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The Absolute Bioavailability of Rabbit Muscle Creatine Phosphokinase after Intramuscular Administration[1)]

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A two-period crossover study was carried out to determine the rate and the extent of bioavailability of rabbit muscle creatine phosphokinase (CPK) after intramuscular administration to six male rabbits. The plasma profile of CPK after intramuscular administration (500 U/kg body weight in 0.8 ml of 2% rabbit serum albumin solution into the vastus lateralis muscle) showed a plateau around 4—12 h. Intravenous administration of CPK was performed at doses of 300 and 500 U/kg body weight. The mean clearance/body weight and Vd_{ss}/body weight were 0.00604 ± 0.00094 l/h/kg (mean $\pm$ S.D., $n=6$) and $5.32 \pm 1.47\%$, respectively. The mean residence time (MRT_{im}) and mean absorption time (MAT_{im}) of CPK after intramuscular administration were 20.7 ± 4.2 and 11.9 ± 5.1 h, respectively. Since MAT_{im} was long relative to MRT_{im}, the rate of bioavailability was slow and the residence time distribution appeared to be skewed. The value of MAT_{im}/MRT_{im}, reflecting the contribution of moment of input function to the plasma activity of CPK, was $55.4 \pm 11.6\%$. The variance of residence time (VRT_{im}) and variance of absorption time (VAT_{im}) were 608 ± 107 and 505 ± 122 h^2. The results imply that the aborption and disposition of CPK after intramuscular administration were rather complicated. The mean fraction of CPK absorbed was 0.311 ± 0.149. Presumably, the incomplete access of CPK to the systemic circulation was due to extravascular inactivation.

Keywords——rabbit muscle creatine phosphokinase; clearance/body weight; intramuscular administration; mean residence time; mean absorption time; variance of residence time; fraction of absorption

Most previous attempts to assess musculoirritancy or muscular lesions due to intramuscular injections using plasma activity of muscle creatine phosphokinase as the indicator were based on the determination of the activity elevation of muscle creatine phosphokinase in the plasma or on estimation of the area under the plasma activity curve. The quantification of muscular lesions by these methods appears to be unsatisfactory because many factors which influence the plasma creatine phosphokinase are still not well understood. In a series of studies, a systematic investigation has been carried out to elucidate the nature of the inactivation of rabbit muscle creatine phosphokinase (CPK),[2)] the disposition of CPK *in vivo* and the important pharmacokinetic parameters.[3)] In a previous report,[4)] the implication of thoracic duct lymph in the distribution and elimination of CPK was presented. The results indicated that CPK is transported to the circulation from a muscle site *via* the lymphatic system, and CPK in the circulation is readily distributed to the lymphatic system. It was also suggested that CPK *in vivo* may be partly inactivated in the lymph. Since it has not been demonstrated what proportion of the CPK liberated at an injured muscle site can gain ingress into the circulation, to draw inferences regarding muscular lesions from the observed plasma activity of CPK remains impracticable.

This report presents the results of a two-period crossover study on the determination of

the absolute bioavailability of CPK after intramuscular administration as a basis for the quantification of muscular lesions by the serial analysis of plasma CPK.

Materials and Methods

Materials——Rabbit muscle creatine phosphokinase (CPK: 90.5±3.7 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.

Rabbits——White male rabbits weighing 3.20—3.87 kg were used. Six rabbits which showed normal uric acid level and elevation of plasma CPK of less than 500 U/l during a blank test[3] involving 12 h fixation and successive blood sample collections were selected for the experiments. Rabbits were handled carefully and were accustomed to the experimental conditions for at least 5 d. Rabbits were lightly fixed at the neck position in an attempt to minimize their anxiety. Experiments were carried out in a room with controlled temperature (23±2 °C) and humidity (RH 70—80%), with moderate illumination and without loud noises. During the intramuscular administration experiment, the rabbits were kept in cages and fed as usual after each blood sample collection; however they were fixed and also fasted for 12 h during the intravenous administration experiment.

Experimental Schedule and Fluctuation of Endogenous CPK——The fluctuation of endogenous CPK during 12 h fixation and successive blood sample collctions in untreated rabbits was determined and used as the blank value for correction of the results in the intravenous administration experiment. Rabbits were allowed to recover for 10 d and then the experiment involving intravenous administration of CPK was carried out. The plasma activity of CPK after the intramuscular administration of 2% RSA solution (0.8 ml, into the vastus lateralis muscle) was used as the blank value for correction of the results after the intramuscular administration of CPK to exclude the contribution of endogenous CPK due to puncture, injection volume and experimental handling. Since the plasma level of endogenous CPK returned to the normal level within a one week rest period, the intramuscular administration of CPK was carried out 10 d after the administration of 2% RSA solution to the opposite side vastus lateralis muscle. The intravenous and intramuscular administrations of CPK to each rabbit were carried out with a one-month interval in a crossover experiment.

Intravenous or Intramuscular Administration of CPK——CPK was dissolved in 2% RSA solution (similar to the protein concentration of the interstitial fluid) and filtered through 0.22 micrometer Millipore filter (Millipore Corp., U.S.A.). The activity of CPK in the filtrate was determined. The injected dose of 300 or 500 U/kg body weight in 0.8 ml (tolerated volume for the vastus lateralis muscle) was administered into the marginal ear vein of rabbits within 20 s or into the vastus lateralis muscle at exactly 1 cm depth with a 23 G hypodermic needle (Top Surgical Manufacturing Co., Japan).

Variability of Pharmacokinetic Parameters of CPK——Replicate intravenous administrations were performed with 300 and 500 U/kg body weight at a two-month interval to check the variability of pharmacokinetic parameters of CPK.

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

Activity Determination——If necessary, the plasma samples were diluted with 5% RSA solution and isotonic saline solution. The activity of CPK was determined at 25 °C with the Merckotest kit (E. Merck Co., West Germany) which is based on the optimized Oliver–Rosalki method[5] using a spectrophotometer (Hitachi model 320, Japan). Duplicate determinations were performed on each sample and the range was less than 6% of the mean in this study.

Estimation of Area under the Plasma Level Curve (AUC) and Fraction of CPK Absorbed (F_{im})——The total AUC (AUC_∞) in the intravenous administration experiment was calculated from the plasma level equation obtained by fitting the corrected plasma profile to a two-compartment body model, whereas the total AUC in the intramuscular administration experiment was calculated by the conventional method. Thus, the AUC from time zero to the last plasma level point determined in the experiment was estimated by the log trapezoidal rule method.[6] The remaining area was calculated by dividing the last plasma level point (56 h after the administration) by the individual terminal rate constant (β_{im}). The fraction of CPK absorbed is represented by the ratio of the total AUC on intramuscular administration to the total AUC on intravenous administration in the same rabbit in the two-period crossover experiment with correction of the dose size and the terminal rate constant (β_{iv} and β_{im}), based on the presumption that the disposition of CPK follows linear pharmacokinetics under the experimental conditions.

Estimation of the Mean Residence Time (MRT), Mean Absorption Time (MAT) and Variance of Residence Time (VRT)——Statistical moments analysis in terms of MRT and VRT[7] was performed on the plasma activity curve. These values, which are respectively the first normal and second central moments of the plasma activity profile, are defined by equations (1) and (2).

$$MRT=\int_0^\infty t\cdot C\cdot dt/AUC_\infty \quad (1)$$

$$VRT=\int_0^\infty (t-MRT)^2\cdot C\cdot dt/AUC_\infty \quad (2)$$

The mean absorption time (MAT_{im}) was calculated from the difference of the MRT between intramuscular and intravenous administrations.[8] The log trapezoidal rule method[6] was used in estimating the area under the first moment curve ($AUMC_\infty=\int_0^\infty t\cdot C\cdot dt$). The VRT was calculated by means of linear trapezoidal integration and extrapolation.

Results

Fluctuation of Endogenous CPK

Figure 1 depicts the fluctuation of endogenous CPK in the blank test and after the intramuscular injection of 2% RSA solution. The data were used for correction of the observed plasma profiles of CPK after intravenous and intramuscular adminitrations.

Disposition of CPK after Intravenous Administration

Figure 2 depicts the corrected plasma profiles of CPK after intravenous and intramuscular administrations. Since it is necessary to use individual pharmacokinetic parameters to elucidate the absolute bioavailability and the variability of pharmacokinetic parameters with subject and dose level, intravenous administrations of CPK at 300 and 500 U/kg body weight were given to each rabbit. The individual plasma profiles of CPK after intravenous administration showed a biphasic pattern and could be fitted to a two-compartment body model. The regression equation, $C_1=Ae^{-\alpha t}+Be^{-\beta t}$ was fittd by the least-squares method to the plasma profile of CPK and the estimated pharmacokinetic parameters

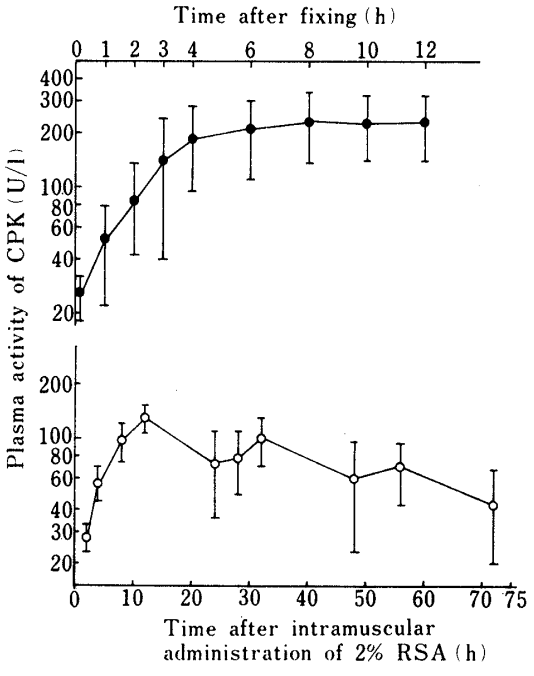

Fig. 1. Fluctuation of Endogenous CPK in the Blank Test (●) and after the Intramuscular Injection of 2% RSA Solution (○)

Mean ± S.D., $n=6$.

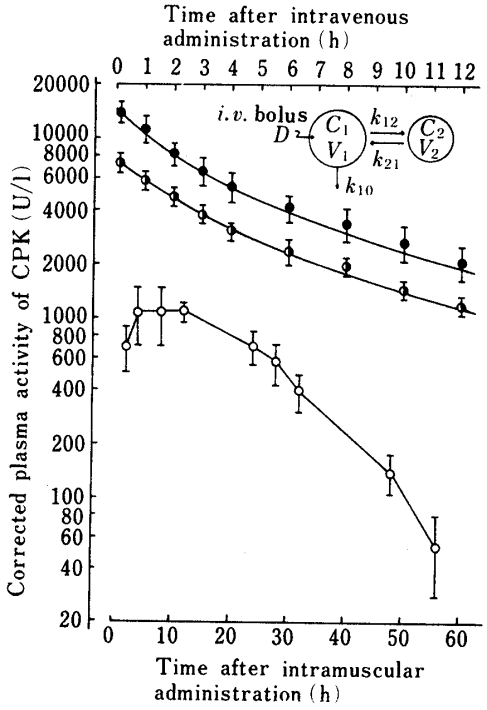

Fig. 2. Corrected Plasma Profiles of CPK after Intravenous (● and ◑) and Intramuscular (○) Administrations

(●), 500 U/kg body weight; (◑), 300 U/kg body weight; (○), 500 U/kg body weight.
Mean ± S.D., $n=6$.

TABLE I. Pharmacokinetic Parameters of CPK after Intravenous Administration (Values for Parameter ± S.E.,[a] $n=6$)

Parameters	Administration dose	
	300 U/kg body weight	500 U/kg body weight
A, U/l	3110 ± 864	7480 ± 1800
B, U/l	4350 ± 938	7150 ± 1900
α, h^{-1}	0.549 ± 0.217	0.620 ± 0.250
β, h^{-1}	0.109 ± 0.020	0.109 ± 0.025
k_{10}, h^{-1}	0.164 ± 0.013	0.188 ± 0.021
k_{12}, h^{-1}	0.128 ± 0.055	0.182 ± 0.085
k_{21}, h^{-1}	0.366 ± 0.188	0.359 ± 0.195
Vd_{ss}/BW, %[b]	5.54 ± 1.24	5.32 ± 1.47
Cl/BW, l/h/kg[c]	0.00644 ± 0.00029	0.00604 ± 0.00038

a) According to the method in the textbook "Statistical Adjustment of Data," ed. by W. E. Deming, John Wiley & Sons, Inc., New York, 1946.
b) $Vd_{ss}=V_1+V_2$; BW: body weight, kg.
c) Cl: clearance = dose/AUC_∞.

TABLE II. Analysis of Variance for Vd_{ss}/Body Weight after Intravenous Administration of CPK (300 and 500 U/kg Body Weight)

Source of variation	df	SS	MS	F
Subject	5	8.04	1.61	6.44[a]
Dose	1	0.14	0.14	0.56
Error	5	1.23	0.25	

df = degree of freedom (subject = $m-1=6-1$, dose = $n-1=2-1$, m = number of rabbits, n = number of dose levels); SS = sum of squares; MS = SS/df; F = F-value ($F_5^5(0.05)=5.05$; $F_5^1(0.05)=6.61$).
a) Significant ($p<0.05$).

are presented in Table I. The results are similar to those in a preceding report.[3]

Variability of Pharmacokinetic Parameters of CPK

The analysis of variance (ANOVA) for the pharmacokinetic parameters, α, β, k_{10}, k_{12}, k_{21}, Vd_{ss}/BW and Cl/BW obtained from the intravenous administrations of CPK (300 and 500 U/kg) revealed that subject and dose level factors were both insignificant for all the parameters except Vd_{ss}/BW. The intersubject variation of Vd_{ss}/BW was significant ($p<0.05$) as shown in Table II. Presumably, the result arises from individual differences of physical and physiological condition among the rabbits studied.

Plasma Profile of CPK after Intramuscular Administration

The corrected plasma CPK profile after intramuscular administration of CPK (500 U/kg body weight) showed a plateau around 4—12 h (Fig. 2). Curve fitting to the plasma profile with the one- or two-compartment body model involving a first-order absorption process by the least-squares method was unsatisfactory, since the values for standard errors were larger than the corresponding estimated parameters. The mean terminal rate constant (β_{im}) obtained from the last four or five points in the individual plasma profiles of CPK was $0.075 \pm 0.039\ h^{-1}$ (mean ± S.E., $n=6$). Student's t-test showed no difference between β_{im} and β_{iv}.

TABLE III. *MRT*, *MAT*, *VRT* and *VAT* after Intramuscular or Intravenous Administration of CPK

Parameters	Mean ± S.D. ($n=6$)
MRT_{im}, h	20.7 ± 4.2
MRT_{iv}, h	8.6 ± 1.4[a]
	8.8 ± 0.9[b]
MAT_{im}, h	12.0 ± 4.8[a]
	11.9 ± 5.1[b]
MAT_{im}/MRT_{im}, %	56.8 ± 11.3[a]
	55.4 ± 11.6[b]
VRT_{im}, h^2	608 ± 107
VRT_{iv}, h^2	92.0 ± 40.2[a]
	103 ± 41.2[b]
VAT_{im}, h^2	516 ± 106[a]
	505 ± 122[b]

a) Calculated with the data from the 300 U/kg dose experiment.
b) Calculated with the data from the 500 U/kg dose experiment.

$$MRT=\int_0^\infty t\cdot C\cdot dt/AUC_\infty;\ VRT=\int_0^\infty (t-MRT)^2\cdot C\cdot dt/AUC_\infty;$$

$$VAT=VRT_{im}-VRT_{iv};\ AUMC_\infty=\int_0^\infty t\cdot C\cdot dt.$$

The *AUC* and *AUMC* values obtained by extrapolation were 2.18 ± 1.18% and 7.13 ± 3.10%.

TABLE IV. Analysis of Variance for MRT_{iv} after Intravenous Administration of CPK (300 and 500 U/kg Body Weight)

Source of variation	df	SS	MS	*F*
Subject	5	10.98	2.20	2.97
Dose	1	0.12	0.12	0.16
Error	5	3.70	0.74	

df = degree of freedom (subject = $m-1=6-1$, dose = $n-1=2-1$, m = number of rabbits, n = number of dose levels); ss = sum of squares; MS = SS/df; F = F-value ($F_5^5(0.05)=5.05$; $F_5^1(0.05)=6.61$).

MRT, *MAT*, *VRT* and Variance of Absorption Time (*VAT*) of CPK

Table III lists the *MRT*, *MAT*, *VRT* and *VAT* after the administration of CPK. The MRT_{im} and MAT_{im} were 20.7 ± 4.2 (mean ± S.D., $n=6$) and 11.9 ± 5.1 h, respectively. The value of MAT_{im}/MRT_{im} reflecting the contribution of moment of input function to the plasma activity of CPK was 55.4 ± 11.6%. The results suggest a slow absorption process of injected CPK, presumably due to its macromolecular protein nature (molecular weight: 81000[9]), as was anticipated in the preceding study.[4] The MRT_{iv} was 8.6 ± 1.4 h (300 U/kg dose) or 8.8 ± 0.9 h (500 U/kg dose), whereas VRT_{iv} was 92.0 ± 40.2 h^2 (300 U/kg dose) or 103 ± 41.2 h^2 (500 U/kg dose). Table IV shows the ANOVA for MRT_{iv} after the intravenous administration of CPK (300 and 500 U/kg). The result shows that the effects of subject and dose factors were insignificant.

AUC and F_{im}

Table V shows the corrected total *AUC* (AUC_∞) after the intravenous or intramuscular administration of CPK (300 or 500 U/kg body weight) along with the calculated fraction of CPK absorbed from the muscle depot. The mean fraction of CPK absorbed after in-

TABLE V. Corrected Total AUC and Fraction of CPK Absorbed (F_{im}) after Administration of CPK

Parameters	Mean ± S.D. ($n=6$)
$AUC_{im\cdot\infty}$, (U/l)·h[a]	31300 ± 6950
$AUC_{iv\cdot\infty}$, (U/l)·h	40200 ± 18200[b]
	84800 ± 14800[c]
$AUC_{iv\cdot\infty}{}^{b)}/AUC_{iv\cdot\infty}{}^{c)}$	0.562 ± 0.061
Fraction of CPK absorbed (F_{im})	0.304 ± 0.118[d]
	0.311 ± 0.149[e]

a) Obtained by log trapezoidal rule method, $D_{im}=500$ U/kg.
b) $D_{iv}=300$ U/kg. c) $D_{iv}=500$ U/kg.
d) $F_{im}=AUC_{im\cdot\infty}D_{iv}\beta_{im}/[AUC_{iv\cdot\infty}D_{im}\beta_{iv}]$, using data (b).
e) $F_{im}=AUC_{im\cdot\infty}\beta_{im}/[AUC_{iv\cdot\infty}\beta_{iv}]$, using data (c).

tramuscular administration was 0.311 ± 0.149 (mean ± S.D., $n=6$). The mean ratio of total AUC after the intravenous administration of 300 U/kg to that for the 500 U/kg dose was 0.562 ± 0.061. The good agreement between the theoretical value ($AUC_{iv\cdot\infty\cdot 300\,U/kg}/AUC_{iv\cdot\infty\cdot 500\,U/kg}=0.60$) and the observed value, as well as the constancy of MRT_{iv} in the experiments with different doses, supports the presumption that the disposition of CPK follows linear pharmacokinetics under our conditions.

Discussion

Incomplete Transfer of CPK from Muscle Depot to the Circulation

Since intramuscularly administered CPK was transferred slowly into the circulation *via* the lymphatic system,[4] extravascular inactivation is a likely explanation for the poor bioavailability. The fraction of CPK absorbed showed a large intersubject variability. Difference in CPK concentration (in 0.8 ml, 2% RSA) during the intramuscular administration is one of the possible variables. However, the mean fraction of CPK absorbed ($F_{im}=0.311$) obtained in this study was larger than the value reported by Neumeier *et al.* for cardiac creatine phosphokinase from an infarct area to the circulation in dogs ($F=0.15$[10]). The difference may be due to the fact that muscle creatine phosphokinase is more stable than cardiac creatine phosphokinase.[11]

Features of the Plasma Profile of CPK after Intramuscular Administration

Statistical moments analysis of the plasma profile of CPK revealed that MAT_{im} was long relative to MRT_{im} ($MAT_{im} \gg MRT_{im}/5$).[8] The result suggests that the rate of bioavailability was slow and the residence time distribution was skewed. Therefore, it is impossible to establish a general definition of the mean time for residence or absorption. It should also be noted that the variance of residence time (VRT_{im}) and the variance of absorption time (VAT_{im}) were large. This might imply that the absorption and disposition of CPK after intramuscular administration are rather complicated. Further studies are in progress in our laboratories.

References and Notes

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