Plasma and Saliva Concentrations of Rifampicin in Man after Oral Administration

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The saliva and plasma concentrations of rifampicin were determined following oral administration of 600mg of the drug to healthy human subjects. Rifampicin is a zwitterion with PKa's of 1.7 and 7.9. The saliva-plasma concentration ratios calculated on the basis of the PKa of 1.7 was 1.6346. The saliva concentrations were much higher than the MICs of a variety of organisms, indicating the possible usefulness of rifampicin in the treatment of susceptible oropharyngeal and nasopharyngeal pathogens. After 24 hrs, when rifampicin was completely absent in the urine, the saliva and plasma concentrations also had fallen almost to zero.

(Key words: rifampicin, plasma & saliva concentration)

INTRODUCTION

Rifampicin is a semi-synthetic derivative of the antibiotic rifamycin. It is bactericidal and is mainly employed in the management of leprosy and tuberculosis. Effective chemotherapy of bacterial infections with antibiotics requires that effective concentrations of the drug be present at the site of infection (1). Devine et al. (2) stated that drugs must be present in saliva in measurable concentrations to be effective in the elimination of Neisseria meningitidis from the nasopharynx. In a similar report, Hoeprich (3) reported that antimicrobials which attain significant concentrations in saliva and tears were those which showed clinical efficacy in eradicating Neisseria meningitidis from the nasopharynx of carriers. From the aforementioned studies, investigations dealing with the concentration of rifampicin in saliva may have added clinical significance with regard to treating oropharyngeal and nasopharyngeal infections.

Drugs administered orally are known to pass from the gastrointestinal tract into the circulatory system, then back into the gastrointestinal tract, by different routes. The major pathway of drugs from the circulatory system back to the gastrointestinal tract is by biliary excretion. Apart from this route, drugs may pass directly into the gastrointestinal tract from the blood stream, depending on the drugs, PKa and the environmental pH. Bases with PKa greater than 5 can pass into the stomach contents in high concentrations, whereas weak acids are found in minimal concentrations. Additionally, drugs can enter the gastrointestinal tract via salivary excretion. The transport mechanism here is primarily by passive diffusion of the non-ionized moiety. The present study investigated the salivary, plasma and urinary levels of rifamicin, with a view to predicting saliva-plasma concentration ratios.

Study Design

Six healthy male volunteers, aged 19 to 28 years, participated in the study after giving informed consent. Subjects' weights ranged from 58 to 75kg. The health status of the subjects was reviewed with a medical history. Prior to the investigation, subjects were not taking rifampicin, reported no sensitivity to the drug, had no history of leprosy or tuberculosis, and...
had not consumed rifampicin within one week before the study.

The study was initiated with a single oral dose of 600mg of rifampicin (Ciba-Geigy), equivalent to two standard doses of rifampicin capsules. This was ingested with 350ml of distilled water. The study commenced at 0800 hours after an overnight fast. Blood samples were collected through an indwelling heparinised catheter just before, and approximately 0.5, 1, 2, 4, 8, 12, and 24 hrs after, rifampicin ingestion. Saliva samples were also collected at the same time intervals. The blood samples were immediately centrifuged at, 3000 r. p. m for 10 minutes and the plasma collected and stored frozen at -20°C until analysis. Urine collections were also made just before, and 0.5, 1, 2, 4, 8, 12, and 24 hours after, rifampicin intake. The volume of urine collected at the various time intervals were recorded. Two ml of each sample was stabilized with 0.2ml of toluene and frozen at -20°C until analysis.

Plasma, saliva, and urine rifampicin concentrations were determined by the spectrometric method of Sunahara and Nakagawa (4). About 0.5ml of each of these biological fluids were added to test tubes containing 1ml of distilled water and 1.5ml of phosphate buffer (pH 7), after which 2.0ml of iso-amyl alcohol was added. The samples were then analysed at 475nm with a Sp/6 Pye Unicam spectrophotometer. The concentration of rifampicin present in each sample was calculated from a calibration curve determined from known concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.125 μg/ml.

Analysis of Data

To analyze the data we assumed that rifampicin elimination followed linear kinetics in the concentrations encountered. For each subject, a plasma and saliva concentration versus time curve was plotted. Elimination rate constants were determined with the least square linear regression in each subject, using the last four points in the terminal phase of the curve (i.e 4-24 hours). The rifampicin half life was calculated from the relationship

\[ t_{1/2} = -\frac{0.693}{K} \]  
(1)

The area under the curve (AUC₀-24 hrs) was determined using the trapezoid rule, while AUC to infinity was calculated as

\[ \text{AUC}_{0-24} = 24 \frac{C_{24 \text{hrs}}}{K} \]  
(2)

Clearance (Cl) was calculated by the product of K and the volume of distribution (vd), while the maximum concentrations and time to attain these concentrations, Cmax and Tmax, were obtained directly from the experimental data. The amount of rifampicin recovered was calculated from the cumulative urinary excretion data. The differences between sample means were analyzed using student’s t-test for paired data, with statistical significance defined at P<0.05. The saliva-plasma concentration ratio was calculated using the equation proposed by Matin et al. (5), for the prediction of saliva-plasma concentration ratios (R) for acids and bases given by

\[ R = \frac{1+10(\text{pH}s-\text{PKa})}{1+10(\text{pHp}-\text{PKa})} \times \frac{\text{fp}}{f_s} \]  
(3)

where pHs = Saliva pH, pHp = Plasma pH, fp = Fraction of unbound drug in plasma, and fs = Fraction of unbound drug in saliva.

This equation has been utilized to predict the R values for the acidic sulphamethoxazole and the basic Trimetoprim by Eatman et al. (1).

RESULTS AND DISCUSSION

Measurable rifampicin concentrations were detected in all plasma and saliva samples. Figure 1 shows the saliva and plasma rifampicin levels. Correlation coefficients for the elimination decay curve from 4 to 24 hrs ranged from 0.958 to 1.00. The different pharmacokinetic parameters are shown in Table 1. The rifampicin half lives, times to reach peak concentration, and elimination rate constants did not show significant differences. The observed experimental half lives (t1/2) in this study are in conformity with those obtained in previous studies by Orisakwe et al. (6) and Furesz et al. (7).

The plasma values for peak concentration, area under the curve from zero to 24 hrs, and zero to infinity were significantly higher than those of saliva. The saliva clearance was, however, significantly higher than the plasma clearance. The mean serum level at 2 hrs of maximum concentration of the drug was
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**Table 1** Rifampicin pharmacokinetic parameters in saliva and plasma after oral administration of 600mg rifampicin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Saliva</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (mcg)</td>
<td>17.8 ± 5.1</td>
<td>11.6 ± 4.9</td>
<td>P ≤ 0.05</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>2.0</td>
<td>2.0</td>
<td>*NS</td>
</tr>
<tr>
<td>t_{1/2} (hr)</td>
<td>2.26 ± 0.3</td>
<td>2.27 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>K</td>
<td>0.31 ± 0.2</td>
<td>0.31 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>CL (ML/kg/hr)</td>
<td>6.2 ± 1.1</td>
<td>12.0 ± 1.9</td>
<td>P ≤ 0.05</td>
</tr>
<tr>
<td>AUC$_{\text{0-24hr}}$ (mcg/hr/ml)</td>
<td>94.15 ± 18</td>
<td>49.68 ± 9</td>
<td>P ≤ 0.05</td>
</tr>
<tr>
<td>AUC$_{\text{0-\infty}}$ (mcg/hr/ml)</td>
<td>96.76 ± 12</td>
<td>50.01 ± 11</td>
<td>P ≤ 0.05</td>
</tr>
</tbody>
</table>

+ = data expressed as Means ± SEM  
*NS = Not significant

Fig. 1 Plasma and saliva rifampicin concentrations at timed interval in 6 subjects following oral administration of 600mg rifampicin.

17.8 ± 5.10 µg/ml compared to 11.6 ± 4.90 µg/ml for the saliva. At 4 hrs, however, the plasma and saliva concentrations were almost identical (Fig. 1). The saliva concentrations reached were much higher than the MICs of a variety of causative pathogens, indicating a possible usefulness of rifampicin in the treatment of susceptible oropharyngeal and nasopharyngeal pathogens. A similar experimental finding has been reported for a fluoroquinolone antibiotic, ofloxacin (8). Here, a correlation of the serum and sputum levels of ofloxacin was taken as an indication of the usefulness of the drug in the pharmaco-therapeutic management of respiratory tract infections.

The relationship between the cumulative
Fig. 2 The relationship of the changes in the plasma and saliva levels of rifampicin and the cumulative urinary excretion of rifampicin after oral administration of 600mg.

amount of rifampicin excreted in the urine and the saliva-plasma level time curve is shown in Figure 2. It is evident from this figure that at 24 hrs, when rifampicin is completely absent in the urine, the saliva and plasma concentrations also are almost zero.

Rifampicin is a zwitterion, with PKa 1.7 related to the 4-hydroxy and PKa 7.9 related to the 3-piperazine nitrogen (9). The saliva-plasma rifampicin concentration ratio was calculated as 1.6346 based on the PKa of 1.7. F_s and f_p in equation 3 were 0.2917 and 0.5644, respectively, and the pH_p was equal to 7.4. The value of pHs was taken to be 6.5, the mean pH of saliva samples reported by Matin et al. (5) These calculated values differ from the value of 1.9348 obtained from the experiment. The Matin equation presumed no partitioning of the drug, whereas Eatman et al. (1) maintained that discrepancies between the experimental and calculated values of the saliva-plasma concentration ratios of sulphamethaxazole and Trimetoprim may have resulted from partitioning of the drugs.

Other experiments have shown that the pH values of frozen saliva samples are approximately 1 pH unit greater than would have obtained had measurements been made immediately after collection, an effect which has been attributed to carbon dioxide loss on freezing (1). The results obtained in the present study further indicate the necessity of studying the pH of freshly collected saliva and investigating buccal partitioning as possible factors influencing the saliva-plasma drug concentration ratio.

REFERENCES


