Effect of Picroliv on the Pharmacokinetics of rifampicin in rats

ANIL KUMAR DWIVEDI, RAVI RASTOGI, SATYAWAN SINGH AND BHOLA NATH DHAWAN
ICMR Centre for Advanced Research in traditional Remedies,
Central Drug Research Institute, Lucknow - 226 001.

The pharmacokinetics of rifampicin (50 mg/kg i.p. daily for 6 days) was studied in control rats and those simultaneously treated with 12 mg/kg Picroliv, a standardised iridoid glycoside mixture from roots and rhizomes of Picrorhiza kurroa. Picroliv did not modify the pharmacokinetics of rifampicin.

Key Words: rifampicin, picroliv, hepatotoxicity, hepatoprotection, pharmacokinetics

RIFAMPICIN is an important drug for the chemotherapy of mycobacterial infections such as tuberculosis and leprosy but with a major side effect of hepatotoxicity. Picroliv is a standardised iridoid glycoside mixture obtained from the roots and rhizomes of Picrorhiza kurroa has demonstrated protective activity against various hepatotoxicants in rats and Plasmodium berghei infection in Mastomys natalensis. Earlier studies also demonstrated that Picroliv protected the levels of DNA, RNA and proteins effectively which were either decreased by monocrotaline and aflatoxin B1 or increased by thioacetamide. It has been recently reported that most of the biochemical changes after rifampicin (50 mg/kg i.p x 6 days), such as changes in γ-glutamyl transpeptidase, succinate dehydrogenase, acid phosphatase, acid ribonuclease, RNA, total proteins, glycogen, total lipids, phospholipids, cholesterol and bilirubin in liver and bilirubin and total proteins in serum, were significantly prevented following simultaneous oral administration of Picroliv (12 mg/kg x 6days) in rats. Therefore to establish that this is not due to the lower blood levels of rifampicin, which are not desirable in clinical situation, the following bioequivalence studies of rifampicin in normal rats and those receiving Picroliv concurrently were taken up.

MATERIAL AND METHODS

Male albino rats (150±10 g, sprague Dawley Strain) obtained from C.D.R.I. animal house were used in this study. The animals were fed a standard pellet diet ad Libitum and had free access of water. Rifampicin I.P. were obtained from Lupin Laboratory, Aurangabad, India. Picroliv Batch No.5K was obtained from the Chemical Technology Division of this Institute. HPLC grade methanol, chloroform and acetonitrile were produced from E. Merck (India) Ltd., Bombay and L-ascorbic acid AR grade from S.D. fine Chem. Ltd., Boisar (India). Triple distilled water from quartz glass apparatus was used. All other reagents like disodium hydrogen phosphate and potassium dihydrogen phosphate obtained from Qunalgens Fine Chem. Bombay were of analytical grade. All glass-ware, were washed with detergent, rinsed thoroughly with triple distilled water and then dried prior to use.

Drug Administration

The rats were divided into two groups each containing three animals. The rats of both groups received a daily i.p. injection of 50 mg/kg rifampicin (50 mg/ml in DMSO) for 6 consecutive days. Picroliv (12 mg/kg in normal saline) was administered orally
to rats of group II concurrent with rifampicin administration. The blood samples from retro-orbital plexus were collected on first day immediately after the first injection for zero hour reading and on 6th day at 1, 2.5, 4, 5.5, 7, 15 and 24 hrs after rifampicin injection and kept for half an hour to obtain serum. This time schedue and route of administration was same as reported in the literature for pharmacokinetic of rifampicin in rats.

Estimation of rifampicin in the blood
(a) Apparatus and chromatographic conditions:

The HPLC instrument consisted of a Perkin Elmer 250 binary pump (Perkin Elmer, USA), a Rheodyne model 7125 injector with a 20 loop (Rheodyne USA) and Perkin Elmer 235 diode array detector (Perkin Elmer, USA). Separation was achieved on a C-18 cartridge column (E. Merck, India Ltd.) preceded by a 2 cm precolumn (Perkin Elmer, USA), with a mobile phase containing acetonitrile and phosphate buffer pH 3.0 in ratio of 40:60 at a flow rate of 1.5 ml/min. The column effluent was monitored at a wavelength of 255 nm, chromatograms were recorded by GP 100 printer plotter (PerkinElmer, USA). Under these conditions the retention time of rifampicin was about 8 minutes.

(b) Stock and standard solutions

A stock solution of rifampicin (100 mg/ml) was prepared by dissolving 2.5 of rifampicin in 25 ml methanol. Working standards were prepared in methanol from stock solution in the range of 0.2 ug/ml to 2 ug/ml by sequential dilution method. Calibration samples of rifampicin were prepared by adding varying volumes of standard solutions of rifampicin to normal rat blood and keeping them for 30 minutes at room temperature.

(c) Assay procedure:

Two hundred and fifty µL of serum was mixed with 0.5 ml of methanol and 0.5 ml of 2% ascorbic acid solution and vortexed. The drug was extracted by shaking with 2x5 ml chloroform for 5 minutes. The chloroform extract was washed with 4 ml of 2% ascorbic acid solution and evaporated to dryness under nitrogen. The residue was dissolved in known amount of methanol and after appropriate dilution, 20 µl aliquot was injected onto the HPLC column. Quantification was done by external standard method.

RESULTS AND DISCUSSION

Calibration curves were obtained by plotting peak heights of rifampicin against their corresponding concentrations in spiked blood. The response was linear over a concentration range of 16-120 ng/mL in the serum. No interfering peaks were detected in control rat serum samples or in samples from the test animals. Reproducibility of the method was checked by calculating percent relative standard deviations (% RSD) of intra and inter assay variations (n=3) of concentrations found of rifampicin. An acceptance limit of 20% irrespective of the concentration level was applied. The values obtained were within acceptable limits. The accuracy of the method was
<table>
<thead>
<tr>
<th>Parameter</th>
<th>PICROLIV UNTREATED (n=3)</th>
<th>PICROLIV TREATED (n=3)</th>
<th>P(0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>ug.h/ml</td>
<td>334.3 ± 24.3</td>
<td>355.3 ± 64.2</td>
</tr>
<tr>
<td>AUMC</td>
<td>ug.h²/ml</td>
<td>1278.7 ± 131.5</td>
<td>1421.9 ± 396.6</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>3.82 ± 0.11</td>
<td>3.96 ± 0.37</td>
</tr>
<tr>
<td>Ke</td>
<td>l/h</td>
<td>0.243 ± 0.044</td>
<td>0.223 ± 0.013</td>
</tr>
<tr>
<td>T (el)</td>
<td>h</td>
<td>2.92 ± 0.58</td>
<td>3.12 ± 0.18</td>
</tr>
<tr>
<td>Cl</td>
<td>ml/h</td>
<td>22.5 ± 1.59</td>
<td>21.5 ± 3.65</td>
</tr>
<tr>
<td>Vd</td>
<td>ml</td>
<td>94.1 ± 11.9</td>
<td>96.6 ± 15.0</td>
</tr>
</tbody>
</table>

assessed by percent deviation from actual concentration. This was also found within the acceptance limit \(^1\) (i.e. 15%).

Basic pharmacokinetic parameters were derived by model independent analysis \(^1\). AUC was calculated by trapezoidal method and extrapolated to infinity. The terminal half-life (t/2) was calculated from the regression line fitted to a semilogarithmic plot of the serum concentration time profile by the method of least squares.

The mean plasma concentration-time curves, which are comparable with the published literature, of rifampicin after i.p. administration of 50 mg/kg dose of rifampicin with and without picroliv treatment is shown in Fig.1 and the relevant pharmacokinetic parameters are listed in Table 1. The mean terminal half-lives are 2.92 hr and 3.12 hrs for picroliv untreated and picroliv treated rats respectively. The AUC are 1278.7 and 1421.9 ug.h/ml for both the groups respectively. Similarly the values of MRT, Ke Cl and Vd are not significantly (p 0.05) different between the two groups of rats (Table 1). This indicates that pharmacokinetics and bioavailability of rifampicin is not altered by simultaneous administration of picroliv.

The results indicate that there is no significant change in pharmacokinetics of rifampicin when administered with Picroliv and the pharmacokinetic parameters show that the protection of liver by picroliv is not due to the altered pharmacokinetics of rifampicin. Therefore picroliv may be useful in management or prevention of rifampicin induced hepatotoxicity in patients without affecting the bioavailability of rifampicin.

ACKNOWLEDGEMENT

The technical assistance of Mrs. M. Chaudhary is acknowledged.

REFERENCES


