Rifampicin and Ketoconazole Modulation of Intestinal Transport of Carbamazepine in Rats

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ABSTRACT

Many drug substances interact with each other and affect the CYP enzyme system and transport properties of other drugs. The present study was aimed to investigate the effect of ketoconazole and rifampicin pre-treatment on the transport of carbamazepine across the intestine in rats. The transport of carbamazepine across different parts of intestine was studied by the everted and non-everted sac methods. The control and ketoconazole (80 mg/kg) and rifampicin (60 mg/kg) pre-treated rats were sacrificed and the intestine was harvested. The sacs of intestine were prepared, treated with carbamazepine solution and then placed in Dulbecco’s buffer. Samples were collected periodically and the drug content was estimated using HPLC method. The results show that there was a significant (p<0.05) difference in the transport of carbamazepine from the intestinal sacs of pretreated with ketoconazole and rifampicin as compared to control. It appears that ketoconazole and rifampicin may affect the intestinal transport of carbamazepine and hence may result in pharmacokinetic interactions.

KEYWORDS: Everted sac; Non-everted sac; Induction; Inhibition; CYP3A4.

Introduction

Many drug substances interact with each other and affect the CYP enzyme system and transport properties of other drugs. This could increase or decrease of the plasma drug concentrations. Drug-drug interaction may alter drug bioavailability through its effect on absorption, distribution and elimination. The CYP3A families of enzymes constitute the most predominant phase I drug metabolizing enzymes estimated to metabolize 50-70% of currently administered drugs. CYP3A4 is the most abundant form present primarily in the hepatocytes and enterocytes (Kolars et al., 1992). Carbamazepine has a potential for drug interactions; caution should be used in combining other medicines with it, including other antiepileptics and mood stabilizers. Lower levels of carbamazepine are seen when administrated with phenobarbital, phenytoin, or primidone. Carbamazepine, as a CYP450 inducer, may increase clearance of many drugs, decreasing their blood levels (Gonzalez Frank et al., 2006).

Ketoconazole is a synthetic antifungal and antiandrogenic drug used to prevent and treat fungal skin infections, especially in immunocompromised patients such as those with AIDS or those on chemotherapy. Likeazole antifungal agents, ketoconazole works principally by inhibiting the enzyme cytochrome P450 (Susan et al., 2005). Rifampicin is a bactericidal antibiotic drug of the rifamycin group. Rifampicin is an effective liver enzyme-inducer, promoting the upregulation of hepatic cytochrome P450 enzymes (such as CYP2C9 and CYP3A4), increasing the rate of metabolism of many other drugs that are cleared by the liver through these enzymes. As a consequence, rifampicin can cause a range of adverse reactions when taken concurrently with other drugs (Douglas, 1985). Hence it is important to assess this effect as there are few reports on CYP3A-mediated drugs interaction. Carbamazepine is an anticonvulsant which is used in the treatment of partial seizures, tonic-clonic seizures and trigeminal neuralgia. The present study was aimed to investigate the effect of ketoconazole and rifampicin pre-treatment on the intestinal transport of carbamazepine in rats.

Materials and Methods

Materials

Carbamazepine, ketoconazole and rifampicin were gifts from Dr. Reddy's Lab Ltd.(Hyderabad, India), Dulbecco's phosphate buffer pH of 7.4 (Hi Media Ltd Mumbai), Methanol, Acetonitrile (E. Merck Ltd Mumbai, India) and all chemicals used in this study were of AR grade.
Experimental animals

Male Wistar rats weighing about 200±25 g were selected and the study was conducted according to the protocol approved by animal ethics committee, Kakatiya University, India.

Study Methods

In vitro transport study

The transport of carbamazepine across rat intestine (duodenum, jejunum and ileum) was studied by using in vitro everted and non-everted sac methods (Ruan et al., 2006; Taher et al., 2004). The rats were treated separately with ketoconazole (80 mg/Kg) and rifampicin (60 mg/Kg) in groups of 3 for 7 days sacrificed, isolated the intestinal segments isolated and then the sacs were prepared. The drug solution (500 µg/ml) was placed in sac and kept in Dulbecco's buffer. Samples were collected at pre-set time points by replacing with fresh buffer and their drug contents were estimated using validated HPLC method. Control experiments were also performed.

Precipitation method

Methanol (100 µl) was added to intestinal sac samples (200 µl) vortexed on cyclomixer for 2 min, centrifuged at 2500 rpm for 15 min using Biofuge Fresco Centrifuge (Heraeus, Germany) and supernatant was separated. Twenty microlitres of the supernatant was taken into Hamilton syringe and injected into HPLC column.

HPLC Assay

Shimadzu HPLC system equipped with a LC-10AT pump and SPD 10 AT UV visible detector and RP C18 column (120 mm x 4.6 mm ID, particle size 5 µ) was used for the analysis of samples. The mobile phase was a mixture of methanol and water (50:50). The flow rate was 1 ml/min and the detection was carried out at 270 nm. The calibration curve was plotted in the range of 3.125-50 µg/ml. A linear relationship was observed between the concentration and the peak area of carbamazepine with a correlation coefficient (r^2 = 0.999). The required studies were carried out to estimate the precision and accuracy of the HPLC method of analysis of carbamazepine. The limit of detection was 0.25 µg/ml. The average recovery of the drug was 95.75%. The intraday R.S.D (%) was less than 7. 29% and the inter day R.S.D (%) was less than 7. 64% (n=5).

Statistical Analysis

The in vitro results were compared by student t-test using Sigma Stat Software (Jandel Scientific Sigma stat version 1, 1992-94). A value of P<0.05 was considered to be statistically significant.

Results and Discussion

In the present study, the amount of transport of carbamazepine from non-everted sac (mucosal to serosal surface) and everted sac (serosal to mucosal surface) was determined in different regions of the rat intestine of the control, ketoconazole and rifampicin treated groups (Table1). In rifampicin-treated group, carbamazepine transport decreasing in non-everted sac to about 19.23, 10.14 and 16.24% and increased in everted sac to about 13.67, 16.32 and 10.56% from duodenum, jejunum and ileum regions, respectively (Fig.1-6). In ketoconazole-treated group, carbamazepine transport increased in non-everted sac to about 19.25, 8.69 and 20.57% and decreased in everted sac to about 8.15, 20.61 and 9.51% from duodenum, jejunum and ileum regions, respectively (Fig.1-6).

| TABLE 1 |
| Cumulative concentrations of carbamazepine in µg/ml (Mean±S.D) transported from different parts of albino wistar rat small intestine in Ketoconazole and Rifampicin treatment (n=3) after 120 min. |

<table>
<thead>
<tr>
<th>Part of Intestine</th>
<th>Control Normal Everted</th>
<th>Rifampicin treatment Normal Everted</th>
<th>Ketoconazole treatment Normal Everted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>12.42±0.14 13.17±0.27</td>
<td>11.09±0.44† 14.23±0.67†</td>
<td>16.11±0.87† 11.58±0.39*</td>
</tr>
<tr>
<td>Jejunum</td>
<td>13.87±0.65 15.23±0.23</td>
<td>12.41±0.66† 17.12±0.75†</td>
<td>15.02±0.63* 11.75±0.42†</td>
</tr>
<tr>
<td>Ileum</td>
<td>9.57±0.78 14.07±0.31</td>
<td>7.94±0.77† 15.71±0.58†</td>
<td>11.44±0.71† 12.99±0.57*</td>
</tr>
</tbody>
</table>

*indicates P<0.05, †indicates P<0.01 when compared with control.

| TABLE 2 |
| The percentage of changes in cumulative amount of carbamazepine transport in Ketoconazole and Rifampicin treated groups. |

<table>
<thead>
<tr>
<th>Part of Intestine</th>
<th>Rifampicin treatment Normal (%) Everted (%)</th>
<th>Ketoconazole treatment Normal (%) Everted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>-19.23 13.67</td>
<td>-19.25 8.15</td>
</tr>
<tr>
<td>Jejunum</td>
<td>-10.14 16.32</td>
<td>8.69 20.61</td>
</tr>
<tr>
<td>Ileum</td>
<td>-16.84 10.56</td>
<td>20.57 -9.51</td>
</tr>
</tbody>
</table>
Fig. 1. Cumulative transport pattern of carbamazepine in duodenum normal sac in Wistar rats.

Fig. 2. Cumulative transport pattern of carbamazepine in jejunum normal sac in Wistar rats.

Fig. 3. Cumulative transport pattern of carbamazepine in ileum normal sac in Wistar rats.
Fig. 4. Cumulative transport pattern of carbamazepine in duodenum everted sac in Wistar rats.

Fig. 5. Cumulative transport pattern of carbamazepine in jejunum everted sac in Wistar rats.

Fig. 6. Cumulative transport pattern of carbamazepine in ileum everted sac in Wistar rats.
The results of this study show that the transport of carbamazepine in non-everted and everted sac methods was moderately reduced by the treatment of rifampicin and ketoconazole as compared to control. A growing number of studies have documented interaction of ketoconazole and rifampicin with drugs that are metabolized by the cytochrome P450 subfamily in particular by the CYP3A4 and CYP3A5 enzymes (Kyoung et al., 2012, Sebastian et al., 2012; Boyce et al., 2012; Mahatthanatrakul et al., 2012). Carbamazepine is mainly metabolized by CYP3A4 to active metabolite carbamazepine-10, 11-epoxide. Some reports have shown that carbamazepine regulates intestinal P-glycoprotein and multi-drug resistance protein and influences disposition of talinolol in humans (Giessmann et al., 2004). On the basis of a linear relationship between in vitro everted sac and the in vivo studies (Yumoto et al., 1998) it has been suggested that in vivo P-gp related drug-drug interactions can be predicted by in vitro everted sac studies.

It also suggested that drug-drug interactions related to P-gp mediated transport in human intestine could be predicted by in vivo exsorption across rat ileum or in vitro (everted rat intestine) transport study using rat ileum which are comparable with the transport studies in Caco-2 cell monolayers. The non-everted sac model was originally used to evaluate drug transport mechanisms (Kaul and Ritschel, 1981). Evered gut sac method also has been used to assess the role of P-glycoprotein on the intestinal secretion of the ivermectin (Ballent et al., 2006). Results of this in vitro study revealed that carbamazepine transport across the small intestine is much affected by ketoconazole and rifampicin pre-treatment. In this study, the mean cumulative absorption and exsorption concentrations of carbamazepine altered at a modest level after pre-treatment with ketoconazole and rifampicin in compared to control in duodenum, jejunum and ileum regions. This observation indicated the role of CYP3A4 on carbamazepine metabolism. The study revealed that pre-treatment with ketoconazole acts as CYP3A4 inhibitor and rifampicin acts as CYP3A4 inducer.

Conclusions

The rifampicin pre-treatment decreased intestinal permeation of carbamazepine which may indicate a likely pharmacokinetic interaction. The ketoconazole pre-treatment modestly increased intestinal permeation of carbamazepine which may be due to pharmacokinetic issues. Further studies are warranted to advance these findings in a large group size and more extensive design.

References


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