ABSTRACT: Studies on oral kinetics (Blood and tissues) after single therapeutic dose of cefpirome (20mg/kg oral) in rats of either sex and on some biochemical parameters, tissue residue, and spermatozoa motility in male rats after cefpirome administration (20mg/kg oral bid 7days) were undertaken so that generated data could be extrapolated to humans. For kinetic studies, 24 Wistar rats of either sex, 3 months of age, (180–210gm) were used (Groups I–IV; n=6). Blood samples collected from each animal of Group I-IV at 0 h to serve as predrug control. All the groups (I-IV) received cefpirome 20mg/kg once orally as a single dose. At the end of 1, 4, 12, and 24 hour post oral administration, Groups I, II, III, and IV were utilized for kinetic studies. Blood samples were collected from each animal and vital organs namely brain, lung, liver, spleen, kidney, and heart, were studied for drug analysis and determination of weight. For biochemical parameters, tissue residue and spermatozoa motility, 12male rats were randomly divided into Groups A and B (n=6). Group B received cefpirome (20mg/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 cefpirome was observed at 2h and reached a maximum at 4h and persisted in blood till 24h. Elimination half-life in lung was highest followed by heart, liver, kidney, and spleen while t½, k in plasma was very low suggesting more affinity of cefpirome 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 cefpirome was highest in liver followed by kidney and other study organs. Therefore, the drug should be used in human beings judiciously.

KEYWORDS: Cefpirome, Kinetics, Tissue residue.

NEED FOR STUDIES ON PHARMACOKINETIC PARAMETERS OF CEFPIROME:

1. This is the study of first kind where all pharmacokinetic parameters like plasma half-life (t½), Clearance (CL), volume of distribution (Vd) are studied.
2. This is the study of first kind where sperm count were compared before and after cefpirome.
3. Kinetics of elimination of cefpirome are discussed which can be extrapolated to humans.

INTRODUCTION: Cephalosporins are bactericidal drugs, which act by inhibition of bacterial cell wall synthesis. Cefpirome is a semi-synthetic 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 cephalosporins. This is attributed to their increased resistance to degradation by the beta-lactamases. Cefpirome was developed as a treatment for bovine clinical
mastitis as it is bactericidal, penetrates tissue well and is rapidly excreted from urine.\cite{1,2} It shows good activity against Klebsiella pneumonia, many members of enterobactericeae and almost all strains of Escherichia coli.\cite{3,4} Cefpirome is extensively used in human beings against infections caused by susceptible organisms for a prolonged period.\cite{5} Although various researchers have worked on the pharmacokinetic aspects of the drug,\cite{6} its effects on biochemical parameters and spermatozoa activity are scarcely available in the literature.\cite{7} 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) were used. The rats were kept at a room temperature of 22±3°C and 12h natural light dark cycle, in the animal house of department of Pharmacology, Govt. Medical College, Nagpur. They were fed on standard laboratory feed and water ad libitum. Male rats (age5 months, weighing between 210 and 220gm) 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 Cefpirome containing 125mg/5ml of Cefpirome was used for the study. One milliliter of the syrup was diluted with 4 ml of distilled water. The resultant suspension contained 5mg/ml of cefpirome. The dose of 20mg/kg\cite{7} for each rat was calculated according to their body weight and the individual dose was administered orally through rat feeding cannula.

**Experimental Study Design: Kinetic Studies:** Twenty-four rats of either sex were used for the blood and tissue kinetic studies. They were randomly selected into four group's I–IV (n+6). Blood samples were collected from each animal of group I–IV at 0 h to serve as pre-drug control. All the groups (I–IV) received cefpirome 20mg/kg once orally as a single dose. At the end of 1,4,12, and 24 h post oral drug administration, animals of groups I, II, III, and IV were examined for kinetic studies.

**Collection of Samples:** Blood-At the end of 1,4,12, and 24 h post oral drug administration, blood samples were collected from the animals of groups I, II, III, and IV, respectively. Samples were kept in heparinized test tubes and centrifuged at 3000 rpm for 30 min to separate the plasma for estimation of the drug.

**Tissue:** At the end of 1,4,12, and 24 h post oral drug administration, animals of groups I,II,III, and IV (n=6), respectively were examined and vital organs, namely brain, lung, liver, spleen, kidney, and heart were studied 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 cefpirome 20mg/kg twice daily for 7 days orally while Group A served as control and received 2ml of distilled water in a similar manner.

**Biochemical Analysis:** Before and after the study period (7 days), blood samples were collected from each animal. Blood samples were centrifuged and the serum was collected and frozen till
analysis by standard methods. Serum protein was measured according to Lowry et al method.\[8\] Hemoglobin content was assessed following the methods of Kolmeret al.\[9\] AST and ALT were measured as per the colorimetric procedure of Wootton.\[10\] Blood sugar was estimated by the glucose oxidase method.\[11\]

**Sperm Collection:** At the end of the study period (7 days), from rats of both groups epididymis sperm fluid was separated from the testes and placed in 0.5 ml of pre warmed saline solution. It was then carefully pressed gently to let the sperms come out.\[12\]

**Residue of Drug in Tissues:** At the end of the study period (7 days), the vital organs, namely brain, lung, liver, spleen, kidney, and heart of each animal of the control and study group were assessed. The mean bodyweight and relative organ weight were calculated in both the control and study groups. The drug was analyzed for pharmacokinetic parameters like volume of distribution (Vd), Bioavailability (F), Clearance (CL) 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 12000rpm for 10min at 4°C. The supernatant was transferred to rotary evaporator and evaporate was reconstituted with 250 μl of HPLC grade acetonitrile. The final volume was measured and 20 μl was injected to the HPLC injection port.

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

**Spermatozoa:** Sperms were mixed with 1% aqueous eosin Y (10:1) and kept for 30 min for the staining. Then an aliquot of stained filtrate was taken in white blood cell pipette up to 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 Vega et al.\[13\]. The sperm count in 8 squares of 1 mm² 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 the Mann–Whitney U test using SPSS software and P<0.05 was considered as the level of statistical significance.

**Instrument and Chromatographic condition:** Cefpirome 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) C18 (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 detector adjusted wave length at 273 nm. Retention time of the drug was 2.45 min. The chromatograms were integrated 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 cefpirome in blood at 1h was found to be 0.22±0.03 µg/ml, which attained a maximum concentration at 4 h (1.07±0.23 µg/ml), its presence has also been recorded [Figure 1]. Cefpirome could not be detected in the blood sample of rats at 24 h. Different pharmacokinetic values were presented in Table 1.

Tissues: The elimination half-life of cefpirome different tissues are depicted in Table 2. There was no significant alteration of mean organ (lung, liver, spleen, kidney, and heart) weight/100gm in the control and study groups. The mean residual concentrations of cefpirome recovered from different tissues of rats are presented in Table 3. The maximum quantity of cefpirome 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 the 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].

Figure 1: Semi logarithmic plot of mean plasma concentration of cefpirome against time in rats following its single dose.
Parameter | Value
--- | ---
C₀B Zero time blood concentration (microgram/ml) | 3.40±0.87
Ka Absorption rate constant (per hour) | 1.018±0.06
tᵋ/₂ Plasma half life | 0.69±0.10
AUC microgram hour per ml | 7.85±1.76
MRT Mean residence time (hour) | 6.13±1.31
Vd Apparent volume of distribution (L/kg) | 68.75±4.01
CL Total body clearance (L/hr/kg) | 12.76±2.64
K Elimination rate constant (per hr) | 0.31±0.05

Table 1: Mean Kinetic Parameter of Cefpirome following single dose oral administration in rats (values are mean±SE; n=6)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Half-life (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>12.26±2.31</td>
</tr>
<tr>
<td>Liver</td>
<td>5.27±1.14</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.99±0.37</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.11±0.78</td>
</tr>
<tr>
<td>Heart</td>
<td>8.21±2.19</td>
</tr>
</tbody>
</table>

Table 2: Mean Half Life of Cefpirome (in hour) in different organs following single oral administration at 20mg/kg in Rats (Values are Mean±SE; n=6)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Concentration (µgm/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.31±0.06</td>
</tr>
<tr>
<td>Lung</td>
<td>0.75±0.24</td>
</tr>
<tr>
<td>Liver</td>
<td>2.80±0.36</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.75±0.04</td>
</tr>
<tr>
<td>Heart</td>
<td>1.71±0.67</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.09±0.02</td>
</tr>
</tbody>
</table>

Table 3: Mean of Tissue Residue of Cefpirome (microgram/gm) in different organs after twice daily oral administration for 7 Consecutive Days (Values are Mean±SE; n=6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (0 day)</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (gm/dl)</td>
<td>11.87±0.64</td>
<td>9.97±0.23</td>
</tr>
<tr>
<td>Glucose (gm/dl)</td>
<td>51.70±6.35</td>
<td>71.33±12.34</td>
</tr>
<tr>
<td>Protein (gm/dl)</td>
<td>5.73±0.52</td>
<td>4.77±0.55</td>
</tr>
<tr>
<td>ALT µgm pyruvic acid/ml/hr</td>
<td>19.41±1.39</td>
<td>22.76±1.78</td>
</tr>
<tr>
<td>AST µgm pyruvic acid/ml/hr</td>
<td>56.02±3.44</td>
<td>65.10±2.91</td>
</tr>
</tbody>
</table>

Table 4: Biochemical parameters after twice daily oral administration of Cefpirome for 7 consecutive days at 20mg/kg body weight in Rats (Values are Mean±SE; n = 6)
Table 5: Sperm Count (x10^6) (Probit) of male rats in control and study group (Values are mean±SE; n = 6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total sperm Count Before</th>
<th>Total sperm Count After</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>9.88±0.67</td>
<td>6.34±0.06</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Group B</td>
<td>9.73±0.564</td>
<td>3.95±0.08</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Statistically Significant.

**DISCUSSION:** The absorption of cefpirome from intestine started at 0.5 h and reached a maximum concentration at 4h with Ka value of 1.018 which suggest slow absorption of drug from gastrointestinal tract,[14] and also reported similar observation after oral administration in rats. The drug persisted in blood till 24h following oral administration.[15] Absorption across the intestinal mucosa appears to occur as a result of an active transport mechanism. Some drug is hydrolyzed in the lumen of the intestine, the proportion increasing from 16% to 25% with increasing concentration which might be due to slow absorption of cefpirome in rats.[16]

The biochemical parameters like glucose, protein, AST, and ALT activity were not significantly altered in rats at therapeutic doses (Cefpirome 20mg/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 anaemic tendency in rats. The result corroborate with the study of Ito et al.[15] 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 are needed for assessing the effects of cefpirome on fertility in experimental animals.

Residual level of cefpirome 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% volume of distribution area value in rats. Elimination half-life of cefpirome in lungs was highest followed by heart, liver, kidney, and spleen while its plasma half-life t½ in plasma was very low suggesting more affinity of cefpirome in tissues in comparison to blood.

The present study thus indicates that the drug should be used in human beings judiciously taking all pros and cones into consideration for a correct therapeutic indication.

**REFERENCES:**


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