REPRINT

Journal of Chemotherapy Vol. 4 - n. 1 (23-26) - 1992

Peritoneal Absorption of Cefoperazone in Rats

T.A. NAJJAR

Summary

The peritoneal absorption of cefoperazone, administered by intraperitoneal (ip) perfusion in a large volume (100mg/40ml), was investigated in rats. Its pharmacokinetics was also studied after ip or intravenous (iv) injection of 100mg/kg in two groups of rats. The peritoneal uptake after the two modes of ip administration was rapid, peaking in less than 20 minutes and the means of peak concentrations were similar. The peak remained high in the group perfused with a large volume for at least 4 hours, which was the end of sample collection. In addition, the absorption half-life and the fraction (F) reaching systemic circulation were calculated and found to be 10.0 ± 2.5 min and 0.93, respectively. A brief distribution phase (t½α 8.0 ± 0.67 minutes) appeared only after the iv bolus. Otherwise the decline in serum concentration was monoexponential with half-lives of 39.0 ± 4.0 and 63.6 ± 7.5 min for the iv and ip injected groups, respectively.

The stability of cefoperazone in plasma was also investigated in this study. It was found to be unstable at physiological pH even at −30° C and the samples collected should be buffered in acidic media to optimize stability. The degradation process is likely to contribute to its elmimination kinetics during *in vivo* administration.

Key words: cefoperazone, ip injection, ip perfusion, peritoneal absorption, pharmacokinetics, stability.

College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh 11451, Saudi Arabia

Address for correspondence: Dr. Tawfeeg A. Najjar, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh 11451, Saudi Arabia

ISSN 1120-009X

INTRODUCTION

Cefoperazone is one of the third generation cephalosporins commonly used for the treatments of peritoneal infection [1]. In this group of patients the peritoneal cavity is a potential route for antibiotic delivery either during peritoneal dialysis [2], lavage [3] or intraperitoneal chemotherapy with anticancer agents [4]. The peritoneal absorption of cefoperazone has been studied in humans only during peritoneal dialysis [2,5,6]. The transfer of solutes across the peritoneal membranes is passive and depends primarily on the physicochemical characteristics of the drug [7,8]. The aim of the present investigation is to study the peritoneal absorption of cefoperazone from an isotonic solution in rats. This can possibly serve as a model for cefoperazone absorption, taken via the same route, in humans [3,4]. This notion is substantiated by the fact that experimental animals have been successfully used in the past to study the peritoneal transport of solutes [7,9]. Furthermore, since there have been no reports on the precautions needed for proper handling of cefoperazone-containing plasma samples during pharmacokinetics studies, conditions for optimal stability had to be established in the course of this study.

MATERIALS AND METHODS

Animal study

Twenty-eight healthy male Wistar rats, weighing between 305 and 425 g were divided in three groups. The rats were fasted overnight, with water allowed *ad-libitum*. Following anesthesia with ether, a segment of heparinized polyethylene glycol tube (PE-50, Jenkins Sci. Ltd, UK) was used to cannulate each of the

animals' right femoral artery. The rats were then individualized to receive one of the following treatments. In one group (10 rats), cefoperazone was injected intravenously via the tail vein. The second group (10 rats) were injected intraperitoneally via the flank muscle. The dose injected in the two groups was 100 mg/kg which was dissolved in 300 µl normal saline and injected within 10 minutes of preparation. The rats in the third group (8 rats) were perfused with 40 ml of normal saline containing 100 mg of cefoperazone. The abdominal skin and muscles were gently extended with a pair of forceps and the drug solution was slowly injected over 2-3 minutes in the peritoneal space using a 21-guage needle. The rats were then placed in special restraining cages until the end of sample collection.

Blood samples were collected at 0, 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes following drug administration. Additional blood samples between 120 and 240 minutes were collected only for the ip groups. The samples were centrifuged and the plasma was buffered to pH 6.15 with 1/15 M phosphate buffer to maintain drug stability. Samples were stored at −30° C until the day of analysis.

Stability study

Freshly collected rat plasma (pH 7.4) was divided into two portions (A and B). Portion (B) was buffered to pH 6.15 by adding (1/15 M) phosphate buffer. The stability of cefoperazone was then studied in each portion as follows. Aliquots of 60 µg/ml of the drug in portion A and B were prepared and analyzed in duplicates at 25° C and at −30° C over 24 hours and 4 weeks, respectively (*Table 1*).

Similarly, 60 µg/ml of the drug in plasma (portion A) was incubated at 37° and the concentration was monitored for 5 hours.

Drug analysis

The high performence liquid chromatography (HPLC) method described by Cowlishaw and Sharman was followed using a Waters Associates HPLC unit (MA, USA) [10]. The system was equipped with a 720 system controller, 730 data module, 481 UV detector and 710 B Wisp (automatic injector). Cefoxitin (800 µg/ml) was used as internal standard and added to the serum before the precipitation step. The solution used for precipitation was a mixture of methanol: acetonitrile (1:1), and 10 µg/ml of the supernatant injected onto a Resolve® C_{18} (5Im, 10 cm) column. The mobile phase applied for the separation was a mixture of 700 ml (0.05M) ammonium dihydrogen phosphate and 300 ml methanol (pH 6.0). The flow rate was 1.5 ml/min and the effluent was monitored at 254 nm.

Data analysis

The data plotted on semi-log graph paper and the terminal half-lives were determined using linear regression analysis. The method of residuals was used in the estimation of absorption and distribution half-lives. Linear trapezoidal rule and the log method were used to estimate the area under the curve (AUC) for ip and iv, respectively. The residual area, volume of distribution (V_d) and total body clearance (TBC) were calculated from the following relationships C_n/K_{el}, dose/C_p^0 and AUC^{0-s}/FD_0, respectively. Statistical analysis was performed using the Student t-test.

RESULTS

The plasma concentrations of cefoperazone were determined for the three groups of rats. The concentration-time profiles are shown in

TABELLA 1 - *The pharmacokinetic parameters (mean ± s.d) for cefoperazone after three different modes of administration.*

Parameter	iv injection (n = 10)	ip injection (n = 10)	ip perfusion (n = 8)
AUC^{t0-tn} (µg.min/ml)	5476 ± 761	4981 ± 759	11820 ± 4492
$AUC^{0-\infty}$ (µg.min/ml)	5547 ± 846	5176 ± 973	—
Half-lives (minutes):			
— terminal	39.0 ± 4.0	63.6 ± 7.5	—
— absorption	—	10.0 ± 2.5	—
— distribution	8.0 ± 0.67	—	—
C_{Pmax} (µg/ml)	—	65 ± 18.2	64.4 ± 36.7
T_{max}(min)	—	5-15	5-15
Vd (L)	0.35 ± 0.06	—	—
TBC (ml/min)	59.5 ± 11.5	54.1 ± 19.2	

AUC^{t0-tn} = between 0 and 240 minutes

from a large volume of an isotonic solution is as rapid as that obtained after simple ip injection. The peak concentrations were reached in less than 20 min and remained steady within a narrow range until the end of sample collection, *Figure 1*, and the pharmacokinetics parameters are presented in *Table 1*. Following the iv bolus of cefoperazone, a large (~70%) drop in the initial concentration occurred within 20 minutes. The elimination half-life was about 39 minutes which is approximately two-thirds the half-life obtained after ip injection. The peritoneal absorption of cefoperazone after both ip injection and perfusion were compared in this study. The absorption for each was fast, where peak concentrations were reached within 20 minutes. The means of the peak concentrations were similar; however, after ip injection, cefoperazone declined monoexponentially while it remained steady after ip perfusion for a minimum of 240 minutes. The ratio of the AUC between 0 and 240 minutes was 42%. The fraction (F) reaching systemic circulation after ip injection was 93%.

The drug is unstable in plasma and the degradation process followed first order kinetics.

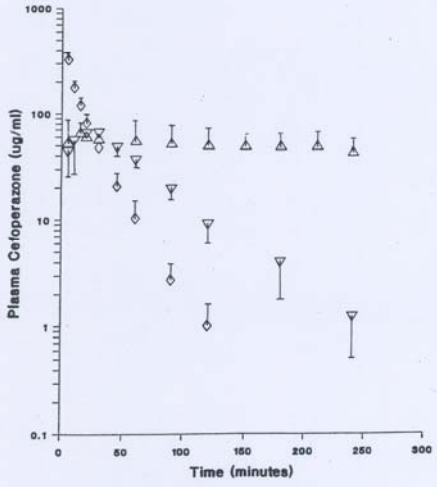

Figure 1. The means ± S.E. of plasma concentration-time profile of cefoperazone after ip (▽) and iv (◇) injections of 100 mg/kg and after ip (△) perfusion of 100mg in 40 ml normal saline.

TABELLA 2 - *Comparison of cefoperazone stability (60 µg/ml in plasma of rat (portion A) and in plasma buffered to pH 6.15 (portion B) with 1/15 M phosphate buffer. The stability in (portion A) was also studied at 37° C for 5 hours.*

	Study period	% Lost during this period
Portion A		
37° C	5 hours	35.1
room temp.	24 hours	73.5
−30° C	4 weeks	27.8
Portion B		
room temp.	24 hours	N.S.
−30° C	4 weeks	N.S.

N.S. = ±4% of the original concentration

The rate constants were 0.042 hours^{-1}, 0.084 weeks^{-1} and 0.087 hours^{-1} for the drug incubated at 25°, -30° and 37° C, respectively. *Table 2* shows the amount lost at the end of the incubation period. The buffered plasma (portion B) retained the plasma concentrations of cefoperazone between 96-104% of the original concentration at both temperatures for the duration of the study.

DISCUSSION

The fluid (40 ml) perfused into the peritoneal cavity was tolerated by the rats without any restriction in their activity. Others have used the same volume[11] and it has been shown that rats can tolerate up to 50 ml[7]. About 24% of this volume, as normal saline, may get into systemic circulation over 4 hours[7]. According to this finding, less than 10 ml of the perfused fluid would be absorbed over 4 hours, which may not have significant influence on the pharmacokinetics of cefoperazone. The dose of cefoperazone which was used in this study was at the same level as that used by others[12,13].

The peritoneal absorption of cefoperazone which was 4 hours. The bioavailability was high since almost 93% of the dose injected intraperitoneally reached systemic circulation. Data on the peritoneal absorption of cefoperazone were only available from human studies during peritoneal dialysis using hypertonic solutions[2,5,6]. In those studies, over 90% of the peak concen-

trations were obtained after one hour, which would then plateau for about 4 hours of the 6-10 hour dialysis interval, and the bioavailability varied between 61-95%. The difference between the peritoneal absorption in this study and that reported in humans is mainly at the rate of absorption. It is not likely to be due to the renal failure in the dialysis patients, because the kinetics of cefoperazone do not change in the presence of severe renal impairment [6,12]. This difference may be explained on the basis of the osmolarity of the perfused fluids. The fluids used during dialysis are hyperosmolar, which may create a force against the absorption of cefoperazone. The similarity between rats and humans has been shown for the transfer of systemic solutes into peritoneal fluids [11]. Therefore, it is not unlikely that this pattern of intraperitoneal absorption of cefoperazone in rats is extendable to humans.

The elimination kinetics of cefoperazone are faster in animals than in humans. The elimination half-life in rats and rabbits[14] was one hour compared to approximately two hours in humans [12,15]. In this study, the iv-injected cefoperazone was eliminated faster than that injected ip: mean half-life 39 minutes compared to 63 minutes after ip injection. Because cefoperazone is mainly excreted via the biliary system [15,16], the extended half-life obtained in the ip group may be due to entero-hepatic recycling of the drug [17]. Although the drug is poorly absorbed when given orally[15], it is possible that the drug excreted in bile may have a better chance for reabsorption.

Cefoperazone was found to be unstable in plasma, and low temperatures (e.g. −30°C) slowed but did not prevent the degradation process. At this temperature, there was a loss of 27.8% of the original concentration over 4 weeks. This should point to the risk of storing plasma samples even at this low temperature for more than a few days. In order to maintain stability for longer periods, plasma samples should be buffered in acidic media before storage. This procedure is deemed necessary for maintaining cefoperazone stability for the purpose of pharmacokinetic study. The degradation process increased at the biological temperature (37°C), since about 35% of the original concentration was lost during 5 hours. This degradation process is likely to contribute significantly to the elimination kinetics of cefoperazone during *in vivo* administration.

ACKNOWLEDGEMENT — The author wishes to thank Mr. R.M.A. Khan, Mr. A. Quraishi, Mr. K. Matar and Dr. A. Takle for their technical assistance at various times.

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Tipografia «Il Sedicesimo», Firenze