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Original research article

Effect of diosmin on the intestinal absorption and pharmacokinetics of fexofenadine in rats

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ABSTRACT

Background: Diosmin is a natural flavone glycoside, a potent P-glycoprotein (P-gp) inhibitor in cultured cells and have the potential to alter the bioavailability of P-gp substrate drugs. However, the interaction between diosmin and fexofenadine is unreported. Hence, the present study was performed to investigate the effect of diosmin on the intestinal absorption and pharmacokinetics of fexofenadine, a P-gp substrate in rats.

Methods: Fexofenadine intestinal transport and permeability were evaluated by *in vitro* non-everted sac and *in situ* single pass intestinal perfusion (SPIP) studies. These results were confirmed by an *in vivo* pharmacokinetic study of oral administered fexofenadine (10 mg/kg) in rats.

Results: The intestinal transport and apparent permeability (P_{app}) of fexofenadine were significantly increased in duodenum, jejunum and ileum of diosmin pretreated group as compared with the control. Similarly effective permeability (P_{eff}) of fexofenadine was increased significantly in ileum of diosmin pretreated group as compared with control. In comparison with control, pretreatment with diosmin significantly increased peak plasma concentration (C_{max}) and area under the concentration–time curve (AUC), while there was no significant change was observed in half life ($T_{1/2}$), time to reach peak plasma concentration (T_{max}) and elimination rate constant (K_{el}) of fexofenadine.

Conclusions: Diosmin significantly enhanced the oral bioavailability of fexofenadine by the inhibition of P-gp mediated drug efflux during the intestinal absorption. Co-administration of diosmin with fexofenadine can reduce the dosage and results in reduced side effects of fexofenadine. The clinical relevance of this interaction should be further evaluated in human subjects.

Introduction

Flavonoids are a group of polyphenolic compounds widely distributed in fruits, vegetables, red wine and tea. Flavonoids have been found to possess various pharmacological properties, such as anti-oxidant, anti-inflammatory, antiviral, antitumor, antibacterial and anti-allergic activities which are beneficial to human health [1–3]. Drug interactions altering pharmacokinetics of drugs with flavonoids have been increasingly reported due to the popular use of dietary supplements containing flavonoids. For example, flavonoids silybinin, baicalein and hesperetin significantly increased the systemic exposure of tamoxifen [4], nimodipine [5] and felodipine [6] respectively in rats. The mechanism underlying in most of the flavonoid–drug interactions have been attributed to P-glycoprotein (P-gp) mediated interaction with drugs [7,8]. Furthermore, it has been reported that berberine and morin significantly altered the pharmacokinetics of P-gp probe substrate drugs digoxin [9] and talinolol [10] respectively by inhibition of P-gp. However, there is far less information available regarding the pharmacokinetic interactions between flavonoids and P-gp substrate drugs.

Diosmin (diosmetin 7-O-rutinoside), is a natural flavone glycoside which is indicated for the treatment of hemorrhoids, lymphedema and varicose veins. It is well known to possess anti-inflammatory, antioxidant, antimutagenic and anti-hyperlipidemic properties [11]. It has been reported that diosmin significantly increased the transport of digoxin and cellular accumulation of rhodamine-123 in Caco-2 cells by inhibition of P-gp-mediated efflux [12]. Since diosmin is a P-gp inhibitor and may potentially inhibit efflux transporter responsible for the poor absorption of P-gp substrates, the effect of diosmin pretreatment on the

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pharmacokinetics of known P-gp substrate is the subject of current investigation.

P-gp is a 170-kDa plasma membrane-associated protein belongs to the adenosine triphosphate (ATP) binding cassette family, which is present in small intestine, liver, kidney and blood-brain barrier of rodents and humans [13]. P-gp is an efflux transporter alters the absorption of many P-gp substrate drugs in animal and human organs and tissues [14]. Fexofenadine is a non-sedating antihistamine drug which is indicated for treatment of seasonal allergic rhinitis [15]. Fexofenadine has low oral bioavailability (approximately 33%) in humans due to P-gp-mediated drug efflux in intestine and it could be used as a suitable probe substrate drug to examine the significance of P-gp mediated drug interactions [16]. The main objective of this study was to investigate the effect of diosmin on the intestinal absorption and pharmacokinetics of P-gp probe substrate, fexofenadine in rats.

To test this hypothesis, the effect of diosmin on the intestinal permeability characteristics of fexofenadine were mechanistically investigated using the *in vitro* non-everted sac and *in situ* single pass intestinal perfusion methods. To confirm these findings, an *in vivo* pharmacokinetic study of oral administered fexofenadine in rats with or without diosmin pretreatment was performed.

Materials and methods

Chemicals and animals

Fexofenadine and propranalol were obtained from Auro Labs Limited (Mumbai, India) and Lupin Labs Limited (Pune, India) respectively. Diosmin and phenol red were purchased from Sigma–Aldrich (Bangalore, India) and Himedia (Mumbai, India) respectively. Solvents used for quantitative analysis were of HPLC grade (Merck, India) and all other chemicals, reagents which were used in the study are of analytical grade.

Male Wistar rats weighing about 220–240 g were used for *in vitro*, *in situ* and *in vivo* pharmacokinetic studies were procured from Mahaveera Enterprises, Hyderabad, India. Rats housed in cages were kept in a room under controlled temperature (20–22 °C) and 12 h day–night cycle. Animals were used for studies after 1-week acclimatization with free access to water and feed. All animal study protocols were approved by Institutional Animal Ethics Committee of Kakatiya University (IAEC, AP, India).

In vitro *non-everted intestinal sac study*

An *in vitro* non-everted intestinal sac study was performed according to the previously described methods [17,18]. Briefly, the rats were divided into two groups control and pretreatment each consisting of four animals. Diosmin suspension (prepared by suspending in 0.25% (w/v) of sodium carboxymethyl cellulose) was administered orally to pretreatment group at a dose of 50 mg kg^{-1} for 7 days and other group was kept as control. Both control and pretreatment group rats were sacrificed on 8th day by using anesthetic ether, the intestine was surgically removed and flushed with 50 mL of ice cold saline. The small intestine was cut into 3 segments, duodenum, jejunum and ileum of equal length (5 cm). The probe drug (fexofenadine 500 μg/mL) was dissolved in pH 7.4 isotonic Dulbecco's PBS (D-PBS) containing 25 mM glucose. The probe drug solution (1 mL) was filled in the normal sac (mucosal side), and both ends of the sac were ligated tightly. The sac containing probe drug solution was placed in a beaker containing 40 mL of D-PBS, containing 25 mM glucose. The medium was pre-warmed at 37 °C and pre-oxygenated with 5% CO_2/95% O_2 for 15 min, under bubbling with a CO_2/O_2 mixture gas, the transport of the fexofenadine from mucosal to serosal direction across the intestinal sacs was measured by sampling the serosal medium periodically for 120 min. The samples of 1 mL were collected from control and pretreatment groups at predetermined time points and stored at −80 °C until analysis. The drug transported from mucosal to serosal direction was measured by high performance liquid chromatography (HPLC).

Calculation of apparent permeability coefficient (P_{app})

The apparent permeability coefficient (P_{app}) of fexofenadine was calculated from the following equation [17]:

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{AC0}$$

where dQ/dt is the transport rate of drug in the serosal medium, A is the surface area of the intestinal sacs and $C0$ is the initial concentration inside the sacs.

In situ *intestinal perfusion study*

The surgical procedure and the *in situ* single-pass intestinal perfusion (SPIP) study were performed according to the previously reported methods [19–21]. Briefly, the rats were divided into two groups control and pretreatment consisting of four animals each. Diosmin suspension was administered orally to pretreatment group at a dose of 50 mg kg^{-1} for 7 days and other group was kept as control. Both control and pretreatment group rats were subjected to surgery on 8th day. Rats were subjected to anesthesia by thiopental sodium (50 mg/kg, *ip*) and they were placed on a hot pad to maintain normal body temperature. A midline incision of 3–4 cm was made on the abdomen of rats and an ileum segment of approximately 8–12 cm was isolated using the ileo-caecal junction as a distal marker. Semi-circular incisions were made at each end of the ileum and the lumen was rinsed with normal saline (37 °C) and the both ends were cannulated with polyethylene tubing and ligated by using silk suture. Then, blank perfusion buffer (Phosphate buffer saline, pH 7.4) was first infused for 5 min at a flow rate of 1 mL/min by using Syringe pump (NE-1600, New Era Syringe Pumps, Inc. NY, USA), followed by perfusion of phosphate buffer saline (pH 7.4) containing fexofenadine (50 μM), propranalol (100 μM) and phenol red (50 mg/mL) at a constant flow rate of 0.2 mL/min for a period of 90 min and perfusate was collected at every 10 min interval. After completion of cannulation the ileum segment was covered with isotonic saline-wet gauze (37 °C). At the end of perfusion the length of the ileum segment was measured following the last sample collection. Perfusion samples were collected from control and pretreatment groups at predetermined time points and stored at −80 °C until analysis. Fexofenadine concentrations in perfusion samples were measured by HPLC.

Phenol red water flux correction

$C_{out\ (corr)}$ was calculated from the following equation [22]:

$$\text{Count(corr)} = \text{Count} \times \frac{\text{Concentration of phenol red in } (CPR_{in})}{\text{Concentration of phenol red out } (CPR_{out})}$$

where $C_{out\ (corr)}$ is corrected outlet concentration of the drug, C_{out} is outlet concentration of the drug, CPR_{in} is concentration of phenol red entering the intestinal segment and CPR_{out} is concentration of phenol red exiting the intestinal segment.