Microbiological assay based biokinetics of cefaclor in male volunteers

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The present study was conducted for evaluation of biokinetics of cefaclor following oral administration of 1 g cefaclor (CCL) tablet in male volunteers. The blood samples of male volunteers were collected for the period of 10 h in heparinized tubes for stipulated period of time, centrifuged (3000xg) and plasma thus separated was stored at -10°C for microbiological assay. The CCL plasma concentration was determined following disc diffusion method against four bacterial strains, namely Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pasteurella multocida. The biokinetics parameter was measured using American Pharmacology Organization (APO) software. Four bacteria were found highly susceptible to CCL and from biokinetics study it is suggested that administration of CCL of 1 g (500 mg q 12 h) daily maintained a considerable concentration that proved it to be very effective for the treatment of bacterial infections understudy in male volunteers.

Keywords: Cefaclor, disc diffusion, biokinetic parameters, dose, antibiotic.

INTRODUCTION

Antibiotics are called the miracle drugs, because before their discovery medicine era was very worse (Francis et al., 1999). For thousands of years, people have used many types of plants, fungi, lichen and other chemicals for curing infections without knowing their actual working. Medicine was more of an experimental practice. Antibiotics are one of the most frequently prescribed medications presently used for curing many diseases. They are used for killing or stopping the growth of bacteria such as bacterial meningitis, neurophilis, endocarditis, burn wounds, skin infections, respiratory and urinary tract infections, pneumonia, anthrax, Lyme disease, bronchitis, diarrhea diseases, abdominal infections, severe acne, gastrointestinal tract infections, blood poisoning, TB, infections and many more (Ashfaq, 2007; Ma et al., 2010).

Hundreds of antibiotics were available for the treatment of infectious diseases, but with the passage of time they became less active. The reason is that microbes have the ability to develop resistance against antibiotics, and thus their biokinetics and dynamics studies are necessary in parallel manner (Walsh, 2003). Cefaclor, IUPAC (International Union of Pure and Applied Chemistry) named 7-[(2-amino-2-phenyl-acetyl) amino]-3-chloro-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid is similar to cephalexin with a substitution of a halogen, which gives cefaclor greater antibacterial activity, especially against Haemophilus influenza. And hence used against various infections in human body especially in upper respiratory tract (Cole, 1997; Novelli, 1998; Meyers, 2000; Gerardus et al., 2000; Cazzola et al., 2003). It does not degrade in body significantly, is excreted with an approximately half life of 2 h (Ashfaq et al., 2010) and the presence of food does not affect the absorption of CCL (Iqbal, 2007). Cefaclor is excreted rapidly in the urine, and is well absorbed without toxicity; it has broad spectrum of activity against gram positive and gram negative bacteria with peak concentrations in serum for 30-60 min (Ashfaq et al., 2010; Karim et al., 2003). So, the present research work was designed to evaluate the biokinetics of 1 g tablet of CCL in human male volunteers with the help of microbiological assay and comparison has also been

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done regarding the susceptibility of four bacterial strains, namely *Escherichia coli*, *Staphylococcus aureus*, *Pasteurella multocida* and *Bacillus subtilis*.

**MATERIAL AND METHODS**

**Experimental subjects**

Eighteen healthy male volunteers were enrolled in this study. Informed written consent was obtained from all volunteers. Demographic data of all participants are given in Table 1. The average age of volunteers was 33 years, within the range of 30-35, whereas height was 71 inches (71-75) and recorded weight was 82 Kg (78-85). On the basis of clinical tests, medical history and laboratory investigations, not any of the members showed any medical liability and involvement in any clinical trials within the 90 days prior to enrolment in the current investigation. In addition, nobody had received any regular course of drug therapy 60 days prior to the present study.

**Cefaclor administration and sample collection**

Each volunteer received a 1 g tablet of CCL (CECLOR®, MR, AGP Ltd, Pakistan) with 400 mL of water. Blood samples (5 mL) form each volunteer were collected in heparinized glass tubes prior to drug administration and at prescheduled time intervals of 0.25, 0.50, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0 and 10.0 h. And samples were centrifuged at 3000xg. The thus separated plasma was stored at -10°C for bioassay analysis.

**Microbial strains**

Concentration of CCL was tested against a set of microorganisms, including two Gram-positive bacteria: *S. aureus* (6736153-APIstaph.tac), *B. subtilis* (JS-2004) and two Gram-negative bacteria: *E. coli* (ATCC 25922) and *P. multocida* (local isolate). Authentic bacterial strains were cultured overnight at 37°C in Nutrient Agar (Oxoid, NA).

**Bioassay procedure**

The concentration of CCL was determined by disc diffusion susceptibility method, performed precisely as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002) against *E. coli*, *S. aureus*, *P. multocida* and *B. subtilis*. Cefaclor standard disks (Wicks No. 319329 (Beckman, U.S.A) and medium (dehydrated powder) were obtained from suppliers of culture media (Oxoid, UK). The medium (40 mL) was used for each glass Petri plate (14 cm in diameter). Plasma (100 µL) was loaded per 10 mm disk. Plates were incubated for 16 to 18 h at 37°C. Zones of inhibition were measured with zone reader in mm and compared with standard. All determinations were performed in triplicate and the results were averaged. The concentration of drug in plasma was measured over time by standard regression seeded from 0.5-180 µg/mL in distilled water. The bioassay procedure was performed in bioassay section, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad.

**Biokinetic analysis**

Plasma concentration data for each subject were manipulated by using American Pharmacology Organization computer software based on the following biokinetic parameters; maximum plasma concentration (Cmax), the Area Under Concentration (AUCt0 to t10h), Mean Residence Time (MRT), Clearance (CL), Volume of Distribution (Vd), Half-life (t1/2), Absorption rate constant (ka), lag time and Time of Peak (Tmax).

**RESULTS**

The CCL plasma concentrations were determined microbiologically up to 10 h for single oral administration. Concentration showed sharp peaks versus time plots (Figure 1) and gradually decreased up to 8 h. The plasma concentration values follow similar plots versus time for
Figure 1: The CCL plasma concentrations (µg/mL) in male volunteer after a single oral administration of 1 g mg tablet measured against four bacterial strains (E. coli, S. aureus, P. multocida and B. subtilis).

DISCUSSION

Plasma CCL concentrations and the calculated biokinetic parameters show significant differences between four microbial strains. B. subtilis was found to be more susceptible, while E. coli found least vulnerable. Absorptions and excretion of CCL is very rapid and this finding was in good agreement with previously reported data (Iqbal et al., 2010). CCL peak concentration was found higher than reported 10.6 µg/mL for male healthy volunteer (Iqbal et al., 2011a), 6.05 and 12.8 µg/mL for normal human volunteer at the rate of 250 mg and 500 mg, respectively and 7.58 µg/mL for human male volunteer (Ashfaq et al., 2010). The serum half life was found to be similarly reported after 60 min versus normal subject and subjects with chronic renal failure (40 and 60 min, respectively) (Bloch et al., 1977). Our finding of the elimination half-life (t₁/₂) was also not in agreement as previously reported for lactating cow of 3.5 h; calves, 3.1 h and foal, 3.26 h (Soback et al., 1991; Halstead et al., 1992; Meyer et al., 1992). This difference might be due to species variation.

Our previous study on dogs indicated less labiality of CCL for human beings (Iqbal et al., 2011a). The AUC values were found to be greater than that reported for sheep (33.7 h.µg/mL) (Ashfaq et al., 2010). The Tₘₚₚₚₚ value indicates greater potency and slower clearance for male volunteers, which makes CCL more active for longer time in serum plasma for treating a susceptible bacterial infection. This characteristic indicates that CCL is very suitable and one of the most important antibacterial agents in use. The biokinetics variable of drug correlated with clinical efficiency, because the plasma concentration level remained high than MIC value (0.78 µg/mL) for young calves until 10 h (Iqbal et al., 2010a). This enables the CCL to treat imperative diseases in human beings due to microbes such as S. aureus, Bacillus subtilis, E. coli and P. multocida.

CONCLUSION

From the results of biokinetics parameters and plasma concentration, it is suggested that oral administration of CCL 1 g tablet (500 mg q 12 h⁻¹) might maintain
Table 2: Biokinetics parameters calculated for cefaclor against tested microbial strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>N</th>
<th>AUC (hmg/L)</th>
<th>AUC(pex) (hmg/L)</th>
<th>AUC (trz) (hmg/L)</th>
<th>CL (L/h)</th>
<th>Vd (L)</th>
<th>Elim. t_1/2 (h)</th>
<th>K_10 (L/h)</th>
<th>MRT (h)</th>
<th>Ka (L/h)</th>
<th>Abs t_1/2 (h)</th>
<th>L.time (h)</th>
<th>T_max (h)</th>
<th>C_max (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>18</td>
<td>137.5 ± 3.55</td>
<td>134.10 ± 3.85</td>
<td>131.6 ± 4.10</td>
<td>6.18 ± 0.92</td>
<td>20.25 ± 4.10</td>
<td>2.10 ± 0.35</td>
<td>0.25 ± 0.01</td>
<td>10.0 ± 0.44</td>
<td>0.21 ± 0.02</td>
<td>1.80 ± 0.07</td>
<td>0.07 ± 0.01</td>
<td>2.95 ± 0.45</td>
<td>17.31 ± 0.77</td>
</tr>
<tr>
<td>S. Aureus</td>
<td>18</td>
<td>125 ± 15.30</td>
<td>123 ± 14.00</td>
<td>120.10 ± 13.5</td>
<td>5.6 ± 1.74</td>
<td>17.5 ± 8.50</td>
<td>2.3 ± 0.88</td>
<td>0.35 ± 0.06</td>
<td>8.10 ± 1.10</td>
<td>0.60 ± 0.22</td>
<td>1.90 ± 0.13</td>
<td>0.07 ± 0.01</td>
<td>1.95 ± 0.05</td>
<td>18.10 ± 3.01</td>
</tr>
<tr>
<td>P. Multocida</td>
<td>18</td>
<td>117.5 ± 13.30</td>
<td>118 ± 12.95</td>
<td>121.5 ± 11.01</td>
<td>6.5 ± 1.90</td>
<td>25.10 ± 9.40</td>
<td>2.3 ± 0.99</td>
<td>0.31 ± 0.07</td>
<td>9.54 ± 1.4</td>
<td>0.30 ± 0.19</td>
<td>1.85 ± 0.18</td>
<td>0.09 ± 0.06</td>
<td>2.01 ± 0.03</td>
<td>20.15 ± 3.01</td>
</tr>
<tr>
<td>B. Subtilis</td>
<td>18</td>
<td>140.0 ± 10.40</td>
<td>139.0 ± 7.56</td>
<td>134.5 ± 8.90</td>
<td>5.5 ± 1.40</td>
<td>23.20 ± 0.77</td>
<td>2.90 ± 0.77</td>
<td>0.21 ± 0.04</td>
<td>10.12 ± 2.01</td>
<td>0.90 ± 0.16</td>
<td>1.10 ± 0.08</td>
<td>0.25 ± 0.07</td>
<td>2.3 ± 0.35</td>
<td>17.31 ± 0.35</td>
</tr>
</tbody>
</table>

AUC, Area Under the Curve; pex, polyexponential (t= 12); trz, trapezoidal rule (t= 12); CL, Clearance; Vd, Volume of distribution; Elim. t_1/2, Elimination Half-life; Abs t_1/2, Absorption Half-life; K_10, Rate constant k10; MRT = Mean Residence Time; ka = Absorption rate constant; L = Lag-time; T_max, peak Time; C_max, Peak concentration.

reasonable concentration that ensures it to be very effective for the treatment of specific infections caused by tested microbes in human beings.

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REFERENCES


Ma J, Bellon M, Wishart JM, Young RL, Jones KL, Horowitz M, Rayner CK (2010). Effects of cefaclor on gastric emptying