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Evidence of Efflux-Mediated and Saturable Absorption of Rifampicin in Rat Intestine Using the Ligated Loop and Everted Gut Sac Techniques

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Abstract: This study was carried out to explore whether efflux-mediated and saturable mechanisms play any role toward poor and variable intestinal absorption of rifampicin. In situ segmental permeability of rifampicin at various residence times was determined in rat gastrointestinal tract using the ligated loop technique. The involvement of efflux-mediated and saturable absorption of rifampicin was studied in rat intestine using the everted sac method. The samples were analyzed by a validated HPLC method. Rifampicin showed decreased permeability in jejunum and ileum with an increase in residence time. The permeation of rifampicin from the serosal to the mucosal side (secretion) was significantly higher than permeation from the mucosal to the serosal side (absorption) of jejunum and ileum. This indicated the involvement of efflux-mediated transport. Addition of verapamil, an inhibitor for P-glycoprotein (Pgp), multidrug resistance associated protein-2 (MRP-2), and other related transporters, increased absorption of rifampicin in jejunum and ileum by 2−3-fold and decreased secretion by almost 4-fold. The permeation rate (flux) of rifampicin through duodenum increased with concentration up to 300 μg/mL, becoming constant thereafter, indicating the existence of saturable absorption. There was no saturable permeation in jejunum and ileum. Thus the present study indicates the involvement of efflux-mediated and saturable absorption mechanisms of rifampicin in rat intestine, which act as barriers to the absorption of the drug. This explains the drug's poor absolute bioavailability. As Pgp varies from person to person to an extent of 2−8-fold, it can be one direct reason for the interindividual variable bioavailability shown by rifampicin.

Keywords: Rifampicin; saturable absorption; p-glycoprotein; efflux; poor and variable bioavailability

Introduction

Rifampicin is one of the major effective broad-spectrum antimicrobial agents used in the chemotherapy of tuberculosis (TB) and leprosy. It inhibits bacterial RNA polymerase by blocking the path of the elongating RNA. The daily dose of rifampicin is 10 mg/kg body weight, and the absolute bioavailability of rifampicin is reported to be 50−68% of this dose.[1−3] Along with the low absolute bioavailability, the drug also shows variable intersubject bioavailability. The C_{max} values have been reported to range between 8 and 24 μg/mL in various bioavailability studies.[4−7] A few studies have shown enhancement of rifampicin bioavailability, when

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the drug was administered along with lipid vehicles or piperine.[8,9] Lipid vehicles such as sesame oil and triglycerides improved bioavailability of the drug by solubilizing the organic anion transporter protein (OATP), which is involved in biliary recirculation of the drugs.[8] The mechanism of action of piperine is not clear yet.[9]

Recently, we reported the permeability behavior of rifampicin in different gastrointestinal segments of rat using the ligated loop technique.[10] Evidence was presented on the saturable absorption mechanism of rifampicin in the duodenum. Also, a decrease was observed in absorption of the drug in parts of intestine distal from stomach and duodenum. The same could be attributed to p-glycoprotein (Pgp) and other efflux transporters, whose concentration increases from duodenum to jejunum and ileum.[11,12] A recent study on TB patients[13] showed that rifampicin serum levels increased to an extent of 158% after pretreatment with a Pgp inhibitor. Otherwise, rifampicin itself is a well-known inducer of Pgp and cytochrome P-450 (CYP).[14,15]

Thus looking into the distinct possibility that efflux-mediated and saturable mechanisms play a negative role in absorption of rifampicin from intestine, studies were carried out to explore their contribution toward low absolute bioavailability of the drug.[1−3] For the purpose, permeability studies were first carried out using the ligated loop technique at different residence times. This experiment is known to provide indication on the involvement of efflux mechanism during absorption of the drugs.[16] Subsequently, studies were conducted using a rat everted gut sac method, which allows determination of permeability of drugs both from mucosal and serosal sides of the intestine at different drug concentrations.[17] No studies exist yet on the use of these or even other models (Ussing chamber, Caco-2 cells, etc.) to determine absorption behavior of rifampicin from intestine.

Materials

Rifampicin and isoniazid were gift samples from Panacea Biotec Ltd., Lalru, India. Verapamil was a gift sample from Torrent India Ltd., Ahmedabad, India. Buffer materials and all other chemicals were of analytical-reagent grade. HPLC grade acetonitrile and methanol were procured from J. T. Baker (Mexico City, Mexico) and Mallinckrodt Baker Inc. (Paris, KY), respectively. Ultrapure water was obtained from an ELGA water purification unit (Elga Ltd., Bucks, England).

Equipment

The following equipment was used in the present study: autopipets (Tripette, Merck KGaA, Darmstadt, Germany), research pH meter (MA 235, Mettler Toledo GmbH, Schwerzenbach, Switzerland), analytical balance (AG 135, Mettler Toledo, Switzerland), digital shaking water bath (Julabo SW21, Seelbach, Germany), homogenizer (PT-MR 3100 POLYTRON, Kinematica AG, Littau, Switzerland), sonicator (Branson Ultra-sonic Corporation, Danbury, CT), centrifuge (Biofuge 15, Hanau, Germany), and vapor pressure osmometer (Vapro, Salt Lake City, UT). The HPLC system consisted of an on-line degasser (DGU-14AM), low-pressure gradient flow control valve (FCV-10AL$_{VP}$), solvent delivery module (LC-10AT$_{VP}$), auto injector (SIL-10AD$_{VP}$), column oven (CTO-10AS$_{VP}$), UV−visible dual-wavelength detector (SPD-10A$_{VP}$), system controller (SCL-10A$_{VP}$), and CLASS-VP software (all from Shimadzu, Kyoto, Japan).

Methods

HPLC Analyses. In all permeability experiments, rifampicin was analyzed by HPLC, employing a validated gradient method reported by us earlier.[18] The separations were

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achieved on a Zorbax XDB C-18 column (250 × 4.6 mm, 5 μm, Agilent Technologies), and the mobile phase used was composed of acetonitrile and a buffer consisting of 0.01 M sodium dihydrogen orthophosphate (pH adjusted to 6.8 with dilute orthophosphoric acid).

Ligated Loop Segmental Permeability Studies at Various Residence Times. For these experiments, the same procedures as described in our previous publication[10] were followed. Studies were conducted for 30 and 90 min in various segments of rat gastrointestinal tract (GIT) (viz., stomach, duodenum, jejunum, and ileum). Sprague−Dawley rats in the weight range 240−260 g were used under approved protocol of the Institutional Animal Ethics Committee (Protocol No. IAEC/01/109). A separate set of animals ($n = 7$) was used for each time period study.

Everted Gut Sac Studies. These studies were also carried out on the same type and weight range of rats, under approved protocol of the Institutional Animal Ethics Committee (Protocol No. IAEC/03/04). The animals were fasted for 12−16 h and were sacrificed by cervical dislocation. A midline incision was made in the abdomen, and the small intestine was removed. After washing with normal saline, underlying muscularis was removed and the intestine was segregated into different segments. The duodenal segment was obtained by cutting 1 cm from the pylorus, the jejunum was obtained by cutting between the duodenum and the ileum, and the ileal segment was cut about 5 cm above the ileo−cecal junction. Each segment was reduced to 5 cm and washed before use.

For estimation of permeation of rifampicin from the serosal to the mucosal side (secretion), the intestinal segments were used directly, while for determining permeation from the mucosal to the serosal side (absorption), the segments were everted and washed with saline. The proximal end of each segment was ligated with a glass receptor tube. The distal end was directly ligated to create a closed compartment. A 1 g stainless steel weight was tied to the distal end to maintain the sac in a vertical position during the experiment. The segments so prepared were suspended in separate permeation assemblies consisting of 25 mL test tubes, which contained 20 mL solutions of the test substances in Krebs Ringer buffer (KRB). The segments were dipped in a manner that the whole of the outer wall of the intestine was exposed to the drug solution. The assemblies were maintained at 37 °C in a water bath, and the donor compartment in each case was bubbled continuously with oxygen. Once the conditions of all three assemblies were equilibrated, 2 mL of the blank KRB was filled into the sacs through the receptor tubes and the same was withdrawn after every 15 min using a hypodermic syringe attached to a long needle, and replaced with fresh buffer. The samples were collected until 90 min. These were filtered and analyzed by HPLC.

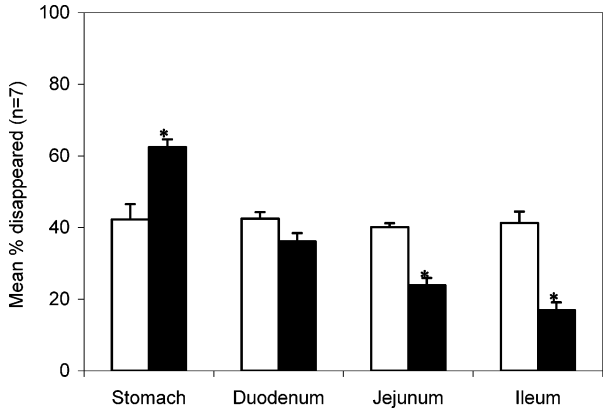

Figure 1. Disappearance of rifampicin from stomach, duodenum, jejunum, and ileum of rat at 30 min (□) and 90 min (■) using the ligated loop technique in rat GIT. Asterisks (*) indicate significant difference from the disappearance of rifampicin from the particular segments at 30 min ($P < 0.05$).

For determining efflux-mediated absorption, rifampicin was used at a concentration of 100 μg/mL. Experiments were also conducted with verapamil, which was used at a concentration of 0.2 mM[19] along with rifampicin. For the study of saturable absorption, rifampicin concentrations were 50, 100, 200, 300, 400, and 500 μg/mL.

Permeation rate (flux) was determined from the slope of the plot of cumulative amount permeated against time. Apparent permeability coefficient (P_{app}) was determined by dividing flux with initial drug concentration.

Results and Discussion

Efflux-Mediated Transport of Rifampicin. Figure 1 shows the permeability behavior of rifampicin in various segments of GIT at residence times of 30 and 90 min using the ligated loop technique. The figure projects that compared to stomach, where the disappearance of rifampicin increased on increase in residence time, there was an opposite trend in duodenum, jejunum, and ileum. Compared to duodenum, the fall differed significantly at each time period in the two distal intestinal segments.

The overall behavior in Figure 1 exactly parallels the concentration of efflux transporters in the GIT. The transporters, particularly Pgp, are absent in stomach and increase from duodenum to ileum.[12] The higher disappearance in intestinal segments at 30 min than 90 min indicates that the drug rapidly enters the intestinal tissues, but effluxes out as the time progresses. This correlates with the efflux transporter-mediated theory, which is said to be behind the poor absorption of drugs from intestine.[16] The existence of the same mechanism during absorption of rifampicin from intestine is also indicated from P_{app} values of rifampicin, determined through the everted sac technique (Figure 2). It

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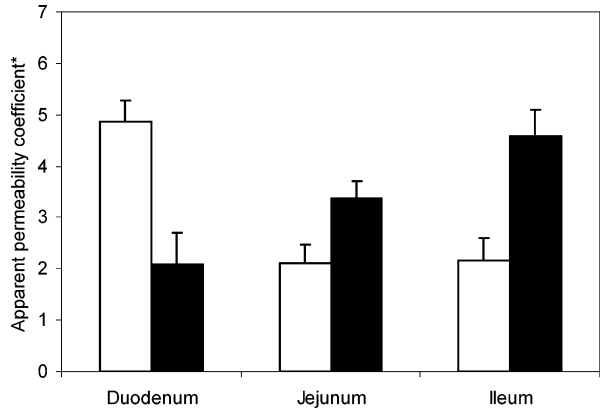

Figure 2. Mucosal to serosal (□) and serosal to mucosal (■) permeation of rifampicin in various segments of rat everted gut sacs. P_{app} values are $\times 10^{-6}$ cm/s.

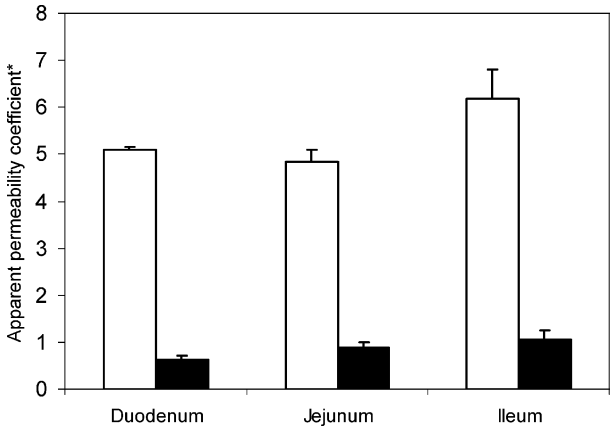

Figure 3. Mucosal to serosal (□) and serosal to mucosal (■) permeation of rifampicin in various segments of rat everted gut sacs in the presence of 0.2 mM verapamil. P_{app} values are $\times 10^{-6}$ cm/s.

is clearly evident that permeation of rifampicin is in the order duodenum > jejunum = ileum, for studies done from the mucosal to the serosal side. However, the order changes to duodenum < jejunum < ileum in serosal to mucosal permeation, which again is in accordance with the concentration of Pgp in different GIT segments.[12]

Figure 3 depicts the behavior of permeation of rifampicin from the mucosal to the serosal and from the serosal to the mucosal sides, in the presence of verapamil, a known inhibitor of Pgp and other efflux transporters. It clearly indicates that the absorption of rifampicin from the mucosal side of the jejunum and the ileum is increased to an extent of 2−3-fold, while the absorption decreases 4-fold from the serosal to the mucosal side in the same segments. This might be due to the inhibition of the transporters by verapamil. This may further confirm that rifampicin absorption in the distal parts of intestine is restricted by transporter-mediated efflux mechanism. Studies on the role of specific transporters, viz., Pgp, BCRP, MRPs, etc.,[20] remain to be done.

No evidence was presented on the presence of site dependent biotransformation of rifampicin, as no metabolites

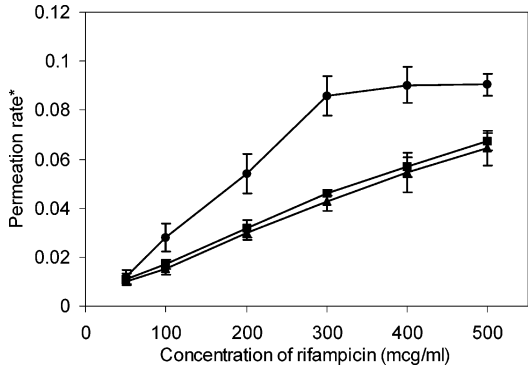

Figure 4. Permeation rates (flux) of rifampicin at different concentrations through rat everted sacs of duodenum (●), jejunum (■), and ileum (▲). Permeation rates are expressed in μg/cm^2.

such as 25-desacetylrifampicin and 3-formylrifamycin were found in the solutions taken from donor or receptor compartments.

Saturable Absorption of Rifampicin in Intestine. Figure 4 shows the flux values of rifampicin in different segments of intestine at various drug concentrations. Evidently, rifampicin permeates to a higher extent through the duodenum in comparison with the jejunum and the ileum. This observation is in agreement with results of a previous report on the segmental permeability of rifampicin employing the ligated-loop technique.[10] But the curve in the duodenal segment reaches a plateau at rifampicin concentrations ≥300 μg/mL, indicating that drug permeation through the duodenum is saturable. It means that to whatever amount rifampicin is available at the duodenum for absorption, only a particular amount of the drug gets absorbed. It means that it acts as another barrier to the absorption of rifampicin, particularly in the proximal part of the intestine. The curves for the jejunum and the ileum are linear, indicating that saturable absorption of rifampicin does not prevail in the distal parts of the intestine.

Conclusion

This study clearly shows that rifampicin undergoes saturable and efflux-mediated absorption in the proximal and distal parts of the intestine, respectively. These reasons, combined with drug decomposition in gastric medium,[21] may

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be responsible for the poor absolute bioavailability of rifampicin when administered alone. Regarding the intersubject variability shown by the drug,[4−7] this can be as a result of differential expression of efflux transporters, like Pgp, in the intestine, which varies in humans to an extent of 2−8-fold.[22] Of course, other variable factors like pH of the stomach, gastric emptying rate, GIT motility, etc. are expected to make their contribution. These findings also explain why rifampicin shows much less intrasubject variability,[4−6] because most of the above-discussed factors remain constant in the same individual.

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