Impact of the changes in P-glycoprotein activity on domperidone pharmacokinetics in rat plasma

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Abstract: The effect of quinidine (QD) and grapefruit juice (GFJ) extract, P-glycoprotein inhibitors, on the domperidone (DOM) concentration in rat plasma was investigated. DOM, a dopamine D₂-receptor antagonist is a substrate for P-glycoprotein. DOM (10 mg/kg) was administered orally 2 h after GFJ extract (0.2 ml/kg) or QD (25 mg/kg). DOM concentration in plasma samples was determined by HPLC with fluorescence detection. The GFJ extract and QD administration significantly increased \(C_{\max}\) of DOM by 19% and 36%, respectively, and the AUC\(_{0-15}\) (area under the concentration-time curve from time zero to 15 min) by 29% and 44%, respectively. In addition, QD significantly increased the DOM AUC\(_{0-15}\) (32%), whereas 19% increase was observed after GFJ extract administration. In conclusion, GFJ and QD significantly influenced DOM rat plasma concentration during the first two hours after DOM administration indicating that interaction takes place during absorption phase.

Key words: domperidone, P-glycoprotein, quinidine, grapefruit juice

Abbreviations: AUC – area under the concentration-time curve, BBB – blood-brain barrier, DOM – domperidone, GFJ – grapefruit juice, PD – Parkinson’s disease, Pgp – P-glycoprotein, QD – quinidine

Introduction

The present outbreak of neurological diseases, including Parkinson’s disease (PD), is perceived as the result of environmental exposure to various chemical substances, e.g. drugs, pesticides, metalorganic compounds, and biphenyl polychlorides. Most of them are substrates of a large number of transporters that control penetration of xenobiotics into body cells and tissues. In clinical pharmacotherapy, ATP-dependent efflux transporters (ATP-binding cassette [ABC] transporters) expressed on the apical membrane of the intestinal epithelial cells determine oral bioavailability, intestinal efflux clearance, and the site of drug-drug interaction of several xenobiotics [8]. P-glycoprotein (Pgp) belongs to the ABC transporters superfamily. It is a product of the MDR1 gene in humans and mdr1a and mdr1b genes in rodents [6]. Pgp is expressed on the luminal surface of the intestinal epithelia, renal proximal tubule, bile canalicular membrane of hepatocytes, placenta, and blood-brain barrier (BBB) [15]. Its anatomical localization, together with its broad substrate profile, contributes to the significant role of Pgp in drug absorption and disposition. Therefore, concomitant administration of substrates and Pgp inhibitors
could modify drug pharmacokinetics by increasing bioavailability and organ uptake, leading to more severe adverse drug reactions and toxicities [4, 11].

Quinidine (QD) is an example of Pgp inhibitor that causes numerous drug interactions [1]. According to Dagenaisa et al. [3], loperamide efflux at the BBB was inhibited by QD. Fullerton et al. [5] study evaluated the ability of loperamide to induce opioid effects in the presence of QD. The combination of loperamide and QD produced moderate central effects. Loperamide may cause respiratory depression after inhibition of Pgp by QD as described by Sadeque et al. [12].

The modulating effect of nutritional constituents on drug kinetics has been described in several articles. Grapefruit juice extract (GFJ) is known to affect the pharmacokinetics of various drugs [1]. Flavonoids, especially flavonols, were found to affect the transport of drugs and other exogenous compounds via interaction with the multidrug-transporter Pgp [9].

These studies indicate that Pgp is involved in pharmacokinetics of numerous drugs. The importance of Pgp is tied to its distribution in the body: on the one hand in the intestine, liver and kidneys, and on the other, in blood-tissue barriers. Thus, it is evident that drug-drug interactions with Pgp are possible. These interactions would lead to increased blood drug levels and higher organ contents with the risk of increase in drug toxicity.

In this study, the effect of QD and GFJ extract, Pgp inhibitors, on the domperidone (DOM) concentration in rat plasma was investigated. DOM, a dopamine D2-receptor antagonist, is a substrate for Pgp.

Materials and Methods

Animals and drug administration

Male Wistar Cmd: (WI) WU rats weighing 340–380 g (Medical Research Center, Polish Academy of Sciences) were used. The rats were housed under controlled environmental conditions and treated according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Local Animal Research Ethics Committee. Twelve rats were randomly divided into three groups in which: (1) DOM was administered alone, (2) DOM was administered in combination with QD, (3) DOM was administered in combination with GFJ extract. Food was withdrawn 12 h before drug administration, free access to water was allowed during the experiment.

DOM powder was dissolved to obtain a concentration of 14 mg/ml in 0.1% water solution of carboxymethylcellulose sodium salt. DOM was administered via gastric gavage at the dose of 10 mg/kg 2 h after QD or GFJ extract pretreatment. QD was dissolved in water at a final concentration of 25 mg/kg (dose calculated as a free base). GFJ extract was dissolved in water at the dose of 2.5 ml/kg (0.2 ml GFJ extract/kg). Rats in control group received DOM 10 mg/kg alone.

Reagents

Domperidone, propranolol hydrochloride and quinidine hydrochloride monohydrate were obtained from Sigma-Aldrich Chem. (Germany). Extract of grapefruit juice, commercially available, Citrosept, was obtained from Cintamani Manufacturing AB (Sweden). Natrium dihydrogenate orthophosphate, orthophosphoric acid, and glacial acetic acid were supplied by POCh Gliwice (Poland). HPLC-grade acetonitrile, dichloromethane and methanol were supplied by Labscan (Ireland). Water was obtained from Mili-Q purification system.

Plasma samples preparation

Blood samples (300 μl) were collected without anticoagulants from tail vein at 15, 120, 240, 360, and 480 min after DOM treatment. The samples were centrifuged at 8000 × g for 5 min and the obtained plasma was stored at –20°C until quantitative determination of DOM. For extraction, to 0.1 ml of each sample, 80 ng of propranolol hydrochloride as internal standard, 0.1 ml of 0.1 M NaOH, and 3 ml of dichloromethane were added. The mixture was shaken for 10 min and centrifuged at 1200 × g for 15 min. Then, organic phase was transferred to another glass tube and evaporated to dryness under gentle nitrogen stream. The dried residue was dissolved in 200 μl of mobile phase, and 20 μl aliquot was injected into the HPLC column.

A recovery study was performed by spiking DOM 20 μl standard solutions into 0.1 ml of plasma and the samples underwent extraction procedure described above.
**Determination of plasma domperidone concentration**

Concentration of DOM in plasma samples was measured by reversed phase HPLC method with fluorescence detection [18] according to the reported method with our modifications. The chromatographic separation was performed using a Discovery HS PEG stainless steel column 150 × 4.6 mm I.D., 5 μm (Supelco, Bellefonte, PA, USA), preceded by a 20 × 4.6 mm I.D., Discovery HS PEG guard column. The column was heated at 40°C. The mobile phase consisted of 0.02 M water solution of natrium dihydrogenate orthophosphate (adjusted to pH = 3.5 with orthophosphoric acid)-methanol (82:18 v/v) and was delivered at a flow rate of 1.0 ml/min. The mobile phase was ultrasonically degassed prior to use. The six point calibration curve was linear ranging between 40 ng/ml and 6000 ng/ml with correlation coefficient ($r^2$) 0.998. The precision was calculated as percent coefficient of variation CV and for each analyzed concentration did not exceed 9.2%. Accuracy of measurement for 6 different concentrations (bias) was 7%.

**Data analysis**

The mean peak DOM concentration ($c_{\text{max}}$) and the time to reach $c_{\text{max}}$ ($t_{\text{max}}$) were derived directly from the observed individual plasma levels. Values for area under the concentration-time curve (AUC) of DOM were calculated by the trapezoidal rule to the last point. For statistical comparison of the AUC data, Student’s $t$-test was used, taking $p < 0.05$ as significant.

**Results**

DOM plasma concentration-time curve profiles after administration of DOM alone, GFJ or QD with DOM to rats are shown in Figures 1, 2.

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**Fig. 1.** Mean (± SD) plasma concentration time profile of domperidone after oral administration of domperidone alone and domperidone 2 h after grapefruit juice (GFJ).

**Fig. 2.** Mean (± SD) plasma concentration time profile of domperidone after oral administration of domperidone alone and domperidone 2 h after quinidine (QD).

**Tab. 1.** Pharmacokinetic parameters of domperidone (DOM) after oral administration of domperidone (10 mg/kg) alone or 2 h after grapefruit juice extract (0.2 ml/kg) and quinidine (QD) (25 mg/kg).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DOM alone 1(^1)</th>
<th>DOM after GFJ 1(^1)</th>
<th>Difference</th>
<th>DOM after QD 1(^1)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_{\text{max}}$ [ng/ml]</td>
<td>301.6 ± 20.9</td>
<td>357.6 ± 36.9</td>
<td>+19%*</td>
<td>409.7 ± 49.5</td>
<td>+36%*</td>
</tr>
<tr>
<td>AUC$_{0-24}$ [ng - h/ml]</td>
<td>34.6 ± 2.5</td>
<td>44.7 ± 4.6</td>
<td>+29%*</td>
<td>49.7 ± 8.6</td>
<td>+44%*</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ [ng - h/ml]</td>
<td>492.7 ± 47.6</td>
<td>584.8 ± 57.0</td>
<td>+19%*</td>
<td>652.2 ± 41.3</td>
<td>+32%*</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ [ng - h/ml]</td>
<td>1067.3 ± 129.6</td>
<td>1222.1 ± 122.1</td>
<td>+14%</td>
<td>1158.3 ± 475.9</td>
<td>+8%</td>
</tr>
</tbody>
</table>

* Statistical significance with $p < 0.05$; 1\(^1\) each value is mean ± SD for $n = 4$
Table 1 lists the pharmacokinetic parameters of DOM. The plasma DOM profiles after GFJ or QD pretreatment, were compared with control group, since 4 h after DOM administration significant differences were not observed. During the absorption phase, the co-administration of GFJ and QD significantly increased the $c_{\text{max}}$ of DOM by 19% and 36%, and enlarged the AUC$_{0-0.25}$ by 29% and 44%, respectively. In addition, GFJ and QD significantly enlarged the AUC$_{0-2}$.

**Discussion**

Several drugs and other xenobiotics interact both with the metabolic enzymes and multidrug transporter Pgp as well as related transporters. The net interaction effect is often unpredictable.

Initially the attention of researchers has focused on cytochrome P450 (CYP) enzyme family. The activity of CYP isoforms that are located in the liver and in the intestinal epithelium is crucial for bioavailability of orally administered drugs. It has been shown that inhibitory activity of xenobiotics can be limited in some cases only to intestinal CYPs. Di Marco et al. [4] demonstrated the inhibitory effect of grapefruit and Seville orange juice (200 ml) on intestinal CYP3A in volunteers. Increased bioavailability of dextromethorphan (30 mg) was observed for 3 to 7 days indicating that juice constituents are long-lasting and perhaps irreversible inhibitors of gut CYP3A.

On the other hand, repeated administration of inhibitors could induce liver cytochrome P450 and increase elimination rate. Mohri et al. [10] administered grapefruit juice (2 ml) orally to rats, twice daily for 10 consecutive days. On the 11th day the pharmacokinetics of nifedipine (3 mg/kg, intravenous or intraduodenal route) was examined again. The nifedipine oxidation activity in microsomes prepared from intestinal mucosa was significantly lower than in control group and the activity in hepatic microsomes were distinctly greater than those in untreated rats. The nifedipine clearance was increased and bioavailability was reduced.

In many instances, the increase in bioavailability is not accompanied by a change in the elimination rate. This means that inhibition of Pgp activity and/or intestinal CYP activity, solely contribute to the observed interaction. QD has no effect on digoxin’s biotransformation but influences digoxin absorption, thereby elevating plasma digoxin levels. Simultaneous oral administration of QD and digoxin in cardiac patients induce increasing plasma digoxin levels from 0.41 ± 0.25 to 0.70 ± 0.31 ng/ml and rises digoxin peak about 1.75 times [11].

Animal study shows that DOM undergoes rapid and extensive hepatic metabolism by hydroxylation and N-dealkylation [7]. *In vitro* metabolism by human liver microsomes with diagnostic inhibitors revealed that CYP3A4 is the major form of cytochrome P-450 involved in the N-dealkylation of DOM, whereas CYP3A4, CYP1A2 and CYP2B6, CYP2C8 and CYP2D6 are involved in DOM aromatic hydroxylation [17]. DOM is a very good substrate for MDR1 and mdr1a Pgp [13].

Both selected inhibitors QD and GFJ interact with CYP. QD is a CYP3A substrate [2] and GFJ components, like bergamottin, inactivate CYP3A4 [16], naringenin, naringin, quercetin, and kaempferol competitively inhibit CYP3A4 [9].

In our experiments, the inhibition pattern of QD and GFJ seems to be limited to absorption phase and may include both inhibition of Pgp activity and destruction or inhibition of intestinal CYP3A isoforms. The inhibition of hepatic CYP3A would result in longer period of inhibitory effect but that we did not observe. Comparing the effect of GFJ on S-talinolol average blood concentration-time profile [14] and on DOM AUC (Fig. 1), it can be concluded that meaningful similarities are present. In Spahn-Langguth at al. [14] experiment, simultaneous oral dose of 10 mg/kg of talinolol and GFJ at 2.5 ml/kg caused approximately 53% increase for S-talinolol AUC. Talinolol was selected as a compound of negligible metabolic clearance and, therefore, it is a suitable model compound for Pgp + mediated transport processes. Quinidine and GFJ influence the DOM pharmacokinetics in the way that suggests inhibition of intestinal secretion. The presented results confirm that in rats, QD 25 mg/kg alters DOM absorption by increasing AUC by 32% during the first two hours after DOM administration. GFJ effects are weaker (19% growth AUC$_{0-2}$) for a given dose (0.2 ml GFJ extract/kg).

In summary, P-glycoprotein inhibitors, quinidine and GFJ extract significantly increased bioavailability of domperidone in the rat. After a single concomitant injection, the interaction takes place in the intestine during absorption phase.
References:


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