Simultaneous and minimally invasive assessment of muscle tolerance and bioavailability of different volumes of an intramuscular formulation in the same animals


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ABSTRACT: Evaluation of skeletal muscle tolerance during development of new drug formulations for i.m. use is most often based on terminal methods performed in the target species after slaughtering. The objective of this study was to evaluate the effect of muscle damage on the pharmacokinetic parameters of the drug delivered into the muscle using an alternative, noninvasive method. Phenylbutazone (PBZ) was used as the test article. Six ewes received increasing volumes of a 20% PBZ i.m. formulation, according to a cross-over design, and an i.v. bolus of the same formulation. Serial blood samples were taken, and a pharmacokinetic analysis of the plasma activity of creatine kinase and plasma PBZ concentrations was carried out. The amount of muscle damage after i.m. administration of 2, 4, or 8 mL of PBZ, calculated from the area under the curve of plasma creatine kinase across time was 36, 76, and 178 g for a 70-kg ewe. The corresponding absolute bioavailability of PBZ was 100 ± 32%, 96 ± 19%, and 100 ± 17%, and the maximal PBZ concentrations were 42 ± 3.4, 74 ± 8.8, and 119 ± 18.2 μg/mL. The plasma clearance of PBZ (i.v.) was 4.2 ± 0.94 mL·kg⁻¹·h⁻¹. In conclusion, the absolute bioavailability of PBZ after i.m. administration was not altered by the increased volume of formulation administered despite the overall increase in the extent of muscle damage.

Key words: bioavailability, creatine kinase, intramuscular administration, muscle damage, ovine, phenylbutazone

INTRODUCTION

Tissue damage induced by the i.m. administration of drugs is a critical issue in drug development, especially in formulations for food-producing animals. Development of well-tolerated formulations by pharmaceutical companies is still the best strategy for limiting the incidence of postinjection blemishes. Although formulation-dependent factors inducing muscle damage after i.m. administration have been identified, a simple way to predict the local tolerance of a formulation from its individual components has not yet been found. The local tolerance of veterinary drugs in target animal species is generally assessed by macroscopic examination of muscle after euthanasia, as stated in the current US and European guidelines for drug development. The drawbacks of such pathologic approaches are numerous: i) these methods are most often qualitative, based on highly subjective scoring systems that differ considerably in the literature and therefore impair any comparison between the various results published; ii) the required euthanasia of a large number of animals presents ethical and financial issues; such investigations are generally performed in food animals along with residue studies, i.e., at the end of drug development, whereas they would be much more useful as screening tests at earlier stages; and iii) comparison of the local tolerance of different formulations of the same drug on the same animal, and simultaneous assessment of the influence of the formulation on drug pharmacokinetics is impossible.

The first aim of this study in sheep was to demonstrate the relevance of a new noninvasive technique for the quantitation of muscle damage, based on the pharmacokinetic analysis of creatine kinase release from the damaged muscle after i.m. administration. The second aim was to assess the effect of different volumes of an intramuscular formulation in the same animals.
volumes administered on the local tolerance and drug bioavailability in the same animals.

MATERIALS AND METHODS

Animals

Experiments were conducted at the National Veterinary School of Toulouse, in facilities that are approved for animal research use by the French Ministry of Agriculture. The research was performed in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996). Six adult Lacaune ewes weighing 76 ± 9.0 kg at the beginning of the study were acclimated to the experimental conditions for 15 d. They were observed daily, fed with hay and pellets (300 g), and provided fresh water ad libitum. During the pharmacokinetic studies, they were placed in individual cages. Body weight was measured before each drug administration.

Experimental Design

Ewes were randomly allocated to 3 groups of 2 animals for a 3-period crossover design. At each of the 3 periods, groups of ewes received 1 of 3 i.m. injection volumes of a phenylbutazone (PBZ) formulation. A washout period of 3 to 4 wk was observed between each injection period. Blood was sampled after each injection to determine plasma PBZ concentration and creatine kinase (CK) activity. To determine the pharmacokinetic parameters of PBZ in each ewe, an i.v. bolus of PBZ was then administered after completion of the crossover experiment and washout period. No treatment other than PBZ was used during the study.

Administration of PBZ

For the i.v. and i.m. injections, a commercially available formulation of 20% PBZ was used (Phenylarthrite, Vetoquinol, Lure, France). Three volumes (2, 4, or 8 mL) of PBZ were administered. The i.m. injections were given between 0800 and 1000 in the longissimus lumborum muscle. The 3 sites of injection within the muscle were all 4 cm lateral to the spinal crest, and 7, 13, or 19 cm cranial to the iliac crest. The volume of formulation injected at each site differed between each group of animals, and each site was injected once only. The hair was clipped the day before, and the skin area was cleaned and disinfected with alcohol (70%) just before each i.m. injection. The alcohol was allowed to dry before the injections, which were given with a 1.1 × 40-mm needle over about 5, 10, or 20 s for the 2-, 4-, or 8-mL volumes of PBZ, respectively. The corresponding dosages administered were 5.2 ± 0.53, 10.5 ± 1.11, and 21.3 ± 2.44 mg/kg, respectively. The i.v. injections of PBZ (8.0 ± 0.08 mg/kg) were given through an indwelling catheter that had been placed in the left jugular vein the previous day. The catheter was flushed with saline after PBZ administration and then removed.

Blood Sampling

An indwelling catheter was placed in the right jugular vein the day before the kinetic analyses and rinsed 3 times each day with heparinized saline. One milliliter of blood was taken before sampling and discarded. Then, 4 mL of blood was collected, and 2 mL of heparinized saline was administered. In each period, the catheter was removed after the last blood sampling. Three blood samples were taken before PBZ administration at 60 and 30 min and just before PBZ administration. Blood samples were then taken 15, 30, 45, and 60 min, and at 2, 4, 6, 8, 10, 12, 24, 32, 48, 72, 96, and 120 h after PBZ administration. The sampled blood was placed in a heparinized tube at 4°C, and centrifuged within 30 min (3,000 × g for 10 min). Plasma was divided into 3 aliquots and stored at −20°C until assay.

Assays

Creatine kinase activity was measured in plasma at 30°C with commercially available reagents (Enzyline CK, ref 63151, BioMerieux, Marcy l’Etoile, France) on a Bio analyzer (Cobas, Roche, Neuilly-sur-Seine, France). The limit of detection was 2 U/L. Quality control was based on pooled animal sera. The between-assay CV was less than 4.5%.

Plasma concentrations of PBZ and its main metabolite, oxyphenylbutazone (OPBZ), were determined by HPLC. To 100 µL of plasma, 50 µL of the internal standard (nimesulide at 10 µg/mL) and 50 µL of acetonitrile were added. The mixture was vortex-mixed for 1 min, then centrifuged at 10,000 × g for 10 min. Ten microliters of the supernatant were injected directly into the chromatographic system. The HPLC apparatus consisted of a pump system equipped with an automatic injector and an ultraviolet detector (254 nm). Separation was achieved by a Lichrospher C18 reverse phase column (6 µm, 125 × 4.0 mm; Interchim, Montluçon, France), using a guard column. The mobile phase was composed of a 40:60 (vol/vol) mixture of 0.2% acetic acid/methanol and was used at a flow rate of 0.4 mL/min. Standard calibration curves were generated in a concentration range between 0.20/0.25 (OPBZ/PBZ) and 20 µg/mL. The within- and between-day CV were less than 15%. The accuracy ranges were 99 to 109% for PBZ and 103 to 110% for OPBZ. The detection limits of the assay were 0.25 µg/mL for PBZ and 0.20 µg/mL OPBZ.

Pharmacokinetic Analysis of PBZ

The pharmacokinetic analysis was carried out using a noncompartmental approach and a pharmacokinetic software package (WinNonLin 3.0, Pharsight Corp, Mountain View, CA). The maximal plasma concentration of PBZ (Cmax) and its time of occurrence (Tmax) were determined from the observed values. Area under the plasma concentration vs. time curves (AUC) of CK, PBZ, and OPBZ were calculated using the linear trape-
zoidal rule with extrapolation to infinity, the extrapolated area representing less than 15% of the total AUC. Elimination half-lives were estimated from the terminal phase by log-linear regression. Phenylbutazone systemic availability from muscle (F, %) and PBZ plasma clearance (Cl, mL·min⁻¹·kg⁻¹) were determined from the i.v. and i.m. administrations of the PBZ formulation using equations (1) and (2):

\[ F = \frac{\text{AUC}_{\text{im}} \times \text{Dose}_{\text{im}}}{\text{AUC}_{\text{iv}} \times \text{Dose}_{\text{iv}}} \times 100; \text{ and} \]

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

in which AUC_{im} (µg·min·mL⁻¹), AUC_{iv} (µg·min·mL⁻¹), Dose_{im} (µg·kg⁻¹), and Dose_{iv} (µg·kg⁻¹) represent the area under the plasma PBZ concentration curve after i.m. administration, the area under the plasma PBZ concentration curve after i.v. administration, the dose of PBZ administered by the i.m. route, and the dose of PBZ administered by the i.v. route, respectively.

**Estimation of Muscle Damage from Plasma CK Activity**

The muscle damage caused by i.m. administration of PBZ was determined as previously described (Lefebvre et al., 1996). Basal plasma CK activity was determined for each animal using the arithmetic mean of the 3 control samples obtained before the i.m. administration of PBZ. This mean value was subtracted from the CK activities observed after each i.m. administration (supersposition principle). The amount A (expressed in grams of damaged muscle/kilogram of BW) was calculated from the following equation, according to Ferré et al. (2001):

\[ A = \frac{\text{Cl} \times \text{AUC}_{\text{CK}}}{\text{F} \times \text{M}}, \]

in which AUC_{CK} (U·min·L⁻¹) represents the AUC of the plasma CK activity (subtracted from the mean basal value) concentration vs. time profile after i.m. administration of PBZ. The values for Cl (L·kg⁻¹·h⁻¹), which represents the plasma clearance of CK; F (%), which represents the muscle bioavailability of CK; and M (U/g of muscle), which represents the muscle content of CK, were those previously determined in ewes in our laboratory (Ferré et al., 2001), and therefore the value of \( \frac{\text{Cl}}{\text{F} \times \text{M}} \) was fixed at 10.4·10⁻⁶·g·L·U⁻¹·h⁻¹·kg⁻¹.

**Statistics**

The results are presented as means ± SEM unless otherwise stated. The following linear model and Systat software (Systat 10.2, SPSS Inc., Chicago, IL) were used for the statistical analysis:

\[ Y_{i,j,k,l} = \mu + \text{Volume}_i + \text{Period}_j + \text{Group}_k(i,j) + \text{Animal}/\text{Group}_k + \epsilon_{i,j,k,l}, \]

in which \( Y_{i,j,k,l} \) represented the response variable (Log-transformed AUC or Log-transformed Cmax), \( \mu \) represented the mean effect, \( \text{Volume}_i \) represented the fixed volume effect (i = 2, 4, or 8 mL), \( \text{Period}_j \) represented the fixed period effect (j = 1, 2, or 3), \( \text{Group}_k(i,j) \) represented the fixed effect of the group of animals receiving Volume at Period, \( \text{Animal}/\text{Group}_k \) represented the random animal effect (i = 1, 2) nested within each group (k = 1, 2, or 3), and \( \epsilon_{i,j,k,l} \) represented the residual error. The effect of the injection site was considered negligible, as all the injection sites were located in the same muscle. Therefore, this potential source of variation was not included in the analysis.

**RESULTS**

**Clinical Observations**

Small and brief skin tremulations were observed at the injection site when inserting the needle into the muscle. During i.m. administration and in the minutes after the end of the injection, tremulations were seen deeper at the injection site, and those tremulations were assumed to be muscle contractions caused by the irritating nature of the formulation. No other clinical sign was observed.

**Plasma CK Activity**

Plasma profiles of CK activity vs. time are shown in Figure 1. The AUC, Tmax, and Cmax for plasma CK activity after i.m. injection of 2, 4, and 8 mL of PBZ are shown in Table 1.

The AUC and the observed Cmax for plasma CK activity increased 2.1- and 2.4-fold, respectively, between the low and greatest volumes of PBZ, and 4.9- and 5.1-fold, respectively, between the low and medium volumes of PBZ, and 4.9- and 5.1-fold, respectively, between the low and greatest volumes of PBZ administered (P < 0.05 and P < 0.01 for log-transformed AUC and log-transformed Cmax, respectively). The corresponding equivalent amounts of muscle damage (in g/kg of BW) were 0.5 ± 0.38, 1.1 ± 0.38, and 2.5 ± 1.23 for 2, 4, and 8 mL of PBZ, respectively, i.e., 36, 76, and 178 g for an average 70-kg ewe. Large interindividual variations were observed between ewes for each volume injected (Figure 2).

**Plasma PBZ Pharmacokinetics**

The plasma profiles of PBZ after i.v. and i.m. injections are shown in Figure 3. The AUC of PBZ and OPBZ after the i.v. injection were 117,614 ± 9,249 and 4,762 ± 349 µg·min/L, respectively. The AUC of OPBZ represented less than 5% of the calculated PBZ AUC and was considered negligible. The plasma clearance for PBZ after i.v. administration was 0.071 ± 0.006 mL·kg⁻¹·min⁻¹, i.e., 4.3 ± 0.38 mL·kg⁻¹·h⁻¹. The maximal
PBZ concentrations after the 2, 4, and 8 mL i.m. administrations were 42 ± 1.4, 74 ± 3.6 and 119 ± 7.4 μg/mL respectively and were observed at 4, 2, and 6 h in mean pharmacokinetic profiles. Individual Tmax values ranged from 1 to 8 h, 1 to 6 h, and 4 to 10 h after the 2, 4, and 8 mL i.m. administrations. The bioavailability of PBZ after i.m. injections of 2, 4, and 8 mL was comparable for all 3 volumes, i.e., 100 ± 13.1%, 96 ± 7.6% and 100 ± 7.1%, respectively.

The terminal half-lives for i.v. and i.m. administrations of 2, 4, and 8 mL were 20.0, 16.3, 18.0 and 19.5 h respectively.

**DISCUSSION**

Several components of drug formulation and mode of administration have been studied and certain formulation-dependent factors identified that induce muscle damage after i.m. administration. Oily vehicles are better tolerated than aqueous ones (Dickson et al., 1986). Other factors affecting the extent of muscle damage include the concentration of drug and vehicles, and the volume injected: the greater the volume injected or the concentration of the formulation, the greater the extent of local damage (Nouws, 1984). The drug itself may be an irritant: about 70% of 107 different injectable drugs were shown to have cytotoxic properties in humans (Oshida et al., 1979). Finally, there is no simple way

**Table 1.** Area under the plasma creatine kinase (CK) activity vs. time curve (AUC, U·min/L), observed peak plasma CK activity (Cmax, U/L), and observed time to peak plasma CK activity (Tmax, h) after a single i.m. injection of 2, 4, or 8 mL of a 20% phenylbutazone formulation in 6 ewes

<table>
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<th>Variable</th>
<th>Volume of i.m. phenylbutazone, mL</th>
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<td>AUC, U·min/L</td>
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Tolerance and extent of muscle absorption

Figure 3. Mean plasma profiles (±SEM) of different phenylbutazone (PBZ) concentrations in 6 ewes. Upper graph: i.v. bolus administration of PBZ at a dose level of 8 mg/kg. Lower graph: single i.m. administration of 2, 4, or 8 mL (5.2, 10.5, or 21.3 mg/kg, respectively) of a 20% PBZ formulation.

Using the pharmacokinetic analysis of plasma CK to quantitatively assess muscle damage has already been proposed (Lefebvre et al., 1994, 1996). The major advantage of this approach is that it is minimally invasive and enables repeated assessments to be performed in the same animal. It also provides a quantitative estimate, which is independent of the operator, in contrast to the pathological approaches based on macroscopic scoring systems and ultrasonographic measurements (Ferré et al., 2001). Although postinjection muscle damage has already been evaluated by determining plasma and serum CK activities at arbitrarily spaced sampling times, these single measurements cannot be used to compare drug formulations. In contrast, the peak plasma CK activity and the AUC of plasma CK vs. time after i.m. injection can be determined by repeated blood sampling. However, these parameters do not provide any information about the amount of CK released or the actual extent of the lesion. The only way to express an AUC as an equivalent amount of muscle damage is to use a pharmacokinetic approach that requires knowing the plasma CK clearance, the systemic availability of CK from muscle, and the AUC of CK activity after the i.m. administration. The equivalent mass of muscle that has been totally depleted of its CK content can then be calculated from the amount of CK released and the CK muscle content (for a review, see Lefebvre et al., 1996). One limit of the current study is that the individual pharmacokinetic parameters were not determined in the tested animals. However, calculation of muscle damage was based on an equation that we had previously validated under the same conditions (adult Lacaune ewe, administration in the longissimus lumborum muscle, same CK assay, Ferre et al., 2001).

For the past 10 yr, this approach has been proposed for the assessment of postinjection damage in cattle (Lefebvre et al., 1994), dogs (Aktas et al., 1995b), horses (Volfinger et al., 1994; Toutain et al., 1995), and sheep (Houpert et al., 1995; Ferré et al., 2001). The equivalent amount of muscle damaged after i.m. administration of different drug formulations in dogs, for example, could vary from 0.04 to 0.30 g/kg of BW (Aktas et al., 1995a). However, the accuracy of this noninvasive approach, i.e., comparison of the equivalent of muscle damaged (estimated from the CK release) with the surrogate endpoint (i.e., the true amount of muscle damage assessed by macroscopic planimetric evaluation) has only recently been validated in sheep (Ferré et al., 2001).

Phenylbutazone, a nonsteroidal antiinflammatory drug, is not commercially available for use in sheep but was used here as a test article because of its very poor local tolerance. The adverse local effects of an i.m. administration of PBZ have previously been documented in rats (Kienel, 1973), horses (Toutain et al., 1995), and sheep (Houpert et al., 1995). The tolerance of i.m. PBZ administration in rats was assessed after euthanasia, using a morphological approach (Kienel, 1973), whereas the same CK pharmacokinetic approach described here to predict the local tolerance of a formulation from its individual components.

Some local tolerance studies in sheep have been published for oxytetracycline (Nouws et al., 1990), vitamins (Behrens et al., 1975; Dickson et al., 1986), chloramphenicol (Dagorn et al., 1990), antibabesial drugs (Mitema, 1985), and PBZ (Houpert et al., 1995). Although the economic impact of postinjection muscle damage (blemishes of the meat and fleece) has not been evaluated in sheep, it is probably not negligible. Sheep were selected in the current study for several reasons: i) numerous i.m. formulations are available for use in this food animal species, ii) the CK pharmacokinetic approach for assessment of postinjection muscle damage has been validated in this species (Ferré et al., 2001), and finally iii) sheep provide a useful animal model for testing drug tolerance. Sheep are easy to handle, blood volume is not a limiting factor for pharmacokinetic studies, and the basal CK activity is stable (Houpert et al., 1995), in contrast to pigs (Steiness et al., 1978) and rabbits (Lefebvre et al., 1992). However, the feasibility of extrapolating data obtained in sheep to other species remains questionable. The i.m. administration of a 10% oxytetracycline formulation, for example, was less tolerated in calves and pigs than in sheep (Nouws et al., 1990).
was used in horses (Toutain et al., 1995) and sheep (Houpert et al., 1995).

In horses, an equivalent of 0.044 and 0.118 g/kg of muscle was destroyed in the neck and gluteal muscles, respectively, after a single administration of the same PBZ formulation (phenylarthritis) at a dose level of 8.8 mg/kg. In the study by Houpert et al. (1995) in sheep, the nominal dose of PBZ was 8 mg/kg and the concentrations of the formulation was 100 mg/mL, which corresponds to an administered volume of 6 mL for a 75-kg sheep. The equivalent amount of muscle damaged after an i.m. administration of PBZ was 0.037 g of muscle/kg of BW. In comparison, the average damage (0.51 g/kg) after a 2-mL i.m. administration was about 14-fold greater in our study. This discrepancy may result from the equation used to calculate the amount of damage from the AUC. In the equation used by Houpert et al. (1995), AUC was multiplied by a coefficient that was 3 times lower than the one we selected from a previous study in very similar conditions to those described here (Ferre et al., 2001). This coefficient takes into account the CK content of the injected muscle, which depends on the anatomical site. The injection site in the study by Houpert et al. (1995), was the gluteal mass (CK content of about 5,114 U/g) (Houpert et al., 1995) which is greater than that of the longissimus lumborum muscle (approximately 2,300 U/g; Ferré et al., 2001). The tested formulations were also different. The formulation used in the current study was twice as concentrated and contained different vehicles, whereas the formulation used by Houpert et al. (1995) only contained PBZ dissolved in sterile water. In pigs, 20% oxytetracycline formulations caused more local irritation than 10% formulations (Nouws, 1984). These differences in tolerance of the same active substance at least confirm that the local tolerance of a new formulation cannot be assessed by extrapolating data obtained with another formulation of the same drug. Another interesting finding in both studies was that the extent of muscle damage showed extremely large interindividual variability, thus emphasizing the non-negligible role of individual sensitivity in the development of postinjection muscle damage.

The pharmacokinetic parameters for PBZ after i.v. and i.m. injections have been published (Houpert et al., 1995; Cheng et al., 1998). The average plasma clearance after i.v. injection ranged from 4.6 (Cheng et al., 1998) to 14.4 mL·kg⁻¹·h⁻¹ (Houpert et al., 1995). The elimination half-life was about 11 (Houpert et al., 1995) and 18 h (Cheng et al., 1998). The plasma clearance and elimination half-life determined in the current study were similar to those obtained by Cheng et al. (1998). The discrepancy with the results obtained by Houpert et al. (1995) could result from the differences in formulation composition. The peak concentration after i.m. injection of PBZ at a dose of 8 mg/kg was 47 μg/mL and was obtained about 30 min after administration (Houpert et al., 1995). This peak value is consistent with our results. The shorter Cmax observed by Houpert et al. (1995) could also be due to the different formulation tested. The terminal half-life determined after i.m. injection was similar to that observed after i.v. administration, as previously described in another study (Houpert et al., 1995) and indicated the absence of a flip-flop process, i.e., longer elimination half-life after i.m. administration due to delayed absorption. The OPBZ data were not analyzed as the AUC ratios of OPBZ to PBZ were very small, as in a previous study (Cheng et al., 1998).

One of our main objectives was to see if the volume of formulation injected affected muscle bioavailability as a result of the increased muscle damage. Previous studies using CK pharmacokinetic analysis only evaluated the postinjection muscle damage after administration of a fixed volume of a given drug formulation. In this study, different volumes of the test formulation were injected and the corresponding lesions evaluated on the same animals for the first time. Muscle PBZ bioavailability was reported to be absolute in sheep (Houpert et al., 1995), as observed here whatever the volume of PBZ formulation administered. The effect of the volume administered by i.m. injection on drug bioavailability has not been documented in sheep. The effect of this volume on local damage has been investigated in humans (Sidell et al., 1974) and animals (Newton et al., 1975; Nouws, 1984; Diness, 1985) for different pharmaceutical compounds. For example, tissue damage was proportional to the injection volume between 0.25 and 1 mL in rabbits and between 0.5 and 3 mL in pigs (Diness, 1985). However, very few studies have investigated the influence of local tissue irritation on the pharmacokinetics of a drug and especially its bioavailability from the injection site. Previous studies (Nouws, 1984; Luthman et al., 1988) suggest an inverse relationship between the extent of muscle damage and drug bioavailability. In pigs, the products that most irritated the tissues, such as the penicillin G preparations, were shown to give lower maximum levels in serum (Luthman et al., 1988), and oxytetracycline residues increased with the extent of local tissue damage (Nouws, 1984). In these studies, increased tissue damage was, in fact, induced by increasing the concentration of the formulation or by the presence of irritant vehicles, but not by changing the volume. It might also be assumed that because i.m. administration of a greater volume of the same formulation increased post-injection muscle damage, it might also alter drug bioavailability. The bioavailability of an oxytetracycline formulation in ruminant calves was slightly lower (about 15%) at the injection site where local irritation was severe (Nouws and Vree, 1983). In contrast, i.m. administration of diazepam formulations that caused a 10-fold difference in the degree of local muscle damage did not seem to affect bioavailability of the drug in rabbits (Brazeau and Fung, 1990). Our results confirm these latter findings. The muscle bioavailability was not influenced by the volume of PBZ formulation injected. Moreover, the corresponding amount of muscle damage, although showing large interindividual variabilities,
tended to be proportional to the volume injected. Further investigations are, however, required to assess this issue more adequately. Although these results cannot be extrapolated to other drug formulations, they confirm that the pharmacokinetic analysis of CK release provides a minimally invasive and quantitative means of evaluating postinjection muscle damage and may enable pharmaceutical companies to simultaneously investigate effects of formulation-dependent factors, such as volume, on local tolerance and drug bioavailability during initial stages of drug development.

**LITERATURE CITED**


