

Effect of ketoconazole and java plum pretreatment on intestinal transport of buspirone across rat intestine

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Abstract

The effect of ketoconazole and java plum on intestinal transport of buspirone, a CYP3A4 substrate across the rat intestine was investigated using normal sac technique. Three groups of rats, one serves as control; treated with ketoconazole and java plum juice for 7 days respectively in second and third group. The rats were sacrificed and intestinal segments (duodenum, jejunum and ileum) and colon were isolated and used for the studies. The buspirone solution was placed in the isolated intestinal sac. Samples were collected at preset time points and buspirone transported was estimated. The transport of buspirone was increased ($p < 0.05$) 12.5, 5, 5.2 and 5.3 times after pretreatment with ketoconazole; 19.5, 8.5, 10.7 and 7.2 times after pretreatment with java plum juice compared to control. It suggests that both ketoconazole and java plum juice might be acting by inhibiting the transporters and enzymes which are responsible for transport/metabolism of buspirone.

Keywords: ketoconazole, java plum, buspirone, normal sac, intestinal transport

Introduction

Natural products, as used by the general population, are usually complex mixtures of many compounds. Both the putative active ingredient(s) and other constituents present in that mixture have the potential to cause interactions with various classes of drugs. Such interactions include induction or inhibition of metabolizing enzymes and drug efflux proteins. Prokaryotes and eukaryotes cannot distinguish between a natural chemical originating from plants and a chemical synthesized in laboratory. Consequently, physiological, pharmacologicals and toxicologicals effects of these chemicals irrespective of their origins remain the same. Ever-increasing use of herbs with western medicines raises the potential for drug-herbal interactions, which may alter drug bioavailability through altered absorption, distribution and metabolism. Primary mechanisms of drug/herb interaction involve either induction or inhibition of intestinal drug efflux pumps [efflux proteins such as P-glycoprotein (P-gp) and multiple resistance proteins (MRPs)] and intestinal and hepatic metabolism by cytochrome (CYP) P450 (Evans 2000,

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Ioannides 2002). A concerted action by both drug efflux pumps and CYPs lower oral bioavailability of many drugs, i.e., protease inhibitors, macrolides and azoles. The most versatile enzyme system involved in the metabolism of xenobiotics is CYP P450. The CYP3A family of enzymes constitutes the most predominant phase-I drug metabolizing enzymes and accounts for approximately 30 % of hepatic CYP and more than 70 % of intestinal CYP activity. Moreover, CYP3A is estimated to metabolize between 50 and 70 % of currently administered drugs (Watkins 1997). A congener of CYP family is CYP3A4, the most abundant form (Kolars et al. 1992). This CYP3A4 enzyme is present primarily in the hepatocytes and enterocytes (Parkinson 1996). It is now fairly established that naturally occurring dietary supplements can modulate hepatic and enterocytic CYP activity. Perhaps the best documented clinically relevant drug interaction is observed with grapefruit juice. Simultaneous consumption of grapefruit juice with a number of therapeutic agents that are subject to first pass intestinal/hepatic metabolism, resulted in higher plasma levels with subsequent adverse effects (Bailey et al. 1998). Grapefruit juice acts through inhibition of intestinal CYP3A4, which regulate pre-systemic metabolism (Guo et al. 2000). Although hepatic biotransformation can make a major contribution to systemic drug elimination, a combination of hepatic and intestinal drug metabolism may cause significant pre-systemic or first-pass drug loss.

Use of herbal drugs has been increased enormously because of their efficacy coupled with decreased risk of side effects. *Syzygium cumini* and *Cleistocalyx nervosum* var. *paniala* are known in Thailand as Waa and Makiang. The fruit of *S. cumini* is also known as Indian black plum or Java plum. It is purplish-black oval-oblong, up to 15 mm, and ripens in May. The juicy fruit-pulp is reported to contain resin, gallic acid and tannins (Martinez and Del Valle 1993). It usually tastes from sour to fairly sweet. The fruit is widely accepted for medicinal purposes, especially for anti-diabetic treatment (Veigas et al. 2007). These fruits can be consumed fresh or can be processed into juice or wine. Wines made from fruits of *S. cumini* were reported to be an excellent source of natural antioxidants (Banerjee and De 2005). Some of the active compounds from the fruit peel of *S. cumini* were identified as anthocyanins (Veigas et al. 2007). Apart from that, other antioxidant components in this plant are still unknown.

In the present study, using non everted sac method, we first investigated whether the components of java pulm could inhibit the CYP3A mediated drug metabolism of buspirone. Buspirone is the first marketed anxiolytic drug from the azaspirone class of compounds (Fulton and Brogden 1997), is as effective as the benzodiazepines for the treatment of anxiety, but it produces fewer adverse side-effects such as sedation, motor impairment, and dependence liability (Hanlon 1991). Unlike benzodiazepine anxiolytics, buspirone has little affinity for the aminobutyric acid benzodiazepine complex. Its primary pharmacological action is believed to be associated with the binding to 5-hydroxytryptamine subtype 1A receptor receptor, resulting in the inhibition of the activity of serotonergic neurons through down-regulation (Goa and Ward 1986). Buspirone undergoes extensive first-pass metabolism in humans, resulting in a bioavailability of less than 5 %, although it is almost completely absorbed after a single oral administration (Gammans et al. 1986).

Materials and Methods

Materials

Buspirone hydrochloride and Ketoconazole are gifted by Dr Reddy's Laboratories, Hyderabad, India and Sun Pharmaceuticals, Baroda, India. Dulbeccos phosphate buffer pH 7.4 (Hi Media Mumbai, India Ltd), Methanol HPLC (Merck Ltd., Mumbai, India), Acetonitrile HPLC (Merck Ltd., Mumbai, India) were purchased. All other chemicals used were of analytical grade.

Preparation of java plum juice

Java plum were collected from the market, the seeds were separated. The pulp was grinded in mixer (Remi, Mumbai, India). The freshly prepared juice was administered to rats. The juice used was collected from same plant.

Noneverted intestinal sac study (Ruan et al. 2006)

The animal study was conducted according to the protocol approved by Animal Ethics Committee, Kakatiya University, India. Male Wistar rats weighing 200 ± 25 gm were selected for experiments. The rats were divided into three groups each consisting of three animals. Java plum juice was administered to one group at a dose of 10 mL Kg^{-1} for seven days. Ketoconazole suspension (prepared by suspending 1 gm of ketoconazole in 0.25 % w/v of sodium carboxymethyl cellulose) was administered at a dose of 50 mg Kg^{-1} to second group for seven days. Third group (untreated) was as control.

The rats were fasted overnight with free access to water before the experiments. Control rats and pretreated rats on seventh day were sacrificed 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; colon of equal length (10 cm). The probe drug (Buspirone, 10 mg mL^{-1}) 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 immersed in 40 mL of D-PBS, containing 25 mM glucose in the mucosal side. The medium was pre-warmed at 37°C and pre-oxygenated with 5% $\text{CO}_2/95\% \text{O}_2$ for 15 min., under bubbling with a CO_2/O_2 mixture gas, the transport of the buspirone from mucosal to serosal surfaces across the intestine was measured by sampling the serosal medium periodically for 120 minutes. The samples of 1 mL were collected at predetermined time intervals from the serosal medium and replenished with fresh buffer. The drug transported was measured using high performance liquid chromatography (HPLC) method (Gannu et al. 2009).

HPLC analysis

Shimadzu HPLC system equipped with a LC-10AT pump and SPD 10 AT UV visible detector and RP C_8 column (Kromosil, 250 mm x 4.6 mm ID, particle size 5 μm) was used for the analysis of samples. The mobile phase used was a mixture of acetonitrile, potassium phosphate buffer (10 mM, pH 4.6) 35:65 v/v, the pH was adjusted to 4.6 with orthophosphoric acid. The elution was monitored at 235 nm, at a flow rate of 1 mL min^{-1} .

Sample preparation

To 200 μL of intestinal sac samples, 100 μL of methanol was added and vortexed for two minutes and centrifuged (Heraeus, Germany) at 5000 rpm for 15 min. The supernatant (20 μL) was injected into HPLC.

Statistical analysis

The efflux results were tested for statistical significance using t-test. The difference in the sample means were considered significant at $p < 0.05$.

Results and Discussion

In the present study, the mean transport of buspirone from mucosal to serosal (normal sac) was determined in duodenum, jejunum, ileum and colon regions of rat intestine in the absence and presence of ketoconazole and java plum juice pretreatment. The time course of buspirone transport at different concentrations across rat small intestine of duodenum, jejunum and ileum was shown in Table 1.

Table 1. Cumulative amount of buspirone transported in intestinal sacs in Wistar rats. Values represented are mean \pm S.D (n=3)

Region	Control	Buspirone + Java Plum	Buspirone + Ketoconazole
Duodenum	55.9 \pm 8.81	1090.7 \pm 36.42	697.2 \pm 32.31
Jejunum	137.9 \pm 16.10	1172.4 \pm 44.88	685.4 \pm 33.32
Ileum	165.2 \pm 7.87	1773.2 \pm 37.68	859.6 \pm 17.33
Colon	176.7 \pm 13.76	1280.4 \pm 44.65	942.2 \pm 36.36

The Ketoconazole pretreatment for 7 days increased the mean cumulative amount of buspirone from 55.9 to 697.2 μg in duodenum; 137.9 to 685.4 μg in jejunum; 165.2 to 859.6 μg in ileum and in colon the mean cumulative amount was increased from 176.7 to 942.2 μg . The java plum juice pretreatment for 7 days showed an increased cumulative amount of buspirone from 55.9 to 1090.7 μg in duodenum; 137.9 to 1172.4 μg in jejunum; 165.23 to 1773.22 μg in ileum and in colon the mean cumulative amount was increased from 176.7 to 1280.4. The transport of buspirone was increased 12.5, 5.0, 5.2 and 5.3 times after pretreatment with ketoconazole; 19.5, 8.5, 10.7 and 7.2 times after pretreatment with java plum juice compared to respective control, there was a statistically significant ($P < 0.05$) difference was observed. The transport was increased from duodenum to ileum. The results suggest that both ketoconazole and java plum pretreatment influences the transport of buspirone. As buspirone is the substrate for CYP3A, java plum pretreatment might have inhibitory effect on the CYP3A, therefore the transport of buspirone was increased compared with known inhibitor ketoconazole (Fig. 1).

The non-everted sac model was originally used to evaluate drug transport mechanisms (Kaul and Ritschel 1981). Genty et al. (2001) compared the permeability values of some actively transported molecules and passively absorbed compounds through everted and non-everted sacs and found that the permeability was higher for actively transported molecules when the sacs were everted. The permeability of passive absorption drug diazepam remained the same whether the sacs were everted or not. These results suggested that the passive permeability of actively transported molecules can be determined through non-everted rat gut sacs (Kivisto et al. 1997).

The results from previous studies (Lamberg et al. 1998) demonstrate that CYP3A inhibitors, verapamil, diltiazem, erythromycin, itraconazole and grapefruit juice, substantially increase the area under the curve (AUC) and the maximum concentration (C_{max}) of buspirone in human

plasma, presumably by inhibiting CYP3A mediated metabolic clearance. In addition a CYP3A inducer, rifampicin decreases the AUC and C_{Max} of buspirone in human plasma by 90 and 84 %, respectively (Kivisto et al. 1999, Venkataramanan et al. 2000). These observations strongly suggest that CYP3A isoforms play an important role in the metabolism of buspirone in humans.

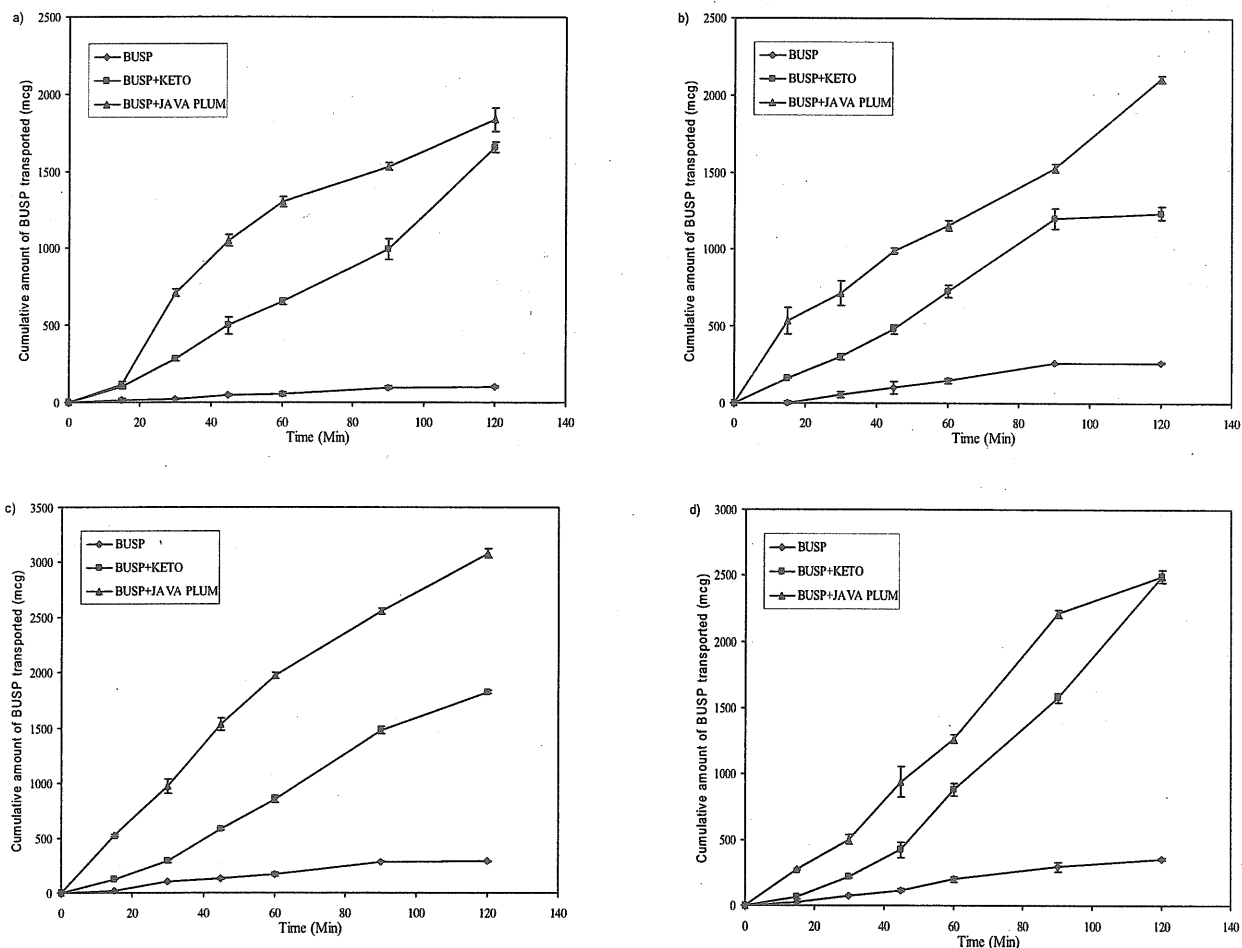


Figure 1. Effect of Ketoconazole and java plum on transport of buspirone across (a) duodenum (b) jejunum (c) ileum and (d) colon

From the present study, it appears that pretreatment with java plum and ketoconazole had an effect on the intestinal transport of buspirone. Preliminary data suggested that ketoconazole influence the metabolic activity of CYP3A4, a CYP450 iso-enzyme responsible for hepatic and intestinal metabolism of many important classes of drugs (Wacher and Benet 1995). Ketoconazole alter drug absorption, distribution and elimination through inhibition of P-gp. Synergistic role of CYP3A4 and P-gp in limiting the oral bioavailability of many drugs proved (Machavaram et al. 2006, Ramesh et al. 2006). Ketoconazole pretreatment appear to have a significant influence on CYP3A4 mediated intestinal metabolism of buspirone (Mingshe et al.

2005), *S. cumini* extract is a rich source of anthocyanins whose content is equivalent to that of blue berries and black currants and higher than that of blackberries, all widely acclaimed anthocyanin-rich edible fruits (Singha et al. 1991). Like grape anthocyanins, which are sold commercially as oenocyanin, the peel powder of *S. cumini* may also be employed as a colorant for foods and pharmaceuticals (Jyothi et al. 2007). Anthocyanins are naturally occurring polyphenols abundant several edible fruits and vegetables and beverages such as red wine and other fruit juices. There is considerable anecdotal and epidemiological evidence that dietary anthocyanins and polyphenols confer preventive and therapeutic roles in a number of human diseases. Several flavonoids, ellagitannins and phenolic acids have been identified from the fruits, seeds, and aerial parts of *S. cumini* (Mahmoud et al. 2001). The components of java plum are similar to that of pomegranate like polyphenols, anthocyanins and gallic acid. In the earlier studies pomegranate component(s) of pomegranate inhibits the CYP3A-mediated metabolism of carbamazepine. Furthermore, pomegranate juice has an influence on the pharmacokinetics in rats (Hidaka et al. 2005). The manner in which inhibition is caused by the components of java plum might be similar to that caused by pomegranate.

Buspirone is an azapirone anxiolytic agent that produces less sedation and impairment of psychomotor performance than do benzodiazepines. It has poor bioavailability due to extensive first-pass metabolism. High consumption of fruits, freshly processed into juice or wine made from fruits of *S. cumini* (Banerjee and De 2005) may influence the pharmacokinetics of buspirone. Java plum juice pretreatment appear to have a significant influence on CYP3A4 mediated intestinal metabolism of buspirone. However, it is difficult to extrapolate our results, which were obtained in rats to humans. Evaluation of java plum buspirone interaction in humans needs to be verified. Further investigations in humans are in progress.

Conclusions

From the results it can be concluded that java plum juice might be acting by inhibiting the enzymes as buspirone is extensively metabolized by CYP3A4. Further studies are recommended to prove their influence in human volunteers or animals *in vivo*.

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