Renal Clearance of Endogenous Creatinine and Levofloxacin in Male Volunteers

Nafeesah Waheed, Tahira Iqbal, Munir Ahmad Sheikh and Faqir Hussain Khan

The objective of the study was to evaluate the renal clearance of levofloxacin and that of the endogenous creatinine, following a single oral dose of 500 mg of levofloxacin to eight healthy male volunteers. After an overnight fasting control and after drug administration blood and urine samples were collected at 0, 30, 60, 90, 120, 150 and 0, 45, 75, 105, 135, 160 minutes time intervals, respectively. The samples were assayed for endogenous creatinine spectrophotometrically and levofloxacin by microbiological assay. Mean ± SE values for the blood and urine pH were 7.548 ± 0.013 and 5.85 ± 0.0039, respectively. The average ± SE value for diuresis was 0.018 ± 0.0056 ml min⁻¹ kg⁻¹. The average ± SE values for renal clearance of endogenous creatinine and levofloxacin were 0.519 ± 0.068 and 0.778 ± 0.110 ml min⁻¹ kg⁻¹. The study performed in summer showed that with an increase in urine flow the renal clearance of levofloxacin also increase, and statistically there is non-significant positive correlation (R² = 0.647) between these two parameters. These variations in results supporting the findings that there is a need to evaluate the imported drugs under indigenous conditions due to environmental difference, for getting optimal therapeutic results.

Key words: Levofloxacin, endogenous creatinine, renal clearance fluoroquinolones antibiotics

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Introduction

Fluoroquinolones, the synthetic antimicrobials, have gained importance in clinical practice over the past few years. Main pharmacological advantages of this group include favorable pharmacokinetic profile high levels being achieved in cerebral, pulmonary, and osseous tissues, and good penetration in phagocyte (Katsung, 1998). The newer fluoroquinolones have broad spectrum bactericidal activity, excellent oral bioavailability, good tissue penetration and favorable safety and tolerability profiles. A new four-generation classification of the quinolone drugs takes into account the expanded antimicrobial spectrum of the more recently introduced fluoroquinolones and their clinical indications (Aminov et al., 1997). The third generation quinolones currently include levofloxacin, gatifloxacin, moxifloxacin and sparfloxacin (Stein and Havlicek, 1998).

Levofloxacin is a chiral fluorinated carboxyquinolone a pure -S enantiomer of the racemic drug substances of ofloxacin. It is useful in the treatment of community-acquired pneumonia, acute sinusitis and acute exacerbations of chronic bronchitis (Aminov et al., 1997). The antibiotic is active against gram-positive organisms, gram-negative organisms, and typical pathogens (Fish and Chow, 1997). It inhibits the bacterial growth and reproduction by blocking the enzyme topoisomerase II (gyrase) and topoisomerase IV (Stein and Havlicek, 1998). The drug being used in Pakistan for health program of man is imported from abroad either in the raw or finished form because of scanty indigenous production.

The research work conducted over several years under indigenous conditions have revealed difference between the foreign and local species explained by an original term “Geometrics”, geographical influences on genetics, manifested by dissimilar biochemical, physiological and pharmacological parameters. (Navaz and Shah, 1986; Navaz et al., 1986; Navaz, 1994). This project was planned to study the renal clearance of endogenous creatinine and levofloxacin in male volunteers following oral administration, under local environmental conditions.

Materials and Methods

The experiments were conducted on 8 healthy male volunteers. The volunteers were apprised of the study protocols and a written consent was taken from all those participating in the study. Additionally, body weight, height, age and blood pressure of each participant was recorded. A commercial preparation of levofloxacin (Cravit tablet 500 mg manufactured by Hilton Pharma Pvt. Ltd.) was given through oral route with a glass of water. After an over night fasting control samples of blood and urine were drawn immediately prior to the drug administration, and after 2.5 hours the volunteers took break fast. The 5 venous blood samples were drawn in heparinized tubes at 30, 60, 90, 120 and 150 min. time interval and centrifuged. The plasma was separated and stored, until it was assayed. The urine samples were collected between blood samples at 45, 75, 105, 135, 165 min. time interval after drug administration. The volume each urine sample was recorded, and a 20 ml aliquot from each collection was frozen at -20 °C until it was assayed.

Analysis: The pH of fresh blood and urine samples were recorded by using glass electrode pH meter by Beckman H8 (Germany). For the estimation of glomerular filtration rate, the endogenous creatinine concentration was measured in plasma and urine samples spectrophotometrically by Jaffe reaction (Bosnes and Tausky, 1945). Concentration of levofloxacin in plasma and urine was determined by microbiological assay by using disk agar diffusion method described by (Aret et al., 1971) using Streptococcus faecalis as test organism.

Calculations: The diuresis, concentration of creatinine and levofloxacin in plasma and urine samples, their clearance values and clearance ratio were calculated as follows:

Diuresis: The rate of urine flow in a time period was calculated as:

\[
\text{Diuresis} = \frac{\text{Urine volume in a collection time period (ml)}}{\text{Time (min.) x body weight (kg)}}
\]

Concentration of creatinine in urine

Concentration in urine (\(\mu\)g ml\(^{-1}\)) = Absorbance x Standard factor x Dilution factor

Concentration in plasma

Concentration in plasma (\(\mu\)g ml\(^{-1}\)) = Absorbance x Standard factor x Dilution factor

Concentration of Levofloxacin in urine: Concentration of levofloxacin in urine samples was calculated by simple linear regression equation:

\[
Y = a + bx
\]

Concentration of drug \(X = Y/a\) x Dilution

Where \(X = \text{Concentration}\)

\(Y = \text{Zone size}\)

Concentration of levofloxacin in plasma: Concentration of levofloxacin in plasma samples was calculated by using linear regression equation:

\[
X = Y/a + b
\]

Renal clearance: Renal clearance was calculated by the following formula:

\[
\text{Renal clearance (ml min}^{-1}\text{Kg}^{-1}) = \frac{\text{Uc x D}}{\text{Pc}}
\]

where:

\(\text{Uc} = \text{Concentration of substance in urine}\)

\(\text{D} = \text{Diuresis}\)

\(\text{Pc} = \text{Concentration of a substance in plasma}\)

Clearance Ratio: It is calculated by dividing renal clearance of drug by creatinine renal clearance.

\[
\text{Clearance ratio} = \frac{\text{Renal clearance of drug}}{\text{Renal clearance of creatinine}}
\]

Statistical analysis: The statistical calculations were done and the result was given as average ± SEM. The correlation between the diuresis, pH and plasma concentration of endogenous creatinine, plasma concentration of levofloxacin with its renal clearance determined by means of regression/correlation analysis (steel and Torrie, 1984).

Results and Discussion

Levofloxacin is a fluoroquinolones with desirable pharmacokinetic characteristics. It is rapidly and essentially 100 percent bioavailable following oral administration. The main elimination route of levofloxacin is known to be the renal excretion. To predict the administration of drug dose, according to local environment, this study was performed. The renal clearance of levofloxacin was investigated on eight human male volunteers after the oral dose of 500 mg tablets of levofloxacin. Reaction of the main body fluid depends on the state of biochemical interior of an organism and is reflected by pH values (Coles, 1997). In the study, the average ± SE value of blood pH was 7.48 ± 0.013 (Table 1). The average ± SE value of urine pH was 5.85 ± 0.0039. In earlier studies the average ± SE value of blood pH were 7.73 ± 0.03 in females.
Table 1: Average data of renal clearance of endogenous creatinine and levofloxacin in male volunteers after oral dose of 250 x 2 mg (500) tablets

<table>
<thead>
<tr>
<th>Volunteers</th>
<th>Body weight (Kg)</th>
<th>Body weight (Kg)</th>
<th>pH Urine</th>
<th>pH Blood</th>
<th>pH Plasma</th>
<th>Concentration (wiug/ml)</th>
<th>Concentration (wiug/ml)</th>
<th>Renal clearance (ml/min kg^-1)</th>
<th>Renal clearance (ml/min kg^-1)</th>
<th>Ratio Ctrun</th>
<th>Ratio Ctrun</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63</td>
<td>0.011</td>
<td>6.28</td>
<td>7.53</td>
<td>11.728</td>
<td>777.35</td>
<td>3.392</td>
<td>166.72</td>
<td>0.529</td>
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<td>63</td>
<td>0.003</td>
<td>5.00</td>
<td>7.51</td>
<td>11.564</td>
<td>1759.32</td>
<td>3.522</td>
<td>644.318</td>
<td>0.442</td>
<td>0.549</td>
<td>1.238</td>
</tr>
<tr>
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<td>7.48</td>
<td>10.588</td>
<td>936.86</td>
<td>4.252</td>
<td>411.66</td>
<td>0.547</td>
<td>0.579</td>
<td>1.368</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
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<td>7.67</td>
<td>11.666</td>
<td>1823.85</td>
<td>3.256</td>
<td>641.56</td>
<td>0.896</td>
<td>0.498</td>
<td>1.299</td>
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<tr>
<td>5</td>
<td>75</td>
<td>0.05</td>
<td>6.87</td>
<td>7.68</td>
<td>11.924</td>
<td>985.54</td>
<td>2.07</td>
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<td>0.379</td>
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<td>0.027</td>
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<td>7.57</td>
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<td>402.04</td>
<td>2.412</td>
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<td>3.379</td>
</tr>
<tr>
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<td>0.017</td>
<td>1.15</td>
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<td>057.11</td>
<td>1.234</td>
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<td>7.63</td>
<td>12.135</td>
<td>367.99</td>
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<td>733.378</td>
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</tr>
<tr>
<td>Mean</td>
<td>63.75</td>
<td>0.018</td>
<td>5.85</td>
<td>7.66</td>
<td>10.818</td>
<td>839.88</td>
<td>2.59</td>
<td>267.749</td>
<td>0.819</td>
<td>0.778</td>
<td>2.360</td>
</tr>
</tbody>
</table>

**Fig. 1:** Relationship between diuresis and levofloxacin renal clearance

**Fig. 2:** Relationship between plasma concentration of drug and renal clearance of levofloxacin

1999). So, the values of blood pH and urine pH of the study is slightly lower than the earlier studies. The average ± SE diuresis value is 0.018 ± 0.0056 ml min^-1 kg^-1, and it is lower than those recorded by Naseem (1999) earlier in winter season which ranged from 0.18 ± 0.048 ml min^-1 kg^-1. The difference is due to environmental difference because in hot climate the evaporation decreases urine flow while, while lower environmental temperature increases the rate of urine drainage in winter (Navaz and Shah, 1984). Creatinine is an end product of creatine metabolism in muscle (Guyon, 1991). Creatinine clearance approximates glomerular filtration rate (GFR), especially when the kidney function is in the normal range (Beirne et al., 1984 and Arant et al., 1972). The clearance of creatinine is only slightly higher than GFR and as a result, creatinine clearance can be used an approximation of filtration rate (Kassiver, 1971). Average ± SE value of creatinine clearance in this studies is 0.519 ± 0.068 ml min^-1 kg^-1. It is slightly lower than the values reported earlier for male volunteers i.e., 0.936 ± 0.172 ml min^-1 kg^-1 (Ghaffar, 1999) and for female volunteers i.e., 1.13 ± 0.073 ml min^-1 kg^-1 (Naseem, 1999). The average ± SE value for the renal clearance of levofloxacin was 0.778 ± 0.110 ml min^-1 kg^-1. The present value is lower than the renal clearance values 1.79 ml min^-1 kg^-1 and 1.74 ml min^-1 kg^-1 body weight as reported by Chein et al. (1997) and Lee et al. (1997), respectively due to difference of genome, environment and temperature. Average ± SE values for the renal clearance ratio was 2.360 ± 0.245. From the data (Table 1), it is clear that clearance of levofloxacin was higher than the endogenous creatinine clearance or (GFR). This indicates that active tubular secretion of drug takes place. With increase in the diuresis, the renal clearance of levofloxacin also increase (Fig 1). It indicates that at slower diuresis, the back diffusion of drug was higher and thus the renal clearance was lower but with an increase in the rate of urine flow, the increase in drug clearance indicates wash out phenomena when the drug gets shorter time period for re-absorption but statistically there is non-significant positive correlation (R^2 = 0.2647) between these two parameters. Plasma concentration of levofloxacin was plotted against the renal clearance of the drug to observe the correlation between the two parameters (Fig 2). The Fig. 2 shows that non-significant (R^2 = 0.0828) negative correlation between the two parameters, but with an increase in plasma concentration there is decrease in the renal clearance, which indicates for active tubular secretion of drug. These variations in results supports the previous findings that there is a need to evaluate the imported drug under indigenous conditions for getting optimal therapeutic results. It is anticipated that such data derived from future studies of levofloxacin, as well as other fluorquinolones, will be directly applicable to maximizing the therapeutic response achieved during clinical use of these agents.
Waheed et al.: Levofloxacin renal clearance

References

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