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Original Article

Pharmacokinetics of Cefpodoxime Proxetil with Special Reference to Biochemical Parameters, Tissue residue and Spermatozoa motility in rats

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ABSTRACT

Studies on oral kinetics (blood and tissues) after single therapeutic dose of cefpodoxime (20 mg/kg oral) in rats of either sex and on some biochemical parameters, tissue residue and spermatozoa motility in male rats after cefpodoxime administration (20 mg/kg oral bid 7days) were undertaken so that generated data could be extrapolated to humans. For kinetic studies, 24 Wister rats of either sex, 3 months of age, (180-210 gm) were used. (Group I-IV; n=6) Blood samples collected from each animal of Group IV through heart puncture at 0 hour to serve as predrug control. All the group (I-IV) received cefpodoxime proxetil 20 mg/kg once orally as a single dose. At the end of 1, 4, 12 and 24 hour post oral administration, Group I, II, III and IV were utilized for kinetic studies. Blood samples were collected from each animal and vital organs viz brain, lung, liver, spleen, kidney and heart were dissected out for drug analysis and determination of weight. For biochemical parameters, tissue residue and spermatozoa motility, twelve male rats were randomly divided into Groups A and B (n=6) Group B received cefpodoxime (20 mg/kg orally bid 7 days) while Group A served as control. Biochemical parameters [Blood glucose, protein, Aspartate transaminase (AST), Alanine transaminase (ALT) and hemoglobin] were measured at 0 and 7th day while sperm count (total, live and dead) and mean organ weight (study and control group) and tissue residue of drug were evaluated at the end of treatment. Absorption of cefpodoxime was observed at 2 hour and reached a maximum at 4 hour and persisted in blood till 24 hour. Elimination half life in lung was highest followed by heart, liver, kidney and spleen while $t_{½}$ k in plasma was very low suggesting more affinity of cefpodoxime for tissues than blood. Blood glucose, protein, AST and ALT activities were not significantly altered but the hemoglobin level and total and live sperm count decreased significantly in the study group compared to the control group. Residual level of cefpodoxime was highest in liver followed by kidney and other study organs. Therefore, the drug should be used in human beings judiciously and further study on human subjects is warranted.

Key words: Kinetics, Cefpodoxime, Tissue residue.

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INTRODUCTION

Cephalosporins are bactericidal drugs, which act by inhibition of bacterial cell wall synthesis. Cefpodoxime is a semisynthetic third generation cephalosporin analogue with a relatively broader spectrum of antimicrobial activity against gram negative and gram positive organisms when compared to the first generation analogues.[1] This is attributed to their somewhat increased resistance to degradation by the betalactamase.[2] Cefpodoxime was developed as a treatment for bovine clinical mastitis as it is bactericidal, penetrates tissue well[3] and is rapidly excreted from urine.[4] Barragry *et al*[5] showed good activity against Klebsiella pneumonia,[6] many members of enterobactericeae[7] and almost all strains of *Escherichia coli*.[8,9] Cefpodoxime is extensively used in human beings against infections caused by susceptible organisms for a prolonged period.[10] Though various researchers have worked on the pharmacokinetic aspects of the drug,[11] its effects on biochemical parameters and spermatozoa activity are scarcely available in literature. Taking all these into considerations, the present study was undertaken to determine the oral kinetic (blood and tissue), tissue half life and certain biochemical parameters such as glucose, hemoglobin, protein, ALT, AST and sperm count in rats with the aim to generate data which could be extrapolated to studies on human beings.

MATERIALS AND METHODS

Animals

Wister rats of either sex, weighing 180-210gm (3 months of age) was used. The rats were kept at a room temperature of 22 ± 3^{0}C and 12 hr natural light dark cycle, in the animal house of department of Pharmacology, B. J. Medical College, Pune. They were fed on standard laboratory feed and water *ad libitum.* Male rats (age 5 months, weighing between 210-220 gm) with proven fertility were used for the sperm count test. The experiments were performed following approval by the institutional animal ethics committee. Different sets of animals were used for the kinetics study (n=24); but the same group of animals were used for the study of biochemical parameters and spermatozoa motility (n=12).

Drug

Syrup Cefpodoxime containing 125 mg/5 ml of cefpodoxime proxetil was used for the study. One ml of the syrup was diluted with 4 ml of distilled water. The resultant suspension contained 5 mg/ml of cefpodoxime proxetil. The dose of 20 mg/kg for each rat was calculated according to their body weight and the individual dose was administered orally through rat feeding cannula.

Experimental protocol

Kinetic studies

Twenty four rats of either sex were used for the blood and tissue Kinetic studies. They were randomly selected into four groups I-IV (n+6). Blood samples were collected from each animal of Group IV through heart puncture at 0 hour to serve as predrug control. All the groups (I-IV) received cefpodoxime proxetil 20 mg/kg once orally as a single dose. At the end of 1, 4, 12 and 24 hour post oral drug administration, animals of groups I, II, III and IV were utilized for kinetic studies respectively.

Collection of samples

Blood: At the end of 1, 4, 12 and 24 hr post oral drug administration, blood samples were collected through heart puncture from the animals of Group I, II, III and IV respectively. Samples were kept in heparinised test tubes and centrifuged at 3000 rpm for 30 minutes to separate the plasma for estimation of the drug.

Tissue: At the end of 1, 4, 12 and 24 hr post oral drug administration, animals of Group I, II, III and IV (n=6) respectively were sacrificed by the cervical dislocation and vital organs viz. brain, lung, liver, spleen, kidney and heart were collected for drug analysis.

Analysis of biochemical parameters, tissue residue and spermatozoa motility

Twelve male rats with proven fertility were randomly divided into Groups A and B (n=6). The animals of Group B received cefpodoxime proxetil 20 mg/kg twice daily for 7 days orally while Group A served as control and received 2 ml of distilled water in a similar manner.

Biochemical analysis

Before and after the study period (7 days), blood samples were collected from each animal by method of heart puncture. Blood samples were centrifuged and the serum was collected and frozen till analysis by standard methods. Serum protein was measured according to.[15] Hemoglobin content was assessed following the methods of.[16] AST and ALT were measured as per the colorimetric procedure of Blood sugar was estimated by glucose oxidase method.[5]

Sperm collection

At the end of the study period (7 days), rats of both groups were anesthetized with diethyl ether and epididymis was separated from the testes and placed in 0.5 ml of pre warmed saline solution. It was then carefully incised and pressed gently to let the sperms come out following the modified method as described by.[9]

Residue of drug in tissues

At the end of the study period (7 days), the vital organs viz. brain, lung, liver, spleen, kidney and heart of each animal of control and study group were collected. The mean body weight and relative organ weight were noted in both the control and study groups. The organ dissected from study group were utilized for analysis of the residual drug.

Sample processing

Blood: One ml of HPLC grade acetonitrile was added to 0.25 ml of plasma and vigorously shaken. The mixture was then centrifuged at 12000 rpm for 10 minutes at 4^0C. The supernatant was transferred to rotary evaporator and the evaporate was reconstituted with 250 microlitre of HPLC grade acetonitreile. The final volume was measured and 20 microlitre was injected to the HPLC injectionport.

Tissue: One gram of tissue was minced thoroughly, 5 ml of acetonitrile added to it and transferred to a small homogenizer cup. Then the mixture was homoigenized properly, filterd through sodium sulphate (4 gm) and transferred to a centrifuge tube at 12000 rpm (4^0C) for 10 minutes. The supernatant was collected and dried with the help of a rotary evaporator and the evaporate was reconstituted with HPLC grade acetonitrile to 1 ml. The reconstituted volume was filtered and 20 microlitre was injected into the HPLC injection port.

Spermatozoa: Sperms were mixed with 1% aqueous eosin Y (10:1) and kept for 30 minutes for the staining. Then an aliquot of stained filtrate was taken in white blood cell pipette upto the 0.5 mark and diluted further upto mark 11 with PBS. The mixture was shaken and charged into Neubauer's chamber and sperm count was performed as per the procedure of.[8] The sperm count in 8 squares of 1 mm^2 each area except the central erythrocyte counting area of Neubauer's chamber was performed and multiplied by 5 into 10^4 factors to calculate the total number of sperms. Data were analyzed by Mann Whitney U test using SPSS software and $P<0.05$ was considered as the level of statistical significance.

Instrument and Chromatographic condition

Cefpodoxime analysis was performed on a HPLC system (SHIMADZU, SPD-M 10 A, JAPAN) fitted with binary pump (LC-20AT), diode array detector, sampler and data station. A 5 micron Luna Phenomenox (250 into 4.6 mm) C_{18} (2) HPLC column was used. The mobile phase consisted of acetonitrile and water with a ratio of 50:50 (V/V). The flow rate of mobile phase was 1 ml/min and the eluent was monitored with a diode array detectoir adjusted wave length at 273 nm. Retention time of the drug was 2.45 min. The chromatograms were integratewd on a data station.

Statistical analysis

Statistical analysis was carried out by one way analysis of variance (ANOVA) and comparison between the control and experiment groups was done using the LSD test. $P<0.05$ was considered significant.

RESULTS

The initial concentration of cefpodoxime in blood at 1 hr was found to be 0.22 ± 0.03 microgram/ml, which attained a maximum concentration at 4 hr (1.07 ± 0.23 microgram/ml), its presence has also been recorded (Figure 1). Cefpodoxime could not be detected in the blood sample of

Table 1: Mean kinetic parameter of cefpodoxime proxetil following single dose oral administration in rats (values are mean ± SE; n=6)

Parameter	Value
C^0_B (microgram/ml)	3.40 ± 0.87
K_a (per hr)	1.018 ± 0.06
T½ K_a (per hr)	0.69 ± 0.10
AUC (microgram hr per ml)	7.85 ± 1.76
MRT (hr)	6.13 ± 1.31
Vd_{area} (L/kg)	68.75 ±4.01
Cl_B (L/h/kg)	12.76 ± 2.64
K_{Cl} (per hr)	0.31 ± 0.05

C^0_B(microgram/ml): Zero time blood concentration
K_a (per hr) : Absorption rate constant
T½ K_a (per hr) :Absorption half life
AUC (microgram hr per ml) :Area under curve
MRT (hr) :Mean residence time
Vd_{area} (L/kg) :Apparent volume of distribution
Cl_B (L/h/kg) :Total body clearance of drug
K_{Cl} (per hr) : Rate constant elimination of drug

Table 2: Mean half life of cefpodoxime proxetil (in hr) in different organs following single oral administration at 20 mg/kg in rats

Organs	Half life (hr)
Lung	12.26 + 2.31
Liver	5.27 +1.14
Spleen	1.99 + 0.37
Kidney	3.11 + 0.78
Heart	8.21 + 2.19

(values are Mean ± SE; n=6).

Table 3: Mean of tissue residue of cefpodoxime proxetil (microgram/gm of tissue) in different organs after twice daily oral administration for 7 consecutive days

Organs	Concentration (microgram/gm of tissue)
Brain	0.31 + 0.06
Lung	0.75 + 0.24
Liver	2.80 + 0.36
Spleen	0.75 + 0.04
Heart	1.71 + 0.67
Muscle	0.09 + 0.02

(values are Mean ± SE; n=6).

Table 4: Biochemical parameters after twice daily oral administration of cefpodoxime proxetil for 7 consecutive days at 20 mg/kg body weight in rats

Parameters	Control (0 day)	7th day
Hemoglobin (gm/dl)	11.87 + 0.64	9.97 + 0.23
Glucose (gm/dl)	51.70 + 6.35	71.33 + 12.34
Protein (gm/dl)	5.73 + 0.52	4.77 + 0.55
ALT (microgram pyruvic acid/ml/hr)	19.41 + 1.39	22.76 + 1.78
AST (microgram pyruvic acid/ml/hr)	56.02 + 3.44	65.10 + 2.91

(values are Mean ± SE; n=6).

Table 5: Sperm count (×10⁶) (probit) of male rats in control and study group

Group	Total sperm count	Live (probit)	Dead (probit)
Control Group A	9.88 + 0.67	6.34 + 0.06	3.56 + 0.09
Treated Group B	9.73 + 0.564	6.05 + 0.07	3.95 + 0.08*

(values are Mean ± SE; n=6).

- Significant compared to respective control

rats at 24 hr. Different pharmacokinetic values were presented in Table 1.

Tissues: The elimination half life of cefpodoxime proxetil in different tissues are depicted in Table 2. There was no significant alteration of mean organ (lung, liver, spleen, kidney and heart) weight/100 gm in the control and study group. The mean residual concentration of cefpodoxime recovered from different tissues of rats are presented in Table 3. The maximum quantity of cefpodoxime proxetil was recovered from liver followed by kidney after twice daily oral administration of the drug for a period of seven consecutive days. Lowest detectable level was observed in muscle.

Biochemical analysis; Mean values of hemoglobin, glucose, protein, ALT and AST before starting the treatment and after thr treatment were compared (Table 4). There was a decrease in hemoglobin and protein values while AST, ALT and glucose were marginally increased.

Sperm count-Live sperm count is decreased significantly (P<0.05) compared to control and the number of dead sperms increased although there was no significant change in the total sperm count after twice daily oral administration of the drug for a period of seven consecutive days (Table 5).

DISCUSSION

The absorption of cefpodoxime proxetil from intestine started at 0.5 hour and reached a maximum concentration at 4 hr with a K_a value of 1.018

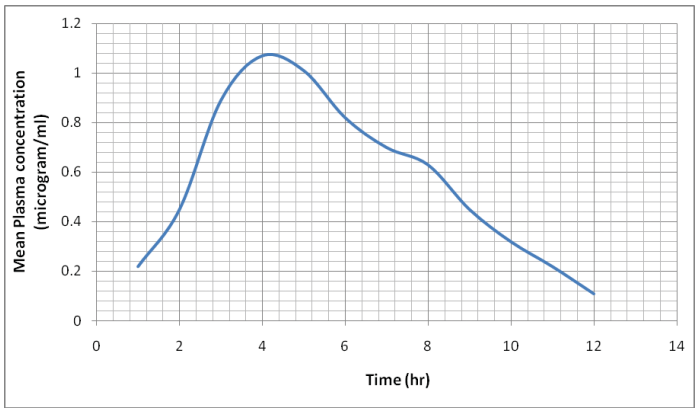

Figure 1: Semilogarithmic plot of mean plasma concentration of cefpodoxime proxetil against time with computerized best fit line in rats following single dose.

which suggest slow absorption of drug from gastrointestinal tract.[12] also reported similar observation after oral administration in rats. The drug persisted in blood till 24 hour following oral administration. Absorption across the intestinal mucosa appear to occur as a result of an active transport mechanism. Some drug is hydrolised in the lumen of the intestine, the proportion increasing from 16 to 25% with increasing concentration[12,13] which might be due to slow absorption of cefpodoxime in rats.[13]

The biochemical parameters like glucose, protein, AST and ALT activity were not significantly altered in rats at therapeutic doses (cefpodoxime proxetil 20 mg/kg twice daily for 7 days orally) but the hemoglobin level decreased significantly (P<0.05) in the study group compared to the control group which suggests anemic tendency in rats. The result corroborate with the study of.[14] Although mild degree of anemia was noted with therapeutic dose selected for the study, there was no sign of overt toxicity or death in any animal during the study period. Although there was no significant difference in total sperm count, the number of variable sperm count decreased significantly (P<0.05) in the study group compared to the control group. Further studies to be needed for assessing the effects of cefpodoxime on fertility in experimental animals.

Residual level of cefpodoxime proxetil was highest in liver followed by kidney and other study organs which indicates that the drug has affinity to accumulate in tissues and is distributed throughout the body as evidenced by its 100% VD area value in rats. Elimination half life of cefpodoxime proxetil in lungs was highest followed by heart, liver, kidney and spleen while $t_{½}k$ in plasma was very low suggesting more affinity of cefpodoxime proxetil in tissues in comparison to blood.

The present study thus indicate that the drug should be used in human beings judiciously and further study on human subjects is required.

What is already known to this topic

- Cefpodoxime proxetil is bactericidal, penetrates tissue well and is rapidly excreted from urine.

What this study adds

- Cefpodoxime proxetil has high affinity to accumulate in tissues like liver, kidneys and is distributed throughout the body.
- Cefpodoxime proxetil has high affinity for tissues in comparison to blood.
- Elimination half life of cefpodoxime proxetil in lungs was highest followed by heart, liver and kidney.
- No significant difference in total sperm count was found however the number of variable sperm count decreased significantly.

CONFLICT OF INTEREST

The authors do not have any conflict of interest financial or otherwise.

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