Cephalotin Penetration into Muscle Tissue Fluid and Muscle Tissue

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SUMMARY. The aim ot fhe present work was to compare the penetration in muscle tissue fluid (MTF) and muscle tissue (MT) of cephalotin administered in rabbits. Animals were distributed in Trial 1 (*n* = 15) and Trial 2 (*n*= 6). In the Trial 1 the animals were implanted in ischiotibial muscle with non reactive material cages. Animals recibed subcutaneously (sc) cephalotin (20 mg/kg) and blood, MTF and MT samples were recollected. In the Trial 2 the animals recibed cephalotin (20 mg/kg) intravenously (iv) and blood samples were recollected. Pharmacokinetic analysis were performed using a non-compartmental model. Results: terminal disposition rate constant (λ_z) (serum iv) 1.43 ± 0.54, (serum sc) 1.90, (MTF) 0.57 and (MT) 1.80 h^{-1}; elimination half-life ($t_{1/2}$) (serum iv) 0.53 ± 0.13, (serum sc) 0.36, (MTF) 1.20 and (MT) 0.38 h; the area under the curve [$AUC_{(0-6)}$] (serum iv) 60.40 ± 38.20, (serum sc) 45.90, (MTF) 21.60 and (MT) 3.00 μg.ml^{-1}.h. and the bioavailability (F) was 76%. The penetration of cephalotin in MTF and MT were 47.70 and 6.43%, respectively. Cephalotin administered subcutaneously in rabbit presented higher bioavailability, distribution in extracellular fluid and low penetration in muscle tissue.

RESUMEN. "Penetración de Cefalotina en Líquido Tisular de Músculo y Tejido Muscular". Los objetivos del presente trabajo fueron comparar la penetración in líquido tisular de músculo (MTF) y tejido muscular (MT) de cefalotina administrada a conejos. Los animales fueron distribuidos en Experimento 1 (*n* = 15) y Experimento 2 (*n* = 6). En el Experimento 1 los conejos fueron implantados en tejido muscular con cajas confeccionadas con material no reactivo. Posteriormente recibieron subcutáneamente (sc) cefalotina (20 mg/kg) y se recolectaron muestras de sangre, MTF y MT. En el Experimento 2 los animales recibieron cefalotina (20 mg/kg) vía endovenosa (iv) y se recolectaron muestras de sangre. El análisis farmacocinético fue realizado utilizando un modelo no compartimental. Resultados: constante de velocidad de eliminación (λ_z) (suero iv) 1,43 ± 0,54, (suero sc) 1,90, (MTF) 0,57 y (MT) 1,80 h^{-1}; tiempo medio de eliminación ($t_{1/2}$) (suero iv) 0,53 ± 0,13, (suero sc) 0,36, (MTF) 1,20 y (MT) 0,38 h; área bajo la curva [$AUC_{(0-6)}$] (suero iv) 60,40 ± 38,20, (suero sc) 45,90, (MTF) 21,60 y (MT) 3,00 μg.ml^{-1}.h y biodisponibilidad (F) 76%. La penetración de cefalotina en MTF y MT fue de 47,70 y 6,43%, respectivamente. La cefalotina administrada subcutáneamente en conejos presentó alta biodisponibilidad, distribución en líquido extracelular y baja penetración en tejido muscular.

INTRODUCTION

Cephalosporins are antibiotics chemically related to penicillins. Both have in common a betalactamic ring as part of their structure. These antibiotics interfere with the normal synthesis of the bacterial cell wall and are effective against the majority of the Gram-positive cocci and some Gram-negative bacilli such as *Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae* [1].

Cephalotin is a first-generation cephalosporin whose pharmacokinetics have been studied in various animal species [2-4] as well as in humans [5,6]. Overall this antibiotic is poorly absorbed in the digestive tract and thus has to be adminis-

tered parenterally to obtain a systemic effect. It is distributed mainly in the extracellular fluid and excreted by the kidneys [1].

The implantation of cages in animals was performed for the first time by Chisholm *et al.* [7] in order to determine the concentration of antibacterial agents into interstitial tissue fluid. This method has further been used to study the mechanisms and courses of the inflammatory responses in tissues and the distribution of drugs [8-10]. There is information about the distribution of betalactamics in muscle tissue fluid [11]. However, there is no bibliographical data on the concentration-time course of cephalotin in muscle tissue fluid, and on how those concentra-

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tions resemble the ones found in muscle tissue. The aim of the present work was to compare the penetration of cephalotin into muscle tissue fluid and muscle tissue, when administered parenterally to rabbits.

MATERIALS AND METHODS
Animals

Twenty-one New Zealand White male rabbits weighing approximately 2 kg. were obtained from the Department of Introduction to Animal Production, Faculty of Agricultural and Forestry Sciences of La Plata University and distributed at random in Trial 1 (n = 15) and Trial 2 (n = 6). Animals were kept in individual cages on a climate-controlled environment and fed with a commercial diet and water ad libitum. The research protocol was approved by the Commission of Scientific Research of the Province of Buenos Aires.

Trial 1

Implantation of the cages in muscle tissue for the collection of muscle tissue fluid (MTF)

Thirty-nine animals were implanted in the ischiotibial muscle non-reactive material cages. Each animal had one cage implanted intramuscularly (semitendinous muscle, left or right). The cages consisted of Silastic (Dow Corning, Midland, MI) rubber tubes, 20.0 mm long and 5.0 mm inner diameter, closed at one end, with 40% of its surface perforated by 1.0 mm holes. For this purpose, the area of the ischiotibial muscle was shaved, washed and disinfected. Implantation sites were anesthetized with Lidocaine 2% and cages were inserted into the muscle, through a skin incision in the mentioned areas.

The percentage of protein binding in MTF fluid was calculated by comparation of antimicrobial activity of cephalotin in presence and absence of protein [12]. For that, were compared the halos of inhibition of standard curves of cephalotin in MTF and buffer.

Biochemical determinations

Muscle tissue fluid and blood/serum samples were collected at days 15, 30 and 60 after the implantation of the cages and postadministration of antibiotic. Samples were analyzed for total proteins by the Biuret method and for albumin by binding sulfobromophthalein in order to determine the reaction to the implanted material.

Antibiotic, route of administration and doses

Animals with stabilized cages received subcutaneously a single dose of cephalotin (20 mg/kg). Blood samples (0.2 ml each one) were obtained (three animals at a time and one sample per animal) by direct venipuncture of the saphenous vein [13] immediately before the administration of the drug and at 0.08, 0.17, 0.25, 0.5, 1.0, 2.0, 4.0 and 6.0 h postadministration of the antibiotic.

Sampling of MTF and muscle tissue

Immediately after taking the blood samples, the animals were sacrificed by cervical dislocation, then samples of MTF and muscle tissue (semitendinous muscle oposited to the implanted muscle) were taken starting at 0.25 hours postadministration of the antibiotic, in equal times to the blood sampling.

Processing and preservation of the samples

Blood samples were allowed to clot and then centrifuged at 3000 g for 15 min in order to separate the serum. Muscle tissue samples were weighted, diluted in phosphate-buffered saline solution and homogenized with a hand homogenizer. All the samples were stored in individual, sterile recipients at -18 °C, until being analyzed.

Trial 2

Blood samples were obtained at the same time and by the same method as in Trial 1, in order to make the biochemical determinations.

Antibiotic, route of administration and doses

Animals received a single intravenous dose of cephalotin (20 mg/kg) (left saphenous vein) and blood samples (0.2 ml each one) were collected in each animal by direct venipuncture of the right saphenous vein at 0.08, 0.17, 0.25, 0.5, 1.0, 2.0, 4.0 and 6.0 h postadministration of the antibiotic. The blood samples were processed and stored in the same way as in Trial 1.

Quantification of the antibiotic

The concentrations of cephalotin in serum, MTF and muscle tissue were determined by means of the microbiological assay technique, using *Bacillus stearothermophilus* var. *calidolactis* as the test organism [14]. The antibiotic solutions used as standard lines in serum, MTF and muscle tissue were prepared in pooled normal rabbits serum, serum diluited 1:2 in phosphate-buffered saline solution and muscle tissue homogenate supernatant diluted 1:2 in phosphate-buffered saline solution (obtained by centrifugation at 3000 g for 15 min), respectively. The correlation coefficient for the standard curves prepared for all experiments was greater than 0.98. The coefficients of variation intra- and inter- assays were lower than 8% and the limit of sensitivity of the assay was 0.01 µg/ml. The pharma-

cokinetic parameters λ_z, and $t_{1/2}$ in MTF and muscle have not been statistically assessed because they were calculated from the concentrations-time means of each group of animals.

Pharmacokinetics

The following pharmacokinetic parameters were determined. The apparent terminal half-life ($t_{1/2}$) and the area under the serum concentration-time curve (AUC_{0-6}) were estimated by noncompartmental analysis with WinNonlin software (version 4.1; Pharsight Corp., Cary, NC).

The percentage of bioavailability (F) was calculated by using the Eq. [1]

$$F = \frac{(AUC_{(0-6)} \; sc)}{(AUC_{(0-6)} \; iv)} \times 100 \qquad [1]$$

The penetration (P) of cephalotin into MTF and muscle tissue (MT) was calculated by the Eq. [2]

$$P = \frac{[AUC_{(0-6)} \; (MTF \; or \; MT)]}{(AUC_{(0-6)} \; serum)} \times 100 \qquad [2]$$

In order to compare the serum and muscle tissue concentrations, the linear regression analysis was employed. The pharmacokinetic data (AUC_{0-6}), the interaction between the serum and muscle tissue concentrations and the biochemical results, were analyzed by means of variance analysis (ANOVA).

RESULTS AND DISCUSSION

Since 15 days untill 60 days postimplantation of the cages to collect MTF were considered as stabilized and the total average protein concentration in MTF was lower to 55% of that obtained in serum samples.

The total protein and albumin levels in serum and MTF at 1, 3 and 6 h postadministration of cephalotin were statistically similar (see Table 1).

The cephalotin percentage of protein binding in MTF was 30%. The time-concentration (means ± standard desviation) values and pharmacokinetic variables in serum, MTF and muscle tissue of cephalotin administered parenterally to rabbits are presented in Figure 1 and Table 2, respectively.

During the period from 0.5 to 4 h after the administration of cephalotin by intravenous and subcutaneous routes, the antibiotic levels in serum were 33.21 ± 26.20 to 0.68 ± 0.40 and 64.11 ± 11.20 to 0.07 ± 0.04 µg/ml, respectively.

Meanwhile, the concentrations of cephalotin in MTF and muscle tissue during the periods from 0.5 to 6.0 h and from 0.5 to 4.0 h were 11.51 ± 6.60 to 0.52 ± 0.42 and 4.60 ± 0.17 to 0.01 µg/ml, respectively.

The half-life of elimination values in serum of cephalotin administered by intravenous and subcutaneous routes to rabbits (0.53 ± 0.13 and 0.36 h, respectively) were similar to those obtained by Bush *et al.* [3] in avian species (from 16 to 54 min) and humans obtained by Kirby & Regamey [5] (from 28 to 51 min) and Barza *et al.* [6] (0.34 h) and greater than the obtained by Rule et al. [4] in goats (0.2 h) and Bergeron *et al.* [2] in rabbits (15.8 min).

The half-lives of elimination of cephalotin in MTF and tissue muscle were 1.20 and 0.38 h, respectively. The higher value in MTF could be explained by means of this formula: SA/V to assess the concentrations of drugs in extracellular fluid [15], where SA is the surface of the available area for the diffusion of the drug and V (volume) represents the distance of diffusion. The low relation between the surface area and the volume of the tissue cages used in our work, could account for the artificial increase in the half-life obtained in MTF compared to the values in serum. The half-lives in serum and muscle tissue were similar, which would indicate a relatively strong relationship between serum and muscle tissue concentrations with equal correlation coefficients (0.901). The P value is lower than 0.01 and there is a statistically significant correlation between concentrations at the 99% confidence level.

Penetration of cephalotin in MTF and muscle tissue were 47.70 and 6.43% of the values obtained in serum, respectively. On the other hand, the obtained volume of distribution of cephalotin (245.60 ± 78.95 ml.kg^{-1}) in our work, would be indicating that its pasagge is mainly realized into extracellular fluid and scarcely to muscle tissue.

Considering that only the free cephalotin in serum and MTF is distributed in muscle tissue,

Parameter	Concentration (g/dl) in serum/ MTF		
	1 h	**3 h**	**6 h**
Total protein	4.9/2.6	5.0/2.9	4.4/2.5
Albumin	3.7/1.6	4.3/1.5	4.0/1.6

Table 1. Mean total protein and albumin concentrations in serum and muscle tissue fluid (MTF) at different times postadministration of cephalotin.
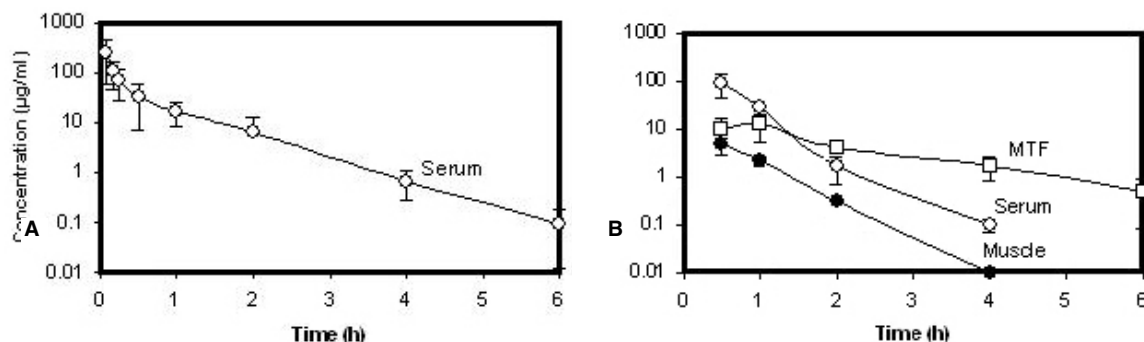
Figure 1. Semilogarithmic plot of cephalotin concentrations (means ± 1 SD) in serum after intravenous administration (**A**) and serum, muscle tissue fluid (MTF) and muscle tissue postadministration by subcutaneous route (**B**) to rabbits.

Pharmacokinetic parameters (units)	Serum (iv) (mean ± SD)	Serum (sc) (mean)	MTF (sc) (mean)	MT (sc) (mean)
λ_z (h^{-1})	1.43 ± 0.54	1.90	0.57	1.80
$t_{1/2}$ (h)	0.53 ± 0.13	0.36	1.20	0.38
V_z (ml.kg^{-1})	245.60 ± 78.95	-	-	-
AUC_{0-6} (µg.ml^{-1}.h)	60.40 ± 38.20	45.90	21.60	3.00
F (%)	-	76.00	-	-
Penetration (%)	-	-	47.70	6.43

Table 2. Pharmacokinetic parameters in serum, muscle tissue fluid (MTF) and muscle tissue (MT) of cephalotin administered by intravenous (iv) and subcutaneous (sc) routes to rabbits. SD: standard deviation; λ_z: terminal disposition rate constant; $t_{1/2}$: elimination half-life; V_z: volume of distribution; AUC_{0-6}: area under the curve; F: bioavailability.

and its binding to serum proteins and MTF in rabbits is 50 [2] and 30%, respectively. The obtained concentrations represented approximately 100 and 20% of antibiotic, respectively.

In conclusion, as cephalotin is a lipophilically-poor drug and has a low distribution its passage to the intracellular fluid becomes restricted; so, if we take into account the concentrations found in extracellular fluid when considering the values in muscle tissue, we can erroneously estimate the concentrations in this tissue.

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