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A non-invasive method of quantification of muscle damage based on the kinetics of plasma creatine-kinase activity vs. time: a review

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Summary

A pharmacokinetic procedure was applied to plasma CK activity in the horse to determine its clearance (0.36±0.10 ml/kg/min), volume of distribution (0.059±0.022 l/kg), and bioavailability from muscle (between 74 and 96 %). These parameters and the PI-CK activity vs time curve were used to evaluate the equivalent amount of muscle damage in 6 horses caused by (i) a race at 200 m/min; (ii) a single intragluteal administration of phenylbutazone (8.8 mg/kg). No effect was observed in (i) up to 30 km but minor damage equivalent to 18.8±4.3 g of muscle per animal was found after 60 km. The muscle damage in (ii) was 0.118±0.048 g/kg BW, i.e. 59 g for a 500-kg horse.

Keywords: muscle damage, horse, creatine-kinase, pharmacokinetics, physical exercise

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Eine nichtinvasive Methode zur quantitativen Erfassung von Muskelschäden basierend auf der Verteilungskurve der Plasmaaktivität der Kreatinkinase pro Zeiteinheit: eine Übersicht

Zur Erfassung von Muskelläsionen beim Pferd wurde eine nichtinvasive Methode getestet, bei der die pharmakokinetischen Daten der CK-Aktivität im Plasma gemessen wurden. Die Kreatinkinase (CK) ist eines der Enzyme, das als „organspezifisch" für das Muskelgewebe angesehen wird. Daneben haben die LDH und die im Zytosol und in den Mitochondrien vorkommende Aspartataminotransferase (ASAT) eine Bedeutung bei der Beurteilung von Erkrankungen des Skelettmuskels.

Die Autoren analysieren die Grenzen der enzymatischen Routinediagnostik bei der Bewertung von Muskelschäden und versuchen, eine rationellere quantitative Meßmethode auf der Basis pharmakokinetischer Untersuchungen zu ermitteln.

Bei der Bestimmung der CK-, LDH- und ASAT-Werte wird nicht berücksichtigt, daß auch physiologische Prozesse im Skelettmuskel zu einer erhöhten Enzymaktivität führen. Außerdem gibt die gemessene Muskelenzymkonzentration nur an, daß Zellschäden in diesem Bereich stattfanden, es kann jedoch bei einmaliger Messung nicht beurteilt werden, ob die Enzymaktivität weiter ansteigt, sich auf dem Höhepunkt befindet oder schon wieder abfällt. Es gelingt ebenfalls nicht immer, aus den CK-, ASAT- und LDH-Konzentrationen zu schließen, ob ein akutes Trauma oder ein chronischer Schaden vorliegt.

Zur quantitativen Diagnostik von Muskelläsionen verwendeten die Autoren eine pharmakokinetische Bestimmung der Kreatinkinase-Clearance, welche Werte von 0,36±0,10 ml/kg/min erbrachte. Das Verteilungsvolumen im Körper betrug 0,059±0,022 l/kg und die Bioverfügbarkeit lag zwischen 74 und 96 %. Diese Parameter wurden in einem Diagramm gegen die Zeit aufgetragen, so daß eine Verteilungskurve der Kreatinkinase entstand, welche das Äquivalent zum Ausmaß der Muskelläsionen nach bestimmten Belastungen darstellte.

In der Studie wurden 6 Pferde getestet. Der erste Teil des Experiments beinhaltete ein Rennen bei 200 m/min. Der zweite Teil bestand in einer einmaligen intramuskulären Injektion von 8,8 mg/kg Phenylbutazon in die Glutäusmuskulatur. Bei jedem Test wurden die CK-Parameter gemessen.

Beim ersten Experiment liefen die Pferde 15, 30 und 60 km mit einer Geschwindigkeit von 200 m/min. Bis zur 30-km-Distanz blieben die CK-Werte konstant, d. h. es konnten keine Muskelläsionen nachgewiesen werden. Nach einer Distanz von 60 km wurden leicht erhöhte CK-Aktivitäten gefunden, entsprechend einer Läsion von 18,8±43 g Muskel pro Pferd. Aufgrund dieser Ergebnisse kann man davon ausgehen, daß solche Rennen ethisch akzeptabel sind und den Muskelapparat eines Sportpferdes nicht überfordern.

Nach der einmaligen Injektion von 8 mg/kg Phenylbutazon ins Muskelgewebe entstanden Läsionen von 0,118±0,048 g/kg KG bezogen auf ein 500 kg schweres Pferd. Es wurden stark erhöhte Plasmaaktivitäten der Kreatinkinase gefunden, wobei die höchsten Werte nach 6 Stunden erreicht wurden. Anhand der Clearance-Kurve der Kreatinkinaseaktivität war jedoch ersichtlich, daß das Enzym innerhalb der ersten 6 Stunden nach der Injektion immer weniger aus dem traumatisierten Muskel freigesetzt wurde und die Kurve längst wieder auf dem Ausgangswert angelangt war, während im Plasma noch hohe CK-Konzentrationen vorhanden waren.

Die Anwendung pharmakokinetischer Gleichungen stellt somit eine gute Möglichkeit dar, das Ausmaß eines Muskelschadens beim Pferd zu erfassen. Die verwendete Gleichung läßt sich in der Sportmedizin, bei akuten Traumen und pharmakologischen Experimenten einsetzen.

Schlüsselwörter: Muskelschaden, Pferd, Kreatinkinase, Pharmakokinetik, körperliche Anstrengung

Organ damage in man and animals is usually evaluated by invasive procedures such as microscopic examination of biopsies and non invasive measurement of the activities of so-called "organ specific" enzymes in the plasma. The use of enzyme analysis has gained wide acceptance and become a routine in clinical biochemistry (*Kramer,* 1989).

Three principal enzymes are used for skeletal muscle investigations in humans and animals: cytosolic creatine-kinase (CK, EC 2.7.3.2) and lactate dehydrogenase (LDH, EC 1.1.1.27), and cytosolic and mitochondrial aspartate aminotransferase (ASAT, EC 2.6.1.1). CK is fairly muscle specific, while LDH and ASAT are more ubiquitous.

Other protein markers of skeletal muscle (e.g. myoglobin, troponin, myosin) are used in humans, but are measured by immunologic techniques inapplicable to animal samples, because species-specific reagents are not available. Muscle damage in the horse is therefore evaluated by measuring the plasma activity of CK and/or LDH and/or ASAT.

This paper deals with: i) an analysis of the limitations of routine clinical enzymology for the evaluation of muscle damage; ii) a proposition for a more rational quantitative approach based on pharmacokinetic tools.

1. Limitations of routine clinical enzymology for the evaluation of skeletal muscle damage in the horse

These limitations stem from the fact that plasma enzyme measurements allow the detection of skeletal muscle damage and monitoring of its evolution but cannot indicate the actual amount of muscle damaged.

As CK, LDH and ASAT mainly originate from skeletal muscles in the horse, the following conclusions can be drawn:

- baseline activities (reference values) reflect the equilibrium between "physiological" skeletal muscle cell damage (i.e. cell turnover and minor reversible damage) and inactivation-elimination processes which are poorly understood and which lead to the clearance of these enzyme activities. These baselines are relatively low irrespective of the activity measurement technique.
- an increase of PI-CK, PI-LDH or PI-ASAT indicates that there has been damage to skeletal muscle cells allowing the escape of intracellular content into the interstitial fluids and plasma.

Plasma enzyme data are generally interpreted as the larger the increase in plasma activity the more severe the muscle damage. This is usually true but cannot be systematized. Clinical signs are usually much more severe when PI-CK exceeds 100.000 U/l, than when it is 1.000 U/l, but at least two important points may severely limit the interpretation of data (Fig. 1):

i) it is often impossible in clinical practice to know precisely when the damage started. It is thus impossible to know if the activity is increasing, peaking or decreasing. Repeated measurements can give some indication.

ii) sudden occurrence of severe damage (e.g. intramuscular injection of an irritant drug) may produce dramatic but temporary muscle damage with very high plasma enzyme activities returning to reference values within a few days. Muscle diseases, in contrast, can produce the continuous release of a limited amount of enzymes with moderately elevated plasma enzymes activities for long periods of time. The question then is: Which damage is more severe? Is it possible to evaluate this damage?

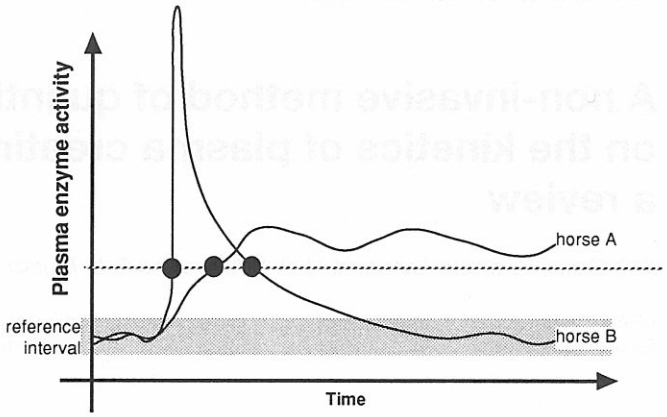

Fig. 1: Comparison of the possible (mis-)interpretations of a given plasma enzyme activity in a suspected muscle damage: i) acute damage beginning *vs.* end horse B: ii) acute *vs.* long-lasting damage in horses B and A respectively.

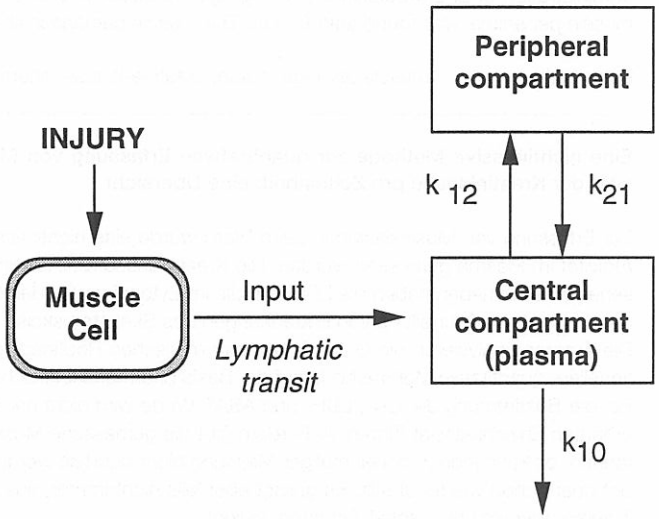

Fig. 2: Bicompartmental model of CK release and disposition after skeletal muscle injury.

2. A method for the quantitative evaluation of skeletal muscle damage

Although such a procedure has already been used to quantify the size of myocardial infarcts in humans, ethical considerations have made it impossible in humans to correctly determine the pharmacokinetic parameters of CK disposition.

Damage is best evaluated from the total amount of enzyme released from the damaged muscle. The evaluation procedure of this damage cannot be applied to clinical situations but is perfectly adapted to experimental or sports medicine when the onset of a damage or physical exercise is known. The following data are required to calculate the total amount of enzyme released into the plasma from a damaged organ:

i) the curve of plasma activity vs time, starting before muscle damage and lasting until the PI-CK returns to base values;

ii) two parameters of CK disposition, i.e. the clearance and the bioavailability.

The plasma concentration of a marker depends on three processes (Fig. 2):

(i) the entry rate of the marker into the plasma: after muscle injury, the damaged muscular site behaves like a pump which perfuses CK into the plasma at a variable rate *via* the lymph. The proportion of released marker which reaches the systemic circulation is the bioavailability.
(ii) marker distribution: CK can diffuse into peripheral compartments other than the plasma (central compartment);
(iii) marker elimination rate, i.e. its body clearance.

Estimation of CK disposition parameters (volume of distribution, clearance, systemic bioavailability) is therefore a prerequisite for correct evaluation of the total quantity of marker released from the damaged muscle. Such a determination necessitates the intravenous administration of a determined quantity of homologous CK (supernatants of horse muscle homogenates), and an adequate pharmacokinetic analysis of the CK profile *vs* time. The quantity of marker released from the muscle can then be calculated from these parameters.

Deconvolution, a more complicated and complementary method, allows determination of the instantaneous entry rate of CK into plasma, thus qualifying the chronological development of the lesion. It consists of a mathematical analysis to remove the influence of CK transport, distribution and elimination so as to reconstruct the actual CK release from the muscle cells. The level of plasma CK activity is dependent on the quantity released, and on the delay for CK to reach the plasma *via* the lymph. Thus a low CK plasma activity may be measured although large quantities of CK are released from the muscle. In contrast, persistent high levels of plasma CK activity can only result from a long half-life, and not from continuous CK release from the damaged site. It should therefore be emphasized that the CK plasma activity profile is not necessarily parallel to the time development of the lesion.

3. Examples of muscle damage quantification in the horse

As already reported (*Toutain* et al., 1995; *Volfinger* et al., 1994), the preceding procedure has been used in horses during physical exercise and after IM injection of an irritative drug and the details of the calculation procedure can be found elsewhere (*Lefebvre* et al., 1996).

3.1 Pharmacokinetics of CK in the horse

Briefly, the whole procedure consists of measuring:

(i) the disposition parameters of CK in the horse following IV injection of horse muscle homogenate. This allows to calculate the clearance of CK and its volume of distribution.
(ii) the disposition parameters of CK following IM injection of horse muscle homogenate, which allows to calculate the bioavailability of CK from skeletal muscle, i.e. the proportion of CK which reaches the plasma compartment.
(iii) the variations of PI-CK vs time after muscle injury in order to calculate the area under the curve, then the total amount of CK released.

The estimated steady-state volume of distribution, clearance and terminal half-life of CK activity in the horse were 0.059±0.022 l/kg, 0.36±0.10 ml/kg/min and 123±28 min respectively (*Volfinger* et al., 1994). The small value of the mean steady state volume of distribution (Vss) indicates that distribution of the marker in the extracellular space is limited. The clearance value is low, representing less than 1 % of the hepatic blood flow. The liver would therefore seem inefficient in eliminating CK from the plasma. The short half-life of plasma CK activity in the horse explains the frequent misinterpretation of this parameter for diagnostic purposes, when samples are collected later after muscle injury.

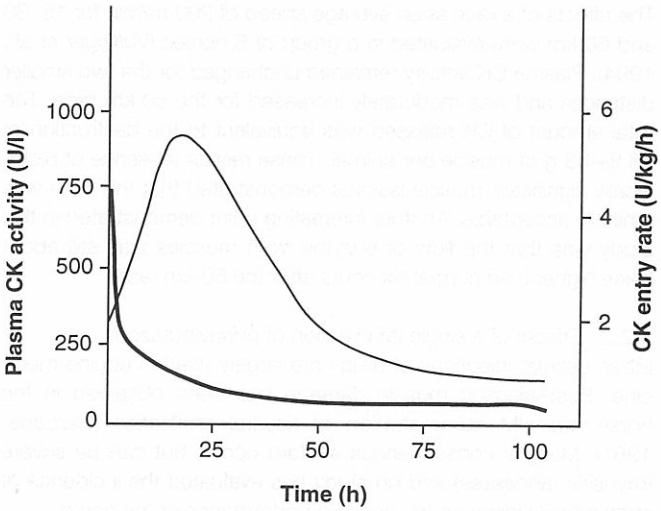

Fig. 3: Example of the time course of plasma CK activity (thin line) and the corresponding instantaneous release rate (thick line) after an intragluteal injection of phenylbutazone at a dose of 8.8 mg/kg to a horse.

The mean bioavailabity of CK is between 74 (*Volfinger* et al., 1994) and 96 % in the horse (*Toutain* et al., 1995), which means that almost all the CK activity which is released from the damaged muscle reaches the systemic circulation.

It may be more evocative to evaluate muscle damage not as units of CK released but as the equivalent mass of muscle which should be totally destroyed to release such units: this can easily be done if the CK activities have been measured with the same analytical procedure.

The equivalent amount of destroyed muscle, QCK, corresponding to the total quantity of CK which has been released can be determined by the following equation (equation 1):

$$Q_{CK} = \frac{Cl \cdot AUC}{F \cdot M}$$

with Q_{CK}, the equivalent amount of destroyed muscle; Cl, the CK clearance; AUC, the area under the curve of CK activity vs time, following the IM administration of the test article; F, the CK bioavailability; and M, the CK content of the injected muscle, i.e. about 3800 U/g of neck muscle (*Toutain* et al., 1995). A more general equation (equation 2) can be used if all measurements are made with the IFCC recommended procedure. Based on mean Cl, F and M values, it allows comparisons but cannot take inter individual variations into account:

$$Q_{CK}\ (g/kg\ BW) = 5.5 \cdot 5.510^{-6} \cdot AUC\ (U \cdot h/l)$$

The entry rate of CK into plasma, after IM administration of CK, was determined using a method of discrete deconvolution as previously described (*Toutain* et al., 1988).

3.2 Examples

3.2.1. Effects of physical exercise

Post-exercise muscle damage has been intensively investigated in human sports medicine. Because of the generalized muscle damage process, it is quite difficult to quantitatively evaluate a lesion by pathological examination. The pharmacokinetic evaluation allows such an approach.

The effects of a race at an average speed of 200 m/min for 15, 30 and 60 km were evaluated in a group of 6 horses (*Volfinger* et al., 1994). Plasma CK activity remained unchanged for the two smaller distances and was moderately increased for the 60 km race. The total amount of CK released was equivalent to the destruction of 18.8±4.3 g of muscle per animal. These results (absence of biologically significant muscle lesions) demonstrated that this race was ethically acceptable. Another interesting point demonstrated in this study was that the flow of enzyme from muscles was still about twice higher than normal six hours after the 60-km race.

3.2.2. Effects of a single IM injection of phenylbutazone

Intramuscular injections of drugs are largely used in equine medicine. Post-injection muscle damage has been observed in the horse after IM administration of several antibiotics (*Garbade,* 1981). Medical consequences seldom occur, but can be severe (myositis, abcesses) and no study has evaluated the incidence of such muscle injury on the sportive performance of the horse.

For these reasons, an evaluation of local tolerance is in order and adequate methods need to be available for the detection of formulations which induce muscle damage. Standardized procedures have been described in several species, all based on scoring of macro- and/or microscopic findings. The major advantage of such an approach is to evaluate the size of the lesion directly. All these morphologic approaches are however semi-quantitative, or even qualitative. They also require euthanasia of a large number of animals to describe the time course of drug effect, which is ethically unacceptable and largely increases the cost of the study. Use of laboratory rodents can lead to erroneous extrapolation to the target species, because of frequent interspecific differences in local reaction. *In vitro* methods, like the hemolysis test or cytotoxicity, are promising, but only screening methods and further studies are still necessary to estimate their predictive values. The pharmacokinetic approach seems particularly promising for such an evaluation.

Significant increases in plasma CK activity were observed after a single IM injection of phenylbutazone at a dose of 8.8 mg/kg either in neck or in hindquarter muscles (*Toutain* et al., 1995). Plasma CK peaked at about the 6th hour reaching 873±365 U/l and 508±109 U/l after intragluteal and intranuchal injections respectively. Plasma CK was back to reference values by day 3. CK activity was higher in gluteal than in the neck muscles: 5441±765 U/g and 3806±706 U/g, respectively ($P<0.001$, Mann and Whitney's test). Thus, the equivalent of muscle destroyed was 0.044±0.029 g/kg and 0.118±0.048 g/kg after intranuchal and intragluteal injection respectively, i.e. about 20 and 60 g for a 500 kg horse. Moreover, the entry rate of CK into the plasma was calculated from the plasma CK *vs* time curve and the parameters of CK distribution (Fig. 3): it can be observed that this rate decreased sharply for the first 6 hours, then slowly for the next day, after which it was very low, while the plasma CK was still elevated.

Conclusion

The application of pharmacokinetic procedures to classical clinical enzymology greatly enhances the diagnostic information collected, thus allowing quantification of the organ damage and evaluation of the time course evolution of muscle damage both in pharmacological tests and sports medicine. The use of the general equation (equation 2) can allow all laboratories using the IFCC recommended technique for CK measurement to determine the equivalent amount of muscle damaged from an adequate measure of the area under the curve of plasma CK *vs.* time. This only provides a means of comparing average muscle damage in sports medicine, spontaneous diseases, pharmacological trials, etc.; moreover, it has the major advantage of being performed in the target species and to be almost non invasive.

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