Comparison of ultrasonography and pharmacokinetic analysis of creatine kinase release for quantitative assessment of postinjection muscle damage in sheep

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Objective—To investigate and validate noninvasive methods for the quantitative evaluation of postinjection muscle damage.

Animals—5 adult sheep.

Procedures—Muscle lesions were induced twice in the lumbar region of the longissimus dorsi muscles (2 sides) by IM administration of a 20% formulation of long-acting oxytetracycline (20 mg/kg of body weight). Clinical signs and local cutaneous temperature above the injection site were recorded. Muscle lesions were quantitatively evaluated by ultrasonography and by use of pharmacokinetic analysis of plasma creatine kinase activity, and both were compared with a comprehensive planimetric computer-assisted analysis of the injection sites after euthanasia.

Results—Transient cutaneous hypothermia (temperature change, -3.9 ± 0.62°C) and subsequent persistent hyperthermia (3.1 ± 1.35°C) were observed after the administrations. Despite coefficient of variation < 10% for precision of ultrasonographic measurement of normal muscle, measurements of the lesions, with coefficient of variation > 60% for precision, were systematically underestimated. Quantitative evaluation of muscle damage by use of pharmacokinetic analysis of creatine kinase (12.1 ± 4.96 g) was in agreement with results of macroscopic planimetric evaluation (10.8 ± 3.64 g).

dilution, performance of ultrasonography, and sampling of the lumbar muscles. The selected test article was a long-acting formulation of oxytetracycline. Three noninvasive methods were chosen and applied at the same time: pharmacokinetic analysis of plasma CK activity, ultrasonography, and cutaneous temperature measurement above the injection site. These noninvasive methods were compared with a reference method of postmortem quantification of muscle lesions by use of macroscopic planimetric analysis.

**Materials and Methods**

**Sheep**—Five adult ewes that weighed (mean ± SD) 50 ± 2.1 kg were acclimated to the experimental conditions for 1 month. Ewes were housed individually in facilities at the Veterinary School of Toulouse, which were approved by the French Ministry of Agriculture and were in accordance with the guidelines for animal care and use. Ewes were fed daily with 1.2 kg of hay and 250 g of pellets. Fresh water was provided ad libitum. The thoracolumbar area was regularly shaved before the IM administrations and the monitoring of the IM injection sites. Clinical observations of all ewes were performed daily throughout the experimental phases.

Oxytetracycline and muscle homogenate—A 20% formulation of long-acting oxytetracycline was used as a test article and was administered IM at the nominal dose of 20 mg/kg of body weight (ie, a volume of 5 ml for a 50-kg sheep).

Homologous muscle homogenate was prepared in saline (0.9% NaCl) solution as described. Briefly, 1 sheep muscle (triceps brachii) was rapidly sampled after a sheep was euthanatized; the sample was minced, homogenized, and centrifuged twice at 13,500 × g for 1 hour. Activity of CK was assessed in the homogenate solution immediately before administration in periods 1 and 3.

**Experimental design**—The same 5 ewes were used during 4 experimental periods. During period 1, pharmacokinetic variables for CK were assessed after IV administration of 3 ml of a homologous muscle homogenate at a dose of 100 U/kg on day 1. In periods 2 and 4, 2 IM administrations of oxytetracycline were performed with a washout interval of 22 days on days 14 (period 2) and 36 (period 4) to evaluate the local muscle reaction. Each IM administration of oxytetracycline was followed by repeated blood samplings, standardized repeated ultrasonographic examinations, and assessment of local cutaneous temperature above the injection site. In period 3, systemic bioavailability of CK from muscle was assessed after IM administration of 5 ml of a homologous muscle homogenate at a dose of 150 U of CK/kg on day 28. The ewes were euthanatized on day 43 (ie, 29 and 7 days after the first and second IM administrations, respectively).

**Drug administration and blood sampling**—Intravenous administration was performed as a bolus through a catheter in the right jugular vein, which was placed 24 hours before administration. The IM administrations were performed in the lumbar area, using 17-gauge sterilized stainless steel needles. Oxytetracycline was administered in the right longissimus dorsi muscle for period 2 and in the left longissimus dorsi for period 4. The needle was inserted 2 cm vertically into the midbelly of the lumbar area. The depth of insertion was calculated from the dorsal spinous process of the adjacent vertebra and the extremity of the transverse vertebral process. In period 3, muscle homogenates were administered 10 cm caudal to the injection sites of the oxytetracycline.

Blood was collected into tubes containing heparin lithium from a left-side jugular catheter that had been placed 24 hours before the experiments. Sampling volume was 5 ml in periods 1 and 3 and 8 ml in periods 2 and 4. Samples were centrifuged at 3,000 × g for 10 minutes at 4°C and plasma was stored at −20°C until analysis. For each period, 3 blood samples were obtained within 2 hours before each administration for estimation of basal plasma CK activity. Time schedule for blood sampling after administration of oxytetracycline was 2, 4, 8, 15, 20, 30, and 45 minutes, and 1, 2, 4, 6, 8, 10, 12, and 24 hours (period 1); 15, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 10, 12, 15, 24, 30, 48, 56, 72, 96, 120, and 144 hours (periods 2 and 4); and 15, 30 and 45 minutes and 1, 2, 3, 4, 6, 8, 10, 12, 24, 30, 48, 56, and 72 hours (period 3).

**Muscle sampling**—After induction of anesthesia with xylazine hydrochloride (0.2 mg/kg, IM, thigh muscle) and ketamine hydrochloride (10 mg/kg, IM, thigh muscle), death was induced by exsanguination. The lumbar areas were rapidly excised and frozen at −20°C. The injection sites were subsequently cut into accurately measured slices 1 to 1.5 cm thick for macroscopic examination. Seven slices of muscle were obtained for each ewe. Two control slices obtained just behind and in front of each injection site were mixed in homogenates as described and used for measurement of basal CK content of longissimus dorsi muscle.

**Creatine kinase assay**—Activity of CK was assessed in plasma and muscle homogenates according to recommendations of the International Federation of Clinical Chemistry. Briefly, an enzymatic kit was used. Activity of CK was determined from the increase in absorbance (340 nm) corresponding to NADP reduction measured at 1 minute intervals for 5 minutes with an automated microplate reader. The level of quantification was 10 U/L and the repeatability and reproducibility coefficients of variation (CV) were < 10%.

**Local cutaneous temperature measurement**—Cutaneous temperature was measured after each IM administration by the same investigator, using an infrared thermometer positioned vertically above the injection site and the contralateral muscles (control site). The difference between temperatures at these 2 sites was calculated and plotted against time. Five measurements were made at each examination.

**Qualitative macroscopic scoring of postinjection muscle damage**—Muscle damage for each slice was classified on a visual basis into 4 exclusive categories (no lesion, necrosis, hemorrhage, fibrosis) according to the predominant lesion. Three investigators performed the classification twice on randomly mixed muscle slices from all the ewes. All the investigators were trained in ultrasonography and in the assessment of postinjection muscle damage. Results of the repeated examinations were used to calculate an index of agreement among and between investigators.

**Macroscopic postmortem evaluation of muscle damage**—Three investigators measured 29- and 7-day-old lesions twice on randomly mixed slices of muscle from all ewes. The area (A) of macroscopically damaged muscle on each slice was calculated from the mathematical product of the height and width measured directly with a graduated ruler. A second method of measurement was used, in which the lesions on each slice were more accurately quantified by computer-assisted analysis. This method included a numeric magnification of the slice of muscle on a monitor screen. The enlarged muscle lesion was then evaluated by use of planimetry. Thickness (T) was measured for each slice (i), and the entire volume of muscle damage (V_{macro}) was computed for
each site of administration according to the following equation, adapted from a described method:  

\[ V_{\text{macro}} = \sum \left( \frac{|A_i + A_{i+1}|}{2} \times T_i \right) \]

The equivalent mass of macroscopically damaged muscle (\( M_{\text{macro}} \)) was deduced from the volume and density of the longissimus dorsi muscle with the following equation:

\[ M_{\text{macro}} = \frac{V_{\text{macro}}}{\text{density}} \]

Results obtained by use of computer-assisted analysis were used as a reference for comparison with in vivo measurements (ultrasonography, plasma CK activity, and local temperature) performed before euthanasia.

**Ultrasonography of the injection site**—Standardized echographic examinations were performed on standing ewes, using a portable ultrasonograph® and a 7.5-MHz linear transducer. The position of the transducer was precisely located on the ewe’s back with a permanent drawing on the skin above the site of IM administration. Transmission gel was applied to the transducer and to the skin prior to cross-sectional examination of the muscle. A 1-cm-thick hydrated polyacrylamide-agar sheet was positioned directly on the skin and used as an aqueous coupling medium to bring the muscle into the focus area of the transducer.  

After 1 ultrasonographic picture was obtained, measurements were immediately taken with electronic calipers.

Prior to any drug administration, the metrologic properties (precision) of the ultrasonographic procedures were assessed. Each investigator performed 5 repeated examinations on the 5 ewes and measured the size of the lumbar portion of the longissimus dorsi muscles. Variables measured were maximum cross-sectional width, height, circumference, and area of the muscle. After the IM drug administration and development of injury, the ability of the method to detect muscle lesions as well as the precision and accuracy of the ultrasonographic measurements of the lesions from the largest cross-sectional image were assessed. This validation study was conducted 24 hours before euthanasia, with 5 repeated examinations by each of the 3 investigators on each ewe.

Ultrasonography was performed in a blinded manner with the investigators unable to view the figures measured on the screen of the ultrasonograph, and the order of examinations for each sheep was randomized.

**Pharmacokinetic analysis of plasma CK activity**—Quantification of muscle damage after a single administration of oxytetracycline was performed as described by use of non-compartmental analysis of plasma CK activity. Pharmacokinetic analysis was performed with commercial software.  

Individual basal plasma CK activity was fixed at the arithmetic mean of the 3 control samples obtained prior to drug administrations. This mean value was subtracted from each plasma CK activity observed after the IM administration. The area under the curve (AUC) for CK activity was calculated by the linear trapezoidal rule without extrapolation to infinity. Bioavailability of CK from muscle (F [%]) and CK plasma clearance (Cl; [L/kg per minute]) were determined from IV and IM administrations of the muscle homogenate, using the following equations:

\[ F = \frac{\text{AUC}_{\text{IV}} \times \text{Dose}_{\text{IV}}}{(\text{AUC}_{\text{IV}} - \text{AUC}_{\text{unt}}) \times 100} \]

\[ \text{Cl} = \frac{\text{Dose}_{\text{IV}}}{\text{AUC}_{\text{IV}}} \]

where \( \text{AUC}_{\text{IV}} \) (U × min/L) was the AUC after IV administration of the muscle homogenate, \( \text{Dose}_{\text{IV}} \) (U/kg) was the dose of CK activity administered by the IV route, and \( \text{Dose}_{\text{IM}} \) (U/kg) was the dose of CK activity administered by the IM route. The CK activity in healthy muscle (M [U/g]) was determined in a sample of longissimus dorsi muscle from each ewe. The equivalent quantity of damaged muscle (Q [g]) for each IM injection of drug could be obtained from Cl, \( \text{AUC}_{\text{CK}} \) (AUC of plasma CK activity), F, and M, using the following equation (F was arbitrarily fixed at 100% when exceeded):

\[ Q = \text{Cl} \times \text{AUC}_{\text{CK}} / (F \times M) \]

**Qualitative macroscopic scoring of lesions**—Investigators were tested 2 × 2 for agreement between the 2 repeated scorings for each slice of muscle. This agreement could be defined as the extent of identical scoring between and among the investigators. For interinvestigator agreement, the final score (Sn) for each slice (n) was the sum of a combination of 1 and 0, obtained for each pair of observations:

\[ S_n = \sum (1 \text{ if } i = j; 0 \text{ if } i \neq j) \]

where i was the first or second observation of 1 investigator and j was the first or second observation of another of the 2 remaining investigators.

For intrainvestigator agreement, the final score for each slice was the sum (Sm):

\[ S_m = \sum (1 \text{ if } k = m; 0 \text{ if } k \neq m) \]

where k was the first observation of 1 investigator and m was the second observation of the same investigator. The \( S_n \) and \( S_m \) were transformed into categoric percentages of the whole agreement between or among the investigators.

**Assessment of accuracy of the measurement methods**—For the ultrasonographic measurements (width, height, circumference, and area), the repeatability (intrainvestigator variability) and reproducibility (interinvestigator variability) CV were computed with an ANOVA with 2 random effects, according to the following model:

\[ Y_{ijk} = \mu + \text{Op}_i + \text{An}_j + (\text{Op} \times \text{An})_{ij} + \varepsilon_{ijk} \]

where \( Y_{ijk} \) was the k\textsuperscript{th} value measured for ewe j by investigator i, \( \mu \) was the mean of the observed values, Op was the differential effect of investigator i, An\textsubscript{j} was the differential effect of ewe j, (Op × An)\textsubscript{ij} was the interaction term, and \( \varepsilon_{ijk} \) was the model error. When no significant interaction occurred, the repeatability CV were calculated with the following equation:

\[ CV_{\text{repeatability}}(\%) = \left( \frac{SD_{\text{R}}}{\mu} \right) \times 100 \]

where SD\textsubscript{R} was the SD for the residual term. Unbiased reproducibility CV were then computed with the following equation:

\[ CV_{\text{reproducibility}}(\%) = \left( \frac{SD_{\text{I}}}{\mu} \right) \times 100 \]

The unbiased SD for the investigator effect,
SD_{investigator}, was obtained with equation:

\[ SD'_{investigator} = (MS_{investigator} - SD_{\varepsilon}) / (n \times p) \]

where MS_{investigator} was the mean square of the deviation from the mean, given by the ANOVA; n was the number of measurements performed per animal and per investigator; and p was the number of ewes.

For macroscopic examination, the precision of the measurement approach was similarly evaluated via CV for repeatability and reproducibility, considering the slice of muscle factor instead of the ewe factor in the statistical model described for ultrasonography; all slices from the different ewes were randomly pooled for macroscopic examination. For cutaneous temperature, a CV for repeatability was calculated in the same manner to evaluate the precision of the method; the model included 2 random effects (ewe and postinjection time) and their interaction.

An ANOVA with the factor ewe was performed to test for the effect of individuals on the AUC of CK obtained after the 2 administrations of oxytetracycline and on the equivalent quantities of muscle determined by the different methods. Tukey multiple comparisons were performed post hoc. Quantity of muscle damage in periods 2 and 4 estimated with all the methods was compared with a Student paired t-test.

A 95% confidence interval (CI) was constructed for the mean ultrasonographic area of muscle damage at each time of observation. When the value 0 was included in the 95% CI, it was concluded that no muscle damage could be detected with ultrasonography.

**Results**

**Clinical signs**—Mild signs of localized pain and swelling were seen in all ewes immediately after IM administration of oxytetracycline.

**Local cutaneous temperature**—The CV repeatability was 0.7%. A biphasic temperature pattern was observed in all ewes after IM administration of oxytetracycline, with an initial hypothermic phase followed by a hyperthermic curve (Fig 1). Maximum decrease in temperature was \(-3.9 \pm 0.62^\circ C\), which occurred at 8.4 \pm 12.07 minutes. Maximum increase in temperature was 3.1 \pm 1.35^\circ C, which occurred at 57 \pm 36 hours. No relationship was detected between temperature profiles and the extent of observed muscle damage.

**Macroscopic postmortem evaluation of muscle damage**—Fibrosis, necrosis, and hemorrhage (Fig 2) were observed in 66, 32, and 2%, respectively, of the 29-day-old lesions and in 16, 31, and 53%, respectively, of the 7-day-old lesions. Interinvestigator agreement was 100% for 53% of the slices and at least 50% for 69.6% of the slices. Intrainvestigator agreement was 100% for 57.6% of the slices, at least 50% for 66.7% of the slices, and 0% for 21% of the slices.

Estimated CV repeatability and CV reproducibility for the direct macroscopic visual measurement of the damaged area were 47.6 and 34.5%, respectively. Results obtained by use of direct macroscopic measurement and planimetry were correlated, with \(R^2 = 0.72\). Mean size of 7-day-old lesions (12.1 \pm 4.96 g) was significantly (\(P = 0.04\)) different from that of the 29-day-old lesions (4.8 \pm 1.89 g), as estimated by use of planimetric image analysis; this difference was not significant (\(P = 0.14\)), as estimated by use of visual macroscopic measurements (7.3 \pm 3.45 g and 4.0 \pm 2.21 g for 7- and 29-day-old lesions, respectively).

**Ultrasonography of the injection site**—Muscle
lesions appeared as hyperechoic areas in the middle of the muscle belly (Fig 3). The CV\text{repeatability} and CV\text{reproducibility} for measurements of the control muscle and muscle lesions were determined (Table 1). After euthanasia, postinjection muscle lesions were clearly apparent at each site of administration. Lesions were detected in 32% of the ultrasonographic examinations performed by the first investigator, 48% of the examinations performed by the second investigator, and 100% of the examinations performed by the third investigator.

Ultrasonography gave a 2.5- to 5.3-fold underestimate of the actual area of the lesions assessed by use of planimetry, depending on the ewe. Many discrepancies between values obtained with the 2 techniques on various days and from individual ewes were observed and could not be explained. Mean lesion measurement by use of ultrasonography was not significantly different from 0 except at 9 and 24 hours after the IM administration, as shown by the 95% CI, which implied that ultrasonography did not detect any lesion except at the 2 noted times.

**Pharmacokinetic analysis of plasma CK activity**—Mean basal CK activity was 27 ± 15.9 U/L and ranged from 16 to 48 U/L. Mean CK activity in control muscles was 2,300 ± 403.9 U/g with a range of 1,621 to 2,601 U/g.

Noncompartmental analysis of CK activity after IV bolus administration of muscle homogenate gave a CK clearance of 0.4 ± 0.17 ml/kg per minute. The apparent mean bioavailability of exogenous CK from muscle after IM administration of the CK solution was 106 ± 31.7%, with a range of 73 to 149%.

The C\text{max} of plasma CK activity after IM administration of oxytetracycline was correlated to the noncompartmental AUC ($R^2 = 0.84$). From CK release, the equivalent amount of muscle damage during periods 2 and 4 after a single IM administration of oxytetracycline was 10.7 ± 4.59 g. The amount of muscle damage did not differ significantly ($P = 0.97$) between the 2 IM administrations (period 2, 10.7 ± 5.85 g; period 4, 10.8 ± 3.64 g). A linear regression was computed between results obtained by use of pharmacokinetic analysis of CK release and planimetric image analysis of the dam-

| **Table 1**—Coefficients of variation for repeatability and reproducibility of ultrasonographic measurements on longissimus dorsi muscle in 5 ewes obtained by 3 operators on normal muscle and muscle lesions induced by a single IM administration of a 20% formulation of long-acting oxytetracycline (20 mg/kg) on day 0. S = Skin. Sc = Subcutaneous tissue. V = Vertebra. Ruler indicates centimeters. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Variable**    | **Repeatability (%)** | **Reproducibility (%)** | **Repeatability (%)** | **Reproducibility (%)** |
| Height          | 6.1              | 1.6              | 51.4            | 92.0            |
| Weight          | 5.6              | 6.6              | 66.7            | 70.0            |
| Area            | 8.5              | 4.1              | 81.4            | 101.4           |
| Circumference   | 5.0              | 2.6              | 60.9            | 81.9            |

Figure 2—Photograph (top) and diagram (bottom) of muscle slice from a ewe with 29-day-old (A) and 7-day-old (B) muscle lesions after 2 successive IM administrations of a 20% formulation of long-acting oxytetracycline (20 mg/kg) on day 0. S = Skin. Sc = Subcutaneous tissue. V = Vertebra. Ruler indicates centimeters.

Figure 3—Transverse ultrasonographic image of a muscle from an ewe 2 days after IM administration of oxytetracycline. Dotted line delineates muscle lesion.
Discussion

Sheep were used as a model in the study reported here, because they represent a target species and are convenient for handling, blood volume is not a limiting factor for pharmacokinetic studies, and the basal CK is stable, compared with pigs and rabbits, in which a stress-induced increase in plasma CK is observed. A long-acting formulation of oxytetracycline was used, because it induces postinjection lesions in sheep, pigs, and cattle. Despite its nonuse in current management of food animals, the selected site of administration in the longissimus dorsi muscle guarantees strict IM deposit of the drug without dispersion into the muscular fascial sheaths, as described in dogs. It also permits adequate standardization of the ultrasonographic examination without disturbing the animals, appropriate conditions for local temperature assessment, and easy delimitation and removal of the whole injection site after euthanasia.

According to current recommendations, assessment of postinjection muscle damage is only made on the basis of clinical recordings and pathologic examination. In this study, only slight swelling and transient pain at the injection site were observed.

Cutaneous temperature was measured above the injected muscles to monitor the time course of the local inflammatory response. Although local temperature could not be used to evaluate the extent of muscle damage, the biphasic temperature profile observed in all the ewes led to 1 hypothesis on the pathophysiologic mechanisms of postinjection muscle damage. Hypothermia could be related to a transient local decrease in blood flow consequent to a compression of muscle bundles and capillaries attributable to the volume and viscosity of the injected material or to reflex vasoconstriction. Hyperthermia could result from reperfusion flow and inflammatory vasodilatation. The successive occurrence of vasoconstriction and vasodilatation could be related to the ischemia-reperfusion syndrome.

Macroscopic examination of muscle damage after slaughter is the current method used to assess the irritancy of different drugs after IM administration. Often, only a single section of affected muscle is examined, and the lesion is assessed according to a scoring system. Lack of standardization of the entire process leads to numerous problems, including inexact location of the site of administration in vivo on the animal, inconsistency in the number of sections obtained from the injection site, and use of many scoring and scaling systems. These are qualitative or semiquantitative, which makes them difficult to analyze for the comparison of different drugs. In the study reported here, the damaged area, determined by use of direct measurement of height and width on the slices, was correlated with results of computer-assisted planimetry. The CV for repeatability and reproducibility indicated that this approach is not sufficiently precise for quantification and should be validated prior to any use, as for any assay in which the CV should be < 15%. A basic recommendation could also be that the same investigator, whose intra- and interday variation should be known, should perform the measurements. Similar comments could apply to qualitative assessment. In our study, the macroscopic examination of 7-day-old lesions with predominance of hemorrhage and 29-day-old lesions with predominance of fibrosis was in agreement with the usual description of the development of postinjection muscle damage. This classification was much simpler than the other scoring systems, which are used without validation. A validation is provided here, with an estimation of repeatability and reproducibility of the method of classification. In 21% of the examinations, there was not any repeatability; the classification was different between 2 successive scorings for all the investigators.

The ultrasonographic approach was also tested, because it was apparent that macroscopic morphologic examination and imaging may give similar information. Ultrasonography of the injection site has been proposed as a promising means of complementing the development, nature, and extent of muscle lesions during the period of drug-induced damage and the healing period. In our experiment, this approach was subjected for the first time to a validation process for its use in quantitative evaluation of postinjection muscle damage. In vivo measurements were compared with direct postmortem examination results. Ultrasonography appears to be reliable for noninvasive prediction of carcass composition, especially for fat and longissimus dorsi muscle measurement in cattle and sheep. Indeed, in our study, ultrasonography had good intrinsic measurement properties, with repeatability and reproducibility CV of 9.5 and 4.1%, respectively, for measurements of the longissimus dorsi muscle area prior to drug administration. In a descriptive study of the ultrasonographic appearance of the structures of the ventral neck region in cattle, the interassay CV, ranging from 3.0 to 12.3%, were quite similar to our results. The ultrasonographic method was reliable for the assessment of dimensions in healthy muscle, but validation could not be extrapolated to damaged muscle. On cross-sectional imaging of normal muscle, the fibro-adipose septae were echogenic, and the muscle bundles were hypoechoic, resulting in a “starry sky” appearance for the whole muscle, which has been described. Our repeated examinations had a sensitivity of 60% for detection of muscle lesions, which is less than that of previous studies in which sensitivity was 82.9% for diagnosis of inflammatory myopathies in humans. When lesions were detected in our study, ultrasonography provided inaccurate measurements of the damaged area, with unacceptable CV for precision and a systematic 2-fold underestimation of the size of the actual lesion, as determined by use of planimetry. In fact, hyperechogenic zones were seen in the muscle and were interpreted to be muscle lesions, but these zones were sometimes multifocal or diffuse areas, which made delimitation of the damage difficult. Conflicting observations concerning the echogenic changes in muscle lesions have been reported; echogenicity has been reported to increase or decrease with edema. The wide range of ultrasono-
graphic findings in muscle makes detection of postinjection lesions difficult and highly dependent on the investigator. Ultrasonography does not appear to be a reliable tool for the assessment of postinjection muscle damage and will underestimate the actual size of the lesion.

Pharmacokinetic analysis of CK has been used to assess local tolerance to different formulations in cattle,10 dogs,11 horses,12 and sheep13 but had not been validated for accuracy. To the authors’ knowledge, this study is the first to reveal positive correlation between the equivalent amount of muscle, determined ante-mortem by use of noncompartmental pharmacokinetic analysis of CK, and the quantity of macroscopically damaged muscle, measured by use of computer-assisted planimetry after euthanasia. The mean mass of muscle damage was within the range of previous calculations in sheep, in which the lesion represented between 1.4 and 83.3 g, depending on the administered oxytetracycline formulation. Some authors have used plasma CK activity at specified times after injection,29 $C_{\text{max}}$ values,30 or AUC of CK to compare tolerance for different formulations.23 In the latter study, the amount of muscle damage in lactating cows after IM administration of flunixin and phenylbutazone at the recommended dose was approximately 80 g. Computing the AUC of CK may be used to compare tolerance to different formulations or drugs administered to the same individuals in a crossover design study, because the AUC in the same sheep in our study were not significantly different between 2 successive administrations of the same drug. Peak value for plasma CK activity may also be used for rough comparisons, because our results indicated that $C_{\text{max}}$ and AUC were correlated. However, differences in absorption or elimination processes between individuals may result in important sources of variation in plasma CK activity or AUC, leading to misinterpretation of tolerance to the formulations. In other words, determination of plasma clearance and bioavailability from muscle is desirable to correctly estimate the amount of muscle damage estimated by use of AUC. Determination of clearance, bioavailability, and CK content of normal muscle allows accurate estimation of the actual amount of muscle damage. The CK clearance in our study was consistent with the plasma CK clearance of $0.3 \pm 0.12 \text{ ml/kg per minute}$ computed in a previous study.33 Systemic bioavailability of CK from muscle exceeded 100% in 2 of the 5 ewes in our study, which may have resulted from partial injury of the muscle cells after IM administration of the exogenous CK solution, leading to a release of endogenous CK. The computed mean bioavailability of CK from muscle was 31% in rabbits,31 42% in sheep,34 51% in cattle,17 and 75% in horses.17 The CK activity in normal lumbar muscle assessed in this study on 5 adult ewes was 2,300 ± 403 U/g. The CK results obtained previously were between 3,941 and 5,940 U/g in triceps brachialis and quadriceps femoralis, respectively, but were only obtained from a single 6-month-old lamb.22 In cattle, the CK activity in neck muscle was reported to be 2,860 ± 796 U/g,4 which is similar to our results in sheep. Quantitative assessment of muscle damage by use of pharmacoki-

netic analysis of CK rather than AUC is more appealing in terms of potential economic loss, because the estimated weight of the damaged muscle is expressed. A similar approach indicated that the damage resulting from an IM administration of imidocarb to a 12-kg dog was equivalent to approximately 3 g of muscle.23

Our results indicated that presently used morphologic approaches may lead to misinterpretations in local tolerance testing. Planimetry should be recommended, although it is tedious and time consuming. Ultrasonography, a noninvasive approach, is not reliable for quantification of muscle damage. Pharmacokinetic analysis of CK release provides a noninvasive and accurate method of quantifying postinjection muscle damage. It can therefore be recommended, especially in companion animals for which macroscopic examination requiring euthanasia is a critical ethical issue, because no residue study is necessary. When individual CK pharmacokinetic values are not available, however, the AUC alone may be used for comparison of several formulations in the same animals.

References


