Compared kinetics of plasma creatine kinase activity in rabbits after intravenous injection of different preparations of skeletal muscle

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Summary — The purpose of this study was to compare the disposition parameters of creatine kinase (CK) in rabbits after intravenous bolus administration of 3 CK preparations: 1) purified CK; 2) CK obtained from a muscular extract after a 3 000 *g* centrifugation (*ie* with cell remains) (3 000 *g* CK); or 3) a 105 000 *g* centrifugation (ie the cytosolic soluble phase) (105 000 *g* CK). The plasma half-lives were not significantly different (≈ 9 h). In contrast, the clearance of 3 000 *g* CK (3.25 ± 0.33 $ml \cdot kg^{-1} \cdot h^{-1}$) was significantly lower than that of the 2 others (7.00 ± 0.49 $ml \cdot kg^{-1} \cdot h^{-1}$ and 4.63 ± 0.65 $ml \cdot kg^{-1} \cdot h^{-1}$ for 105 000 *g* and purified CK, respectively). These findings suggest that purified CK preparation is not the most appropriate form for determination of enzyme pharmacokinetic parameters following muscle damage.

creatine kinase / kinetics / muscle damage / rabbit

Résumé — Cinétiques comparées de l'activité plasmatique de la créatine kinase chez le lapin après injection IV de différentes préparations de muscle strié squelettique. *Le but de cette étude était de comparer les paramètres pharmacocinétiques de la créatine kinase (CK) chez le lapin après administration intraveineuse d'un bolus de 3 préparations différentes de CK: i) une solution de CK purifiée; ii) une solution de CK obtenue à partir d'un surnageant musculaire après centrifugation à 3 000* g *(c'est-à-dire renfermant des fragments d'organites) (CK 3 000* g*); ou iii) à 105 000* g *(c'est-à-dire la phase cytosoluble) (CK 105 000* g*). Les temps de demi-vie (environ 9 h) ne différaient pas de façon significative. La clairance de la CK 3 000* g *(3,25 ± 0,33* $ml \cdot kg^{-1} \cdot h^{-1}$*) était de façon significative plus faible que la clairance des 2 autres préparations (7,00 ± 0,49* $ml \cdot kg^{-1} \cdot h^{-1}$ *et 4,63 ± 0,65* $ml \cdot kg^{-1} \cdot h^{-1}$ *pour la CK 105 000* g *et la CK purifiée respectivement). Ces résultats montrent que la préparation de CK purifiée n'est pas nécessairement la forme la mieux adaptée pour déterminer les paramètres pharmacocinétiques de l'enzyme lors de lésion musculaire.*

créatine–kinase / pharmacocinétique / lésion musculaire / lapin

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INTRODUCTION

Measurement of plasma creatine kinase (CK) activity has proved useful in diagnosing or monitoring the results of drug treatments in a wide variety of neuromuscular diseases in man and animals. Plasma CK is a marker of muscle damage, but is not specific to a particular type of lesion; it increases in cases of membrane permeability alteration, *ie* both in degenerative processes (muscular dystrophy) and acute trauma (rhabdomyolysis). The latter includes the effects of intramuscular injection (Meltzer *et al,* 1970), and increased plasma CK activity has been extensively used as an index of local myotoxicity (Brazeau and Fung, 1989).

The magnitude of plasma CK activity is not directly related to the number of damaged cells. CK plasma activity depends on 4 determinants: the amount and rate of CK released from an injured muscle into the plasma, its distribution volume, and its elimination rate (Friedel *et al,* 1979).

One of the most promising approaches to the quantification of muscle damage is to estimate the amount of muscle CK activity which is actually released from the injured cell into the circulation. This requires prior determination of the disposition parameters of the enzyme and of its factors of variation.

The intrinsic rate at which an enzyme is eliminated from the blood can only be determined after intravenous (iv) administration of the enzyme. For ethical reasons, such an administration is impossible in man, but can be easily carried out in animals.

When performing a CK kinetic study, the immediate problem arising is the nature of the test-article preparation to be administered. CK can be administered as a purified formulation as in many animal investigations including rabbit (Hsu and Watanabe, 1983a), rat (Friedel *et al,* 1976), and dog (Kotoku *et al,* 1971). However, it must be considered that after an acute rhabdomyolysis (characterized by disruption of the cell membrane) or during the development of a chronic process (consisting only of an alteration in membrane permeability), other muscle cell remains may or may not be co-liberated with CK. As these cell remains may influence CK disposition, it is of importance to document CK disposition both in the in presence and absence.

The aim of the present study was to determine the pharmacokinetic parameters of skeletal muscle CK in the rabbit following iv administration of 3 types of skeletal muscle CK: a commercially available rabbit purified CK, CK obtained from a rabbit muscular extract after a 3 000 *g* centrifugation (3 000 *g* CK), *ie* CK and muscle remains, and CK obtained after a 105 000 *g* centrifugation (105 000 *g* CK), *ie* essentially the cytosoluble phase of the muscle cell.

MATERIALS AND METHODS

Animals

New Zealand White female rabbits (n = 5), weighing 3–4 kg were obtained from the Elevage du Lapin (INRA, Auzeville, France). They were housed in individual cages and received a commercial dry chow (UFAC, Rental Languedoc, Colomiers, France) and tap water *ad libitum.* The animals were made familiar with laboratory procedures before any experiment, as previously described (Lefebvre *et al,* 1992).

Test articles

Lyophilized rabbit muscle CK (163 U/mg proteins) was obtained from Sigma Chimie (Saint Quentin Fallavier, France). It was dissolved just

before iv administration with 0.9% NaCl at room temperature.

Longissimus dorsi muscles from a single rabbit, anaesthetized by sodium penthiobarbital (iv, 20 mg/kg) and bled immediately, were rapidly excised and rinsed in physiological saline. Muscle samples (5 g) were freed from connective tissue, weighed and stored at –80°C. After <1 month, samples were thawed at room temperature. After mincing, the samples were placed in saline (15 ml) and homogenized without detergent. Each sample was then centrifuged at 3 000 *g* or 105 000 *g* for 30 min at 4°C. Supernatants were stored at –30°C until administration, which was performed within a maximum delay of 3 d after preparation.

Ten replicates of CK activity measurement were performed on the supernatant just before administration to determine the exact dose given.

Test article administration and blood sampling

All CK preparations were administered by iv route at a dose of 500 U/kg. Each administration was separated by a washout period of at least 15 d. The purified, 3 000 *g*, and 105 000 *g* preparations were successively administered to all animals without cross-over.

One-ml blood samples were taken from the right marginal vein of the ear into heparinized tubes with a syringe and catheter (Venofix, 5/10, Bruneau, Boulogne-Billancourt, France). Blood samples were drawn regularly before (–90, –45 and 0 min) and after (2, 4, 8, 10, 15, 20, 30, 45, 60 min, 2, 4, 6, 8, 10, 24, 36 and 48 h) CK administration. After 10 min centrifugation at 3 000 *g*, the plasma was separated and 2 aliquots stored at –20°C until analysis within 3 d.

CK analysis

Frozen plasma samples were thawed at room temperature and diluted (1/16) in 0.9% NaCl before analysis. CK activity was measured at 30°C with a commercially available reagent (Enzyline CK, 63151 BioMerieux, Marcy l'Etoile, France) on a Roche Cobas Bio analyzer. Quality control was based on pooled animal sera: the level of quantification (as mean + 10 SD of a blank with distilled water) was 10 U/l; the intraday coefficient of variation was < 5.8%.

Pharmacokinetic analysis of plasma CK activity

Pharmacokinetic analysis of plasma CK activity was performed using a program for non-linear regression analysis adapted from Multi (Yamaoka *et al*, 1981).

Plasma CK concentrations obtained after CK iv administration were fitted to a general polyexponential equation (eq [1]) :

$$C(t) = \sum_{i=1}^{N} Y_i \exp(-\lambda_i t) + Co \qquad [1]$$

In eq [1], $C(t)$ is the plasma CK activity at time t, Y_i (U/l) is the coefficient of the ith exponential term. The baseline value (Co, U/l) was incorporated into eq [1] to take into account the basal plasma CK activity. Initial estimates of plasma CK activity were obtained using the method of residuals (Gibaldi and Perrier, 1982). These initial estimates were refined by non-linear regression analysis using an extended least-squares (ELS) method (Peck *et al*, 1984). Initial estimates of Co value were obtained from the arithmetic mean of the plasma CK concentrations of the control samples. In order to avoid an unphysiological estimate of Co, Bayesian constraints were used to fit Co; the mean and variance of Co were known, *a priori*, from the control samples preceding test-article administration; this information was used to influence the parameter estimate by adding a penalty to the objective function. Based on the Akaike's information criterion (Yamaoka *et al*, 1978), biexponential equations were selected for all rabbits (eq [2]):

$$C(t) = Y_1 \exp(-\lambda_1 t) + Y_2 \exp(-\lambda_2 t) + Co \qquad [2]$$

where $C(t)$ is the plasma CK activity (U/l) at time t; Y_1 and Y_2 are preexponential coefficients, λ_1 and λ_2 (min^{-1}) are exponents of the equation, and Co (U/l) is the baseline CK value. Data were therefore analyzed by a 2-compartment open model with CK elimination from the central compartment.

The parameters (Y_1, Y_2, λ_1, λ_2) were used to solve for the first-order rate constants of transfer from central to peripheral compartments (k_{12}, k_{21}) according to the classical equations. The volume of the central compartment (V_c), the CK steady-state volume of distribution (V_{ss}), the total body CK clearance *(Cl)*, the area under the curve (*AUC*), the mean residence time (*MRT*) were determined as previously described, according to a compartmental approach (Gibaldi and Perrier, 1982).

Statistical analysis

All calculations were performed using a STATGRAPHICS package (Mercia, Birmingham, UK). Statistical analysis of results was based on a multifactorial analysis of variance (ANOVA). Controlled factors were the rabbit and the test article formulation. Planned comparison among formulations was carried out using the least significant difference (LSD) when the *F* ratio was significant (protected LSD). Values are reported as mean ± SD.

RESULTS

The visual aspect of the arithmetic plots of plasma CK activity *vs* time (fig 1) after iv CK administration indicates that the kinetics between the CK preparations are not similar. The individual pharmacokinetic parameters describing CK disposition are given in tables I–III for the purified, 3 000 *g* and 105 000 *g* CK respectively.

The estimated basal CK values varied from 85 to 369 U/l and presented fluctuations within animals. The plasma half-lives were not significantly different (ANOVA, $P > 0.05$). Similarly, no significant difference was observed for the mean residence times (ANOVA, $P > 0.05$).

For the 3 000 *g* CK, the mean V_c had a value similar to that of the plasma volume. It was significantly lower than for the 2 other forms of CK (ANOVA, $P < 0.01$). The mean steady-state volume of distribution (V_{ss}) of the 3 000 *g* CK was significantly lower than the V_{ss} of purified CK, itself significantly lower than the value obtained with the CK from a 105 000 *g* muscle supernatant (ANOVA, multiple comparison, $P < 0.01$). The clearances were significantly different between the 3 forms (ANOVA, $P < 0.01$), the clearance of 105 000 *g* CK being twice as high as that of 3 000 *g* CK. The clearance of purified CK had an intermediate value.

DISCUSSION

The results of the present experiment clearly indicate that differences exist in rabbit for the disposition kinetics of different CK preparations. Such differences have previously been observed in rats in

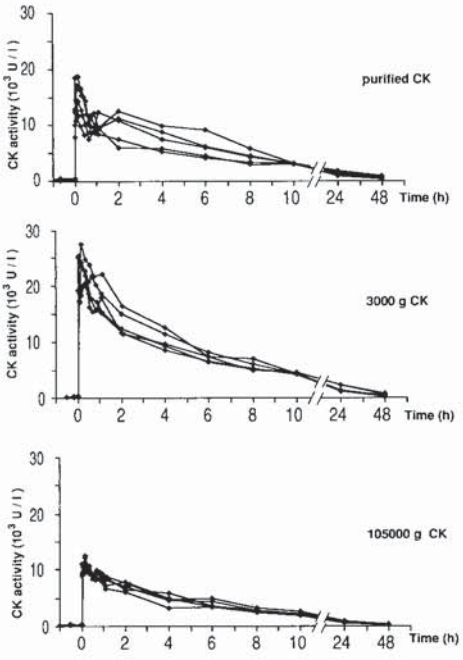

Fig 1. Arithmetic plot of individual plasma CK activities (U/l) *vs* time (h) after iv administration (at a dose of 500 U/kg) of CK to 5 rabbits.

Table I. Pharmacokinetic parameters describing the disposition kinetics of CK in plasma after a bolus administration of purified CK (500 U/kg) to 5 rabbits.

Parameters (units)	*Rabbits*					*Mean ± SD*
	1	*2*	*3*	*4*	*5*	
Y_1 (U/l)	13 365	6 745	6 343	5 682	8 575	8 142 ± 3 111
Y_2 (U/l)	7 021	4 925	4 327	6 446	5 784	5 701 ± 1 095
Baseline (U/l)	245	96	85	469	155	210 ± 158
λ_1 (min^{-1})	0.0269	0.00307	0.00677	0.00430	0.00273	0.00875 ± 0.0103
λ_2 (min^{-1})	0.00158	0.00136	0.00068	0.00137	0.00121	0.00124 ± 0.00034
t1/2 λ_2 (min)	439	225	1 019	505	575	553 ± 292
V_c (l/kg)	0.0245	0.0428	0.0468	0.0412	0.0348	0.0380 ± 0.00871
V_{ss} (l/kg)	0.0580	0.0498	0.0891	0.0515	0.0407	0.0578 ± 0.0186
Cl (ml/kg/h)	5.58	4.70	4.49	4.64	3.75	4.63 ± 0.652
MRT (min)	573	578	1 162	620	646	716 ± 251
AUC (U•min/lx 10^3)	5 378	6 377	6 683	6 459	8 001	6 580 ± 939

Y_1 and Y_2 : intercepts; baseline: control CK value; λ_1 and λ_2: exponents; $t_{1/2}$ λ_2: plasma half-life; *MRT*: mean residence time; V_c: volume of the central compartment; V_{ss}: steady-state volume of distribution; *Cl*: body clearance; *AUC*: area under the curve obtained by integrating equation describing the fate of CK.

which the mean half-lives of intravenously injected CK of heterologous, homologous and autologous origin were equal to 78.9. 34.8 and 33.0 min respectively (Friedel *et al,* 1976).

Rabbits are widely used in local tolerance studies (Steiness *et al,* 1978; Svendsen *et al,* 1979; Gray, 1981) and vitamin E-deficient rabbits are a model of muscular dystrophy (Read and Nehorayan, 1959), justifying extensive CK disposition studies in this species. In rabbits selected for low endogenous CK fluctuations, the distribution volumes and rate of elimination of a rabbit purified muscle CK preparation were previously determined from a 2-compartment open model. After an iv administration, the terminal half-lives were 6.2 ± 0.4, 5.9 ± 0.4, 7.0 ± 2.2 h for low (270 U/kg), medium (384 U/kg) and high (860 U/kg) CK doses, respectively, indicating the linearity of CK disposition (Hsu and Watanabe, 1983a). For the medium dose level, the V_{ss} was 56.4 ml/kg, and the plas-

Table II. Pharmacokinetic parameters describing the disposition kinetics of CK in plasma after a bolus administration of CK from a 3 000 *g* muscle homogenate supernatant (500 U/kg) to 5 rabbits.

Parameters (units)	*Rabbits*					*Mean ± SD*
	1	*2*	*3*	*4*	*5*	
Y_1 (U/l)	12 049	9 211	12 795	16 876	6 891	11 564 ± 3 786
Y_2 (U/l)	7 414	10 228	7 783	9 305	18 153	10 577 ± 4 386
Baseline (U/l)	286	138	208	326	207	233 ± 74
λ_1 (min^{-1})	0.00629	0.00586	0.00763	0.00502	0.0134	0.00764 ± 0.00336
λ_2 (min^{-1})	0.000977	0.00169	0.000921	0.00153	0.00232	0.00149 ± 0.000574
$t1/2\ \lambda_2$ (min)	709	411	753	454	298	525 ± 197
V_c (l/kg)	0.0257	0.0257	0.0243	0.0191	0.0200	0.0230 ± 0.00318
V_{ss} (l/kg)	0.0447	0.0331	0.0458	0.0261	0.0245	0.0348 ± 0.0100
Cl (ml/kg/h)	3.08	3.70	2.97	3.00	3.50	3.25 ± 0.330
MRT (min)	849	507	928	494	408	637 ± 234
AUC (U•min/l x 10^3)	9 730	8 108	10 109	9 994	8 570	9 302 ± 905

ma clearance was 6.87 ml/kg/h. These different parameters are in agreement with those of the present experiment.

The form of CK released following skeletal muscle damage was not assessed, but was unlikely to be only free and pure CK, so it was relevant to test the disposition of preparations mimicking either moderate or intense muscle damage. In these preparations, many other cell components can interact with the distribution and elimination of CK.

The plasma clearance, which reflects the metabolic or transport ability of the clearing organs, was 2-fold higher for the 105 000 *g* than for the 3 000 *g* supernatant, indicating that cell remains significantly impaired the process of CK elimination. It should be noted that the CK clearance for both preparations was low (< 1.5% of the cardiac output), indicating that both the kidneys (≈ 20% of the cardiac output) and the liver (≈ 25% of the cardiac output) have only a minor role in clearing CK from the plasma. It is well established that in the kidney, glomerular filtration can only be observed for proteins with an Mw of < 60 000 Da (Friedel *et al*, 1976), *ie* lower than CK (Mw: 81 000), which accounts for the absence of CK in urine (Hsu and Watanabe,

Table III. Pharmacokinetic parameters describing the disposition kinetics of CK in plasma after a bolus administration of CK from a 105 000 *g* muscle homogenate supernatant (500 U/kg) to 5 rabbits.

Parameters (units)	*Rabbits*					*Mean ± SD*
	1	*2*	*3*	*4*	*5*	
Y_1 (U/l)	6 320	6 473	5 812	5 559	9 368	6 706 ± 1 533
Y_2 (U/l)	3 617	3 819	4 286	5 053	1 113	3 578 ± 1 484
Baseline (U/l)	327	196	217	241	195	235 ± 55
λ_1 (min^{-1})	0.00352	0.00536	0.0103	0.00634	0.00360	0.00582 ± 0.00277
λ_2 (min^{-1})	0.00132	0.00150	0.00126	0.00153	0.000734	0.00127 ± 0.00032
$t1/2\ \lambda_2$ (min)	525	463	551	454	944	587 ± 204
V_c (l/kg)	0.0503	0.0486	0.0495	0.0471	0.0477	0.0486 ± 0.00130
V_{ss} (l/kg)	0.0629	0.0683	0.0876	0.0658	0.0823	0.0734 ± 0.0109
Cl (ml/kg/h)	6.26	7.40	7.41	6.75	7.19	7.00 ± 0.494
MRT (min)	571	514	695	551	678	602 ± 80
AUC (U•min/l x 10^3)	4 790	4 053	4 050	4 444	4 175	4 302 ± 316

1983b). The hepatic clearance appeared to be low, which confirms results in the dog, in which the estimated hepatic CK extraction ratio was 2.5% (Carlsen *et al*, 1982). In the liver, protein uptake results mainly from internalization (*ie* endocytosis) or passage through endothelial and epithelial barriers (*ie* transcytosis) (Shen *et al*, 1992). The lower clearance of 3 000 *g* CK could be explained by competition between CK and other constituents of the muscle homogenate.

The estimated approximate plasma volume of rabbits was 3.2% of total body weight (Hsu and Watanabe, 1983a). In our study, the estimated steady-state volume of distribution (V_{ss}) ranged from 3.5% (3 000 *g* CK) to 7.3% (105 000 *g* CK). Therefore, 3 000 *g* CK was essentially located in the central compartment (plasma), while 105 000 *g* CK was also distributed in a peripheral compartment. The extravascular distribution of CK could result from passive transvascular diffusion through relatively large pores in the endothelial layer and basement membrane, as has been reported for another plasma protein, albumin (Bent-Hansen, 1991). In the 3 000 *g* preparation, CK may be bound to cell fragments, as under intracellular conditions; such complexes are probably unable to diffuse as easily as the 105 000 *g* CK.

The half-life was proportional to the distribution volume, but inversely proportional to the clearance. This explains the absence of significant difference between the half-lives of the 3 000 *g* and 105 000 *g* CK, the lower CK clearance for the 3 000 *g* CK being compensated by a lower volume of distribution. This result indicates that measurement of CK plasma half-life after an acute injury or exercise is not a relevant approach to estimate CK elimination.

Such studies to investigate the influence of disposition factors have previously been carried out in man (Janssen *et al,* 1989; Horie *et al,* 1990). However, CK activities were measured after an acute event (myocardial infarction or exercise) and CK disappearance rates were calculated from the terminal exponential phase of the plasma activity profile. To be correct, such a determination should confirm, among other things, that enzyme leakage from the damaged cells had been totally arrested and that all available released enzyme had reached the plasma. This was not the case, which implies that these authors most likely evaluated a rate constant of CK invasion rather than of CK elimination (Friedel *et al,* 1979).

The last point which merits attention is the fact that purified CK curiously exhibited intermediate values, compared with the 2 other forms. Hsu and Watanabe (1981, 1982) investigated the inactivation profile of purified rabbit muscle CK in biological fluids and Tris–acetate buffer solutions. They suggested that CK molecules in such solutions were dispersed in the form of microaggregates. This probably also occurred with the purified CK dissolved in saline that we used. It is possible that the aggregates have different kinetic properties compared with the soluble CK from the 105 000 *g* supernatant (*ie* associated with other cytosoluble proteins), or from the 3 000 *g* supernatant, which contains the cytosoluble phase and cell organelles.

In conclusion, these results clearly demonstrate that the commercial purified preparation is not necessarily the most relevant form of CK for the determination of kinetic parameters of the enzyme to quantify muscle damage, even if it is more convenient and can be considered as a "standard" for inter-laboratory comparisons. Enzyme disposition should also be expected to differ according to the mechanism of muscle release (acute or progressive). Thus, depending on the origin of muscle disorder, a given CK plasma activity may not have the same meaning in terms of muscle destruction. Further studies are, however, needed to determine the real clinical significance of these considerations in different human or animal muscle disorders.

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