 270 U/kg dose, $n=2$, mean±range. Solid lines represent the regression lines obtained by the least-squares method.

and the estimated pharmacokinetic parameters are presented in Table I.

Figure 5 depicts the corrected elimination profiles of CPK administered at various low doses in three rabbits which showed elevation of plasma CPK to above 1000 U/l in the blank test. The result is different from the profile of the low dose experiment demonstrated in Fig. 3 for selected rabbits in which the elevation of plasma CPK was less than 500 U/l in the blank test.

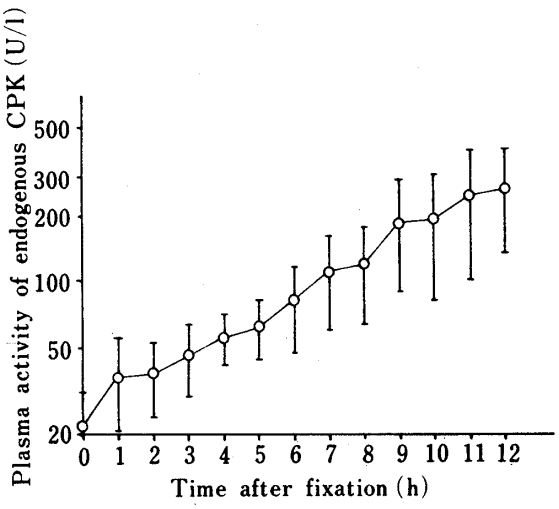

Fig. 4. Plasma Activity of Endogenous CPK in the Blank Test

n=9, mean±S.D.

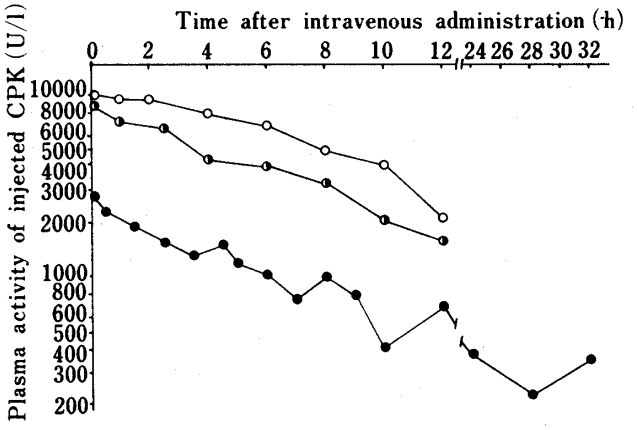

Fig. 5. Elimination Profiles of Injected CPK in Rabbits having High Endogenous CPK Fluctuation Character after Intravenous Administration

(○), 290 U/kg dose; (◑), 270 U/kg dose; (●), 100 U/kg dose. Triplicate determination were made for each point, and the coefficient of variation was less than 6%.

TABLE I. Pharmacokinetic Parameters of Rabbit Muscle Creatine Phosphokinase Following Intravenous Administration (Value for Parameter±Standard Error[a])

Parameters	Dose 860±21.7 U/kg[b] B. W. 2.94±0.30 kg (n=9)	384±11.6 U/kg[b] 3.11±0.26 kg (n=3)	270±7.5 U/kg[c] 2.71±0.04 kg (n=2)
A, U/l	9610±5570	6100±342	689±139
B, U/l	16100±6080	4920±308	1610±95.3
α, h^{-1}	0.432±0.274	0.756±0.079	1.08±0.41
β, h^{-1}	0.099±0.031	0.118±0.007	0.111±0.007
k_{10}, h^{-1}	0.140±0.016	0.221±0.008	0.152±0.009
k_{12}, h^{-1}	0.084±0.046	0.250±0.034	0.250±0.118
k_{21}, h^{-1}	0.308±0.261	0.403±0.053	0.791±0.312
Vd_{ss}, ml[d]	125.5±32.3	175.4±15.8	418±79.1
Vd_{ss}/B. W., %[e]	4.3±1.1	5.6±0.5	15.4±2.9
$t_{1/2\beta}$, h	7.0±2.2	5.9±0.4	6.2±0.4

a) According to the method in the textbook "Statistical Adjustment of Data" ed. by W. E. Deming, John Wiley and Sons, Inc., New York, 1946. *b*) Mean±S.D. *c*) Mean±range. *d*) $Vd_{ss}=V_1+V_2$. *e*) B. W.: body weight.

Discussion

Fluctuation of Endogenous CPK in Rabbits

It is evident from Fig. 1 that the initial plasma CPK levels of rabbits used in the experiments (10.5—370 U/l, n=20) were lower than the normal values reported by Steiness *et al.*[9] (44—390 U/l, n=32) and Gray[10] (241±96 U/l, n=109). However, the plasma CPK of some rabbits can rise above 1000 U/l after a certain period of fixation and successive blood sample collections. Presumably, the fluctuation of endogenous CPK level is due to emotional distress, unease caused by fixing, locomotive escaping reactions and successive punctures. This observation is consistent with our preceding findings in preliminary studies.[2,5] Ogiso *et al.*[11] reported the unexplained phenomenon that plasma CPK levels higher than 2500 U/l were found in rabbits after intramuscular injection of 1 ml of isotonic saline solution. On the other hand, increases of about 300 U/l was reported by Gray[10] after the same treatment. It seems likely that some rabbits which show high CPK elevation in the blank test may cause the anomaly and may be responsible for the deformation of the elimination profile of injected CPK, as can be seen in Fig. 5.

The sedative and tranquilizing actions of diazepam failed to depress the elevation of endgenous CPK during the experiments. Also, it is difficult to correlate body weight or age of rabbits to their fluctuation character. Meltzer *et al.*[2] reported that serum CPK activity in humans is, to some degree, under genetic control. The between-subject variability of the fluctuation character of CPK in rabbits might thus be complicated in nature.

Rate of Elimination of CPK in Rabbits

The pharmacokinetic parameter β for injected CPK in rabbits is estimated to be approximately 0.099—0.118 h^{-1} (Table I). Yasmineh *et al.*[13] observed a slightly smaller apparent rate constant with low dose administration in a study of cardiac CPK in the baboon. Thus, they found the apparent elimination rate constant decreased from 0.00175 min^{-1} (eq to 0.105 h^{-1}, 691 U/head baboon, $n=7$) to 0.00154 min^{-1} (96 and 116 U/head baboon, $n=2$) depending on the dose. However, our study suggests that the rate constant β for injected CPK is unlikely to be affected by the dose, but is influenced significantly by the intersubject variability; the coefficient of variation was as high as 31.3% (Table I, high dose experiment).

The elimination of some enzymes has been established to be independent of the absolute concentration of enzymes.[14] Although such independency has not yet been demonstrated with a sensitive and variable enzyme such as CPK, in view of the fluctuation of endogenus CPK during the experiments, it is probable that the deformation of the elimination profile after low dose administration in rabbits which showed elevation of plasma CPK to above 1000 U/l in the blank test is not an indication of dose-dependency (Fig. 5). The fluctuation of endogenous CPK may be one of the variables contributing to the wide variation of elimination rate constants of human CPK given in clinical reports[15] and the apparent inconsistency between the elevation of plasma CPK and muscular lesions.[10,11,16] The result for nine selected rabbits followig high dose administration of CPK may serve as a guide to the approximate value of the hybrid rate constant β *in vivo* for CPK (0.099 ± 0.031 h^{-1}). This value is significantly larger than the inactivation rate constant of CPK *in vitro*; therefore, there must be some other mechanism involved in *in vivo* elimination in addition to simple inactivation in the circulation by the body temperature.

The β value obtained in this study is approximately 42% less than that calculated from the reported values for half-life in rabbits by Qureshi and Wilkinson[4] (0.170 ± 0.007 h^{-1}, $n=3$), but is very close to the rate constant reported for cardiac CPK in the baboon (0.105 h^{-1}, $n=7$).[13] The difference may be due to the more sensitive method used in this study for the determination of CPK activity as compared with the method used by Qureshi and Wilkinson, and to the effect of intersubject variability in relation to the number of rabbits used.

Distribution Volume of CPK

The blood volume of rabbits has been estimated to be 5.46% of the body weight by the Welcker method, and the hematocrit value is about 41.5%.[6] Hence, the approximate value of plasma volume in rabbits can be estimated to be around 3.2% of body weight. The distribution volume of CPK in the steady state (Vd_{ss}) is estimated to be 4.3 ± 1.1% of body weight at high injected dose (Table I). This value is slightly higher than the plasma volume and is very close to that of the total CPK in dogs reported by Roberts *et al.*[17] (4.5—5.5% of body weight). Also, a distribution volume of cardiac CPK slightly higher than the plasma volume in the baboon was reported by Yasmineh *et al.*[13] The authors have demonstrated that CPK is unlikely to be bound to plasma albumin[5] or blood cells.[2] Therefore, the results suggest that the injected CPK at high dose is distributed into a major compartment (plasma) and a minor one (assumed to be interstitium).

However, Vd_{ss} seems to increase with decrease of the injected dose (Table I). It has been demonstrated that the transfer of injected GOT and GPT from serum to lymph is quite fast in

dogs, and the rate of decrease in serum approximately equalled the rate of increase in lymph.[18] Also, the transport of macromolecules from blood to lymph has been found to be mainly *via* the hepatic lymph system.[19,20] It is probable that the reticuloendothelial system is involved in the elimination of CPK *in vivo*. A study on the implication of thoracic duct lymph in the inactivation of CPK *in vitro* and *in situ* is in progress in our laboratories.

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References and Notes

1) This paper constitutes Part IV of the series entitled "Biopharmaceutical Studies on Muscle Creatine Phosphokinase."
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