Pharmacokinetics and penetration into tissue fluid of ceftizoxime in normal and hyperthermic sheep

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Abstract

The pharmacokinetics of ceftizoxime was studied in six sheep before and after inducing hyperthermia using Escherichia coli endotoxin. Sheep implanted subcutaneously with cages of non-reactive material for collecting tissue cage fluid (TCF) were used to conduct two trials. In Trial 1 animals with normal basal temperature (normal sheep (NS)) were given intravenous (i.v.) and intramuscular (i.m.) monodoses of ceftizoxime (20 mg/kg BW) at 1 week interval. One and 5 weeks later (Trial 2) each sheep were injected 1 mg/kg BW of endotoxin to produce hyperthermia (hyperthermic sheep (HS)) previously to i.v. administration (HSi.v.) and i.m. (HSi.m.) of ceftizoxime (20 mg/kg BW), respectively. Serum and TCF samples were collected over 6 h post-administration. Ceftizoxime concentrations in serum and TCF were determined by a microbiological assay. The concentrations in serum and TCF of ceftizoxime were analyzed through compartmental and non-compartmental models.

Rectal temperature were significantly incremented in all animals during Trial 2. The half-time and constant of elimination in serum of ceftizoxime in NSi.v. ($t_{1/2} = 1.1 \pm 0.4 \text{ h}; \ z = 0.7 \pm 0.2 \text{ h}^{-1}$) were statistically different those observed in HSi.v. ($t_{1/2} = 1.4 \pm 0.4 \text{ h}; \ z = 0.5 \pm 0.2 \text{ h}^{-1}$). The constants of distribution in NSi.v. and HSi.V were $5.1 \pm 4.6$ and $4.1 \pm 3.4 \text{ h}^{-1}$, respectively. The time to reach the maximum concentrations in TCF was latter ($p < 0.05$) in NS ($t_{\text{max}} = 2.3 \pm 0.7 \text{ h}$) than in HS ($t_{\text{max}} = 1.3 \pm 0.6 \text{ h}$). After i.m. administration in NS the absorption half-life (0.12 ± 0.19 h) was latter ($p < 0.05$) than in HS (0.06 ± 0.007 h) with greater areas under the curve (AUC in NS = 65.4 ± 20.8 and AUC in HS = 34.7 ± 7.5 (mg/ml) h). The maximum value of concentration in serum ($C_{\text{max}}$) and AUC in TCF were greater ($p < 0.05$) in NS ($C_{\text{max}} = 46.1 \pm 10.6 \text{ mg/ml}$ and AUC = 84.4 ± 17.4 (mg/ml) h) as compared to same HS ($C_{\text{max}} = 27.0 \pm 12.9 \text{ mg/ml}$ and AUC = 47.9 ± 3.9 (mg/ml) h). The concentrations of ceftizoxime in TCF after i.v. and i.m. in NS and HS were elevated during a 6 h period after administration. The bioavailability of ceftizoxime in NS (101.6 ± 59.9%) and HS (87.4 ± 63.3%) was suitable for its use by the i.m. route. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ceftizoxime is a third-generation cephalosporin, active against Gram-negative microorganism and characterized by its low toxicity and stability to β-lactamases. (Caprile, 1988).

The pharmacokinetic properties of ceftizoxime have been studied in human beings (Nakashima et al., 1981; Dubb et al., 1982) and animals (Murray et al., 1980). Despite there is information about the effect of fever on the behaviour of some antibiotics (Pennington et al., 1975; Wilson et al., 1983 1984; Jernigan et al., 1988; Fauvelle et al., 1994), it is still unknown the pharmacokinetic behaviour and its passage to tissular fluid of ceftizoxime in sheep with and without hyperthermia. The purpose of the present investigation was to study the pharmacokinetics and the tissue penetration of ceftizoxime administered by intravenous and intramuscular routes in sheep with and without hyperthermia.

2. Materials and methods

The experiment was conducted in two trials. Each trial was divided in two parts as follows: Trial 1 was performed using sheep without hyperthermia (NS), while Trial 2 was performed using hyperthermic sheep (HS). Within each trial the first part of it was designed to study pharmacokinetics and tissue penetration after intravenous (i.v.) route of administration of ceftizoxime, while the second part was conducted to study the pharmacokinetics and tissue penetration after intramuscular (i.m.) route of administration of ceftizoxime.

2.1. Animals

Six healthy sheep, Corriedale breed, weighing 45 ± 13 kg were implanted subcutaneously in the flanks, ribs, and neck areas with six cages of Silastic to collect tissue cage fluid (TCF) (Rule et al., 1994).

Tissue cage fluid and blood serum samples were collected once a week during 6 weeks after implantation. Samples were analysed for total protein (by the Biuret reaction method) and albumin (by binding sulfobromophthalein) to determine the body reaction to implanted material and stability of the composition of TCF.

2.2. Trial 1

2.2.1. Doses and routes of antibiotic administration

In the first part of the trial, monodose of ceftizoxime (20 mg/kg BW) by i.v. route (right jugular vein) was administered to NS. After 1 week, the second part of the trial was conducted administering through i.m. route (isqiotibial muscles) to the same group of sheep a monodose of ceftizoxime (20 mg/kg BW).

2.2.2. Sampling

After i.v. and i.m. administration blood samples were collected (5 ml each one) from the left jugular vein at 0.083, 0.17, 0.25, 0.5, 1, 2, 3, 4, 5 and 6 h post-administration of the antibiotic. Tissue cage fluid samples were collected (approximately 0.5 ml each one) starting at 0.25 h and following the blood sampling strategy.

2.2.3. Processing and preservation of samples

Blood samples were allowed to clot and serum was obtained by centrifugation at 1500 × g. Serum and TCF were frozen at −18°C until quantification of antibiotic concentration.

2.2.4. Body temperature

Rectal temperature of sheep was recorded previous and after antibiotic administration following the strategy of the blood sampling.

2.3. Trial 2

After 1 week from Trial 1 was completed, in order to start with the first part of Trial 2 hyperthermia was provoked injecting i.v. 1 µg/kg BW of Escherichia coli 0.27:B8 Lipopolysacharide (LPS) (Sigma Chemical, USA). One hour later, the antibiotic administration scheme was followed as for the first part of Trial 1.

After 4 weeks of being clinically normal, animals were used to complete the second part of Trial 2. Hyperthermia was provoked following the same protocol as for the first part of the Trial 2 and the antibiotic administration was conducting following the same protocol as for the second part of the Trial 1.

Sampling and collection strategy, processing, and preservation of samples was performed as describe for Trial 1.
2.3.1. Quantification of the antibiotic

Concentrations of ceftizoxime in serum and TCF were determined by a microbiological assay (Lennette, 1987) using *Bacillus subtilis* strain ATCC 6633. The limit of quantification was 0.25 µg/ml. The correlation coefficients for the regression lines of the standard solutions were not less than 0.995. The intra-assay and inter-assay coefficients of variation were 3.8 and 5.4%, respectively.

2.3.2. Pharmacokinetic study

The ceftizoxime concentration data were used to perform a pharmacokinetic analysis following an interactive and weighted-non-linear least-squares regression analysis (Metzler and Tong, 1981). The Akaike information criterion (AIC) (Akaike, 1978) was used to determine the compartmental model best adapted to the data set. The concentration-time data were fitted a biexponential equation according to an open two-compartment model for i.v. administration and an open one-compartment model for i.m. administration and the concentration in TCF (Gibaldi and Perrier, 1982). Hybrid constant $C_1$ and $C_z$ (extrapolation at zero time of the experimental terms), the slope of the rapid phase of distribution ($\lambda_1$) and the slope of elimination phase ($\lambda_2$) were used to calculate the rate constants from the central to peripheral compartment ($k_{12}$) and vice versa ($k_{21}$) and the rate constants of elimination ($k_{10}$) (Gibaldi and Perrier, 1982). The apparent volume of the central compartment ($V_C$) was calculated from the following equation:

$$V_C = \frac{\text{Dose (µg/ml)}}{C(0)}$$

where the concentration at zero time $C(0) = C_1 + C_z$ in a two-compartment open model. The distribution volume at steady state ($V_{ss}$) and the volume of distribution by the area method ($V_z$) were calculated as follows:

$$V_{ss} = \left( \frac{k_{12} + k_{21}}{k_{21}} \right) V_C$$

$$V_z = \frac{\text{Dose (µg/ml)}}{\lambda_2 \text{ AUC}}$$

(Ritschel, 1986), respectively. The apparent volume of the central compartment ($V_C$) was calculated from the following equation:

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where the concentration at zero time $C(0) = C_1 + C_z$ in a two-compartment open model. The distribution volume at steady state ($V_{ss}$) and the volume of distribution by the area method ($V_z$) were calculated as follows:

$$V_{ss} = \left( \frac{k_{12} + k_{21}}{k_{21}} \right) V_C$$

The absorption (0.693/absorption rate constant ($k_a$)), the rapid distribution (0.693/$\lambda_1$) and the elimination (0.693/$\lambda_2$) half-lives ($t_{1/2}$) were calculated using equations given by Gibaldi and Perrier (1982).

The i.v. and i.m. serum and TCF data were also analyzed by using non-compartmental methods (Ritschel, 1986). The mean residence time (MRT) was calculated according to the equation:

$$MRT = \frac{\text{AUC}}{\text{AUMC}}$$

where AUC is the area under the concentration versus time curve and AUMC is the area under the first moment curve of the product of time and serum or TCF drug concentration versus time from time zero to infinity. Both, were calculated by the trapezoidal method and extrapolated to infinity from the last measured concentration (Gibaldi and Perrier, 1982).

The penetration (P) in TCF and the bioavailability (F) of ceftizoxime were assessed using the following equations:

$$P_{TCF} = \frac{\text{AUC (i.m.)}}{\lambda_2 \text{ (i.m.)}} \frac{\text{AUC (i.v.)}}{\lambda_2 \text{ (i.v.)}}$$

(Baggot, 1977).

2.4. Statistical analysis

Paired *t*-test was used to obtain the significance of the differences between mean values.

3. Results

At the 6 week post-implantation of the cages the total average of protein concentrations in TCF was lower (56%) than those obtained in serum samples. At that point implanted cages in sheep were considered to be stable and ready to be used in studies to evaluate the pharmacokinetics and the penetration in the tissular fluid of ceftizoxime. Rectal temperature of the sheep during Trial 1 ranged from 39.2–39.5°C and ranged from 39.2–39.4°C after i.v. and i.m. injection of antibiotic, respectively. There was statistically lower temperature ($p < 0.05$) during 5 h post-administration of the antibiotic compared with result from Trial 2 (see Fig. 1).
The serum and TCF concentration (mean ± 1 SD) of ceftizoxime administered i.v. and i.m. in HS and NS are shown in Fig. 2.

Data concerning the pharmacokinetics and the penetration into TCF (mean ± SD) of ceftizoxime administered by i.v. and i.m. route during Trial 1 and 2, based on compartmental and non-compartmental analysis, are presented in Tables 1 and 2, respectively.

3.1. Intravenous administration

After i.v. administration of ceftizoxime, the elimination \( (t_{1/2} \text{ and } \lambda_z) \) in NS \( (t_{1/2} = 1.1 \pm 0.4 \text{ h} \) and

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**Fig. 1.** Thermic curve (mean) after administration of ceftizoxime

**Fig. 2.** Semi-logarithmic plot of ceftizoxime concentrations (means ± SD) in serum and tissue cage fluid (TCF) versus time after intravenous (i.v.) and intramuscular (i.m.) administration in sheep with normal rectal temperature (NS) (A) and same animals with hyperthermia (HS) (B).
3.2. Intramuscular administration

Pharmacokinetic disposition of ceftizoxime in sheep following a one-compartmental open model with maximum value of concentration of antibiotic in serum were statistically greater ($p < 0.05$) in NS ($C_{\text{max}} = 46.1 \pm 10.6 \mu g/ml$) as compared to the same HS ($C_{\text{max}} = 27.0 \pm 12.9 \mu g/ml$). However, the AUC and absorption half-life ($t_{1/2a}$) were also greater ($p < 0.05$) in NS (AUC = 65.4 ± 20.8 (μg/ml) h and $t_{1/2a} = 0.12 \pm 0.19$ h) in comparison to the HS (AUC = 34.7 ± 7.5 (μg/ml) h and $t_{1/2a} = 0.06 \pm 0.007$ h). The AUC in TCF were different ($p < 0.05$) in NS (AUC = 47.9 ± 3.9 (μg/ml) h) compared to the same HS (AUC = 47.9 ± 3.9 (μg/ml) h).

4. Discussion

Using Akaike’s information criterion (AIC) in HS and NS serum concentration versus time data curve best fitted a open two-compartment model, same as those in animals (dogs, mice, rats and monkeys) (Murakawa et al., 1980) and in human beings (Nakashima et al., 1981; Dubb et al., 1982).
The behaviour and the response to elevated temperatures in sheep after the injection of endotoxin were similar to those previously reported for sheep (Wilson et al., 1984). As it was described before by this author, the rectal temperature diminution to baseline in the trial where the animals received endotoxin allowed to study the distribution phase taking into account that the distribution equilibrium should be completed within five distribution half-lives.

Pharmacokinetic variables presented in NS and HS after i.v. administration of ceftizoxime showed that maximum concentrations in the tissular fluid were faster and with lower distribution constant and clearance total in HS that modified the slope and the half-time elimination in serum of the HS contrasting with results from the NS. The mean half-life of ceftizoxime i.v. administered in HS (1.4 h) was similar to that found in humans (1.4 h) (Nakashima et al., 1981; Dubb et al., 1982) and greater to those animals of the Trial 1 (NS) (1.1 h) and also with values found by Murakawa et al. (1980) in dogs (1.06 h), mouse (0.267 h), rat (0.333 h) and monkey (0.738 h).

Therefore, the total clearance of ceftizoxime in HS (218.0 (ml/h) kg) was lower, although it was no statistically shown, than that found in the NS (242.9 (ml/h) kg), mouse (220.02 (ml/h) kg) and monkey (356 (ml/h) kg), and greater than those found in dogs (195 (ml/h) kg) and rats (69.6 (ml/h) kg) (Murakawa et al., 1980).

The tachycardia associated to an increment in the cardiac output could contribute to an increase in perfusion of the musculoskeletal system as a result of an increase in the perfusion of this structure, increasing the absorption of ceftizoxime administered by i.m. route in hyperthermic animals, also found in rabbits that the absorption of gentamicin administered by i.m. route was enhanced by the effect of pyrogen-induced fever (Halkin et al., 1981). A faster $t_{1/2a}$ in HS (0.06 h) respect to NS (0.12 h) allowed a faster arrival of the drug to the blood flow compared with NS. The absence of such variable, when ceftizoxime is i.v. administered, could be one of the causes (another could be its distribution) that modify the half-time of elimination of the antibiotic in the HS treated by i.v., in fact, it can be observed when compared between them, the half-time of the i.m. route.

As it was reported by Wilson et al. (1984) in ewes administered with gentamicin, the fraction of antibiotic present in the central compartment increase in response to endotoxin-induce fever and as the consequence the levels of antibiotic decrease in the peripheral or tissue compartment.

It is known that the penetration of the drug in biological systems is it often related to lipid-solubility. Eventhough ceftizoxime has a low lipid-solubility (Bergan, 1987), the passage to tissular fluid is as good as the other cephalosporins of the third generation (Bergan et al., 1982, 1987; Walstad et al., 1983; Rule et al., 1991, 1994), rapidly diffused and in high concentrations to the extravascular space. The relative biodispositionility (Fr) (calculated as Fr = AUC of TCF (or serum) in HS/AUC of TCF (or serum) in NS) in the tissular fluid was lower in the HS (Fr TCF (i.v.) = 51%); (Fr TCF (i.m.) = 56.6%). As reported before by Jernigan et al. (1988) using gentamicin administered to cats given endotoxin versus cats without endotoxin, in the present work serum Fr was lower in the animals that received endotoxin (Fr serum (i.v.) = 93.6%), than in those that did not received endotoxin (Fr serum (i.m.) = 42.2%). Although, as it was previously shown, the passage to TCF was important either in HS or NS, the changes in serum and TCF concentrations may affect the efficacy in HS to consider the posologic regimens where the antibiotic was administered at intervals for up to 6 h.

In the present study, we could demonstrate that the ceftizoxime was distributed more rapidly into the TCF in the HS versus NS. However, in both trials (1 and 2) constant and substained levels of ceftizoxime were observed in periods between 2 and 6 h post-administration of the antibiotic.

Finally, over the time course of ceftizoxime in TCF and serum obtained after a single dose by i.v. and i.m. routes in HS and NS exceeded the MIC for susceptible organisms at least 6 h post-administration.

References


