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Effect of Resveratrol Pre-treatment on the Oral Bioavailability of Buspirone in Male Albino Rabbits

Mohammed Abdul Aziz Shahid[1], Y Shravan Kumar[*], Syed Umar Farooq[2]

[1]Department of Pharmacology, Singhania University, Jhunjhunu, Rajasthan, India.
[*]Department of Pharmaceutics, Vaagdevi College of Pharmacy, Warangal, Telangana, India.
[2]Department of Pharmaceutics, Singhania University, Jhunjhunu, Rajasthan, India.

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Corresponding Author Email: shravanyamsani@gmail.com

Abstract

Many drug substances and variety of naturally occurring dietary or herbal components are capable of interaction with the CYP enzyme system. The aim of the study was to investigate the effect of Resveratrol pre-treatment on the bioavailability of buspirone in rabbits. White New Zealand rabbits weighing 2.1±0.13 Kg were selected for study. The bioavailability of buspirone after pre-treatment with resveratrol (5 mg Kg-1 for seven days) was compared with an oral solution (4 mL of 0.25 % w/v BUSP in distilled water). Animals were allowed free access to food and water, until night prior to dosing and were fasted for 10 hrs. In the first phase oral solution (2.5 mg ml^{-1}) was administered through feeding tube followed by rinsing with 10 ml of water. In the second phase, the group was pre-treated with resveratrol for 7 days and study was conducted after 15 days of washout period. The results showed that there was a significant ($p<0.05$) difference in the bioavailability of buspirone after pre-treatment with resveratrol. This increase in bioavailability might be due to inhibition of CYP3A4. Further studies are required to prove this mechanism in humans.

Keywords
Buspirone, Resveratrol, CYP3A4, Bioavailability, Pharmacokinetic.

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INTRODUCTION

Buspirone is the first marketed anxiolytic drug from the azapirone class of compounds [1]. It is as effective as the benzodiazepines for the treatment of anxiety, but buspirone produces fewer adverse side-effects such as sedation, motor impairment, and dependence liability [2]. 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 (5-HT1A) receptor, resulting in the inhibition of the activity of serotonergic neurons through down-regulation [3, 1]. Buspirone, originally

approved by the Food and Drug Administration (FDA) for the treatment of generalized anxiety disorder in 1986, has been shown to be efficacious for the treatment of a variety of mental disorders, including panic disorder, major depression, obsessive-compulsive disorder, and social phobia [1, 4, 5]. 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 [6, 7].

Buspirone hydrochloride [BUSP] is an anxiolytic drug that has dopaminergic, noradrenergic and serotonin-modulating properties. BUSP is rapidly absorbed from the gastrointestinal tract but systemic bioavailability is low (4 %) because of extensive first pass metabolism [8]. Most metabolites are inactive, although oxidative dealkylation produces an active metabolite, 1-(2–pyrimidinyl)-piperazine which is about 20 to 25% as potent as parent drug. The major metabolite is 5–hydroxybuspirone. The metabolites are excreted mainly in urine (65%) and faeces (35%) [9,10]. The mean elimination half life of unchanged BUSP after a single 10-40 mg oral dose is merely 2–3 h. The low oral bioavailability restricts its use. Therefore, current BUSP treatment generally involves taking three daily oral doses between 5 and 20 mg each. Due to the chronic nature of therapy required, a decrease in the number of daily doses would be desirable, as it would greatly enhance patient compliance. To overcome the problem of first pass metabolism, improved bioavailability and for effective treatment of anxiety, coadministration with CYP3A inhibitors may be a good alternative to circumvent these problems.

MATERIALS AND METHODS

Buspirone and Diltiazem was kindly gifted from Sun pharmaceuticals. Methanol, Acetonirile and sodium chloride was purchased from E. Merk India Pvt Ltd. Resveratrol was acquired from Medizen Labs Pvt Ltd. All the animals were grouped and treated with the following regimens.

Reseveratrol suspension was prepared by suspending 1 gm of Resveratrol in 0.25 % w/v of sodium carboxy methyl cellulose and was administered at a dose of 5 mg Kg^{-1} to rats for seven days.

In vivo bioavailability study in rabbits

The animal study protocol was reviewed and approved by the institutional animal ethical committee, Anwarul Uloom College of Pharmacy, Hyderabad, India. White New Zealand rabbits weighing 2.1 ± 0.13 Kg were selected for the study. The bioavailability of buspirone after pretreatment with Resveratrol (dose of 5 mg Kg^{-1} for seven days) was compared with an oral solution (4 mL of 0.25 % w/v BUSP in distilled water). They were allowed free access to food and water, until night prior to dosing and were fasted for 10 h. In first phase oral solution (2.5 mg mL^{-1}) was administered through feeding tube followed by rinsing with 10 mL of water. In second phase the group was pretreated with Resveratrol suspension for 7 days and study was conducted after 15 days of wash out period. Blood samples (1.5 mL) from marginal ear vein were collected at preset intervals of 0.0, 0.5, 1, 2, 4, 8, 12, and 24 h respectively, after administration of oral solution and after pretreatment with Resveratrol suspension. All blood samples were allowed to clot and centrifuged for 10 min at 4000 rpm. The serum was separated and transferred into clean micro centrifuge tubes and stored at -20° C until HPLC analysis. The amount of BUSP in the samples was estimated using HPLC [11].

Preparation of calibration curve

Primary stock solutions of each of buspirone and diltiazem hydrochloride (Internal standard) were prepared in methanol at a concentration of 1.0 mg ml^{-1}. The working solutions of 10µg ml-1 and 1.5 µg ml^{-1} were prepared by appropriately diluting the stock solutions of buspirone and diltiazem hydrochloride, respectively. Different concentrations (1, 5, 10, 50, 100, 500, 1000, 2000 and 3000 ng/ml) of buspirone in serum were prepared for calibration curve. The samples were treated as stated in extraction procedure. The peak area ratios obtained by examination of different concentrations of the drug and internal standard were plotted against the concentration of drug. The slope of the plot was determined by the method of least square regression analysis and was used to calculate the buspirone concentration in the unknown sample. The calibration curve in the range of 1-3000 ng ml-1 resulted in the regression equation y = 0.0066x-0.1098 (R2 = 0.9971) in serum.

Extraction and sample preparation

Aliquot (0.5 ml) of the rabbit serum containing buspirone was pipetted into screw capped tubes and 100 µl of an internal standard (1500 ng ml-1 of internal standard) was added and vortexed for 2 min. Phosphate buffer (500 mM of potassium dihydrogen phosphate) saturated with sodium chloride solution of 250 µl was added, vortexed for 3 min and treated with 5 ml of dichloromethane. Vortexed again for 5 min and, centrifuged at 5000 rpm for 15 min. The dichloromethane layer (4.5 ml) was separated and allowed to evaporate under vacuum oven. The

evaporated residue was re-constituted with 150 µl of mobile phase and 50 µl of the re-constituted sample was injected in to the HPLC system.

Pharmacokinetic analysis

Pharmacokinetic parameters of buspirone before and after pre-treatment with pomegranate juice were estimated in each rabbit using a computer program, KINETICA 2000. Non-compartmental analysis with three terminal points were selected for calculation of the pharmacokinetic parameters Cmax, Tmax and area under the curve (AUC). Cmax (ng ml^{-1}) and Tmax (h) were the observed maximal drug concentration and its time, respectively.

Statistical analysis

Statistical comparisons were made using Student's t-test using Sigmastat software (Jandel Corp., CA, USA). Results were considered significant at 95 % confidence interval ($p<0.05$).

RESULTS

All the rabbits tolerated the treatments well and there were no cases of severe adverse affects during the study period. There was a statistically significant difference in pharmacokinetic parameters, C_{max}, $T_{1/2}$, $Auc_{0-\infty}$ and AUC_{0-24}. No statistically significant difference was observed in pharmacokinetic parameter, Tmax.

After pretreatment with Resveratrol C_{max} increased from 44.30 ± 3.49 to 53.13 ±3.98 ng / mL, $AUC_{0-\infty}$ increased from 466.62 ± 50.08 to 766.34 ± 81.16 ng – hr / mL. AUC_{0-24} increased from 433.53 ± 38.74 to 620.88 ± 53.04 and T ½ increased from 6.01 ± 0.97 to 9.6 ± 1.38 hrs. Tmax decreased from 4 to 2 hrs. Reveratrol pretreatment increased the C max, $Auc_{0-\infty}$, AUC_{0-24} and $T_{1/2}$ by statistical significance. The two-tailed P value equals 0.0022 By conventional criteria, this difference is considered to be very statistically significant. Though, there was no statistically significant difference in T_{max}, it increased.

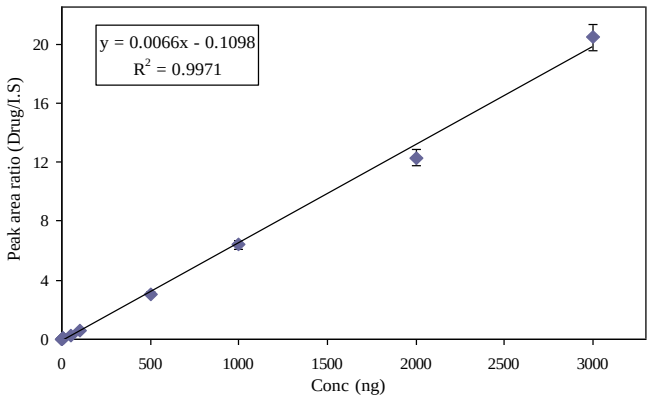

Fig: 1 Standard graph of buspirone in rabbit serum.

Fig: 2. (a) **Fig: 2. (b)**

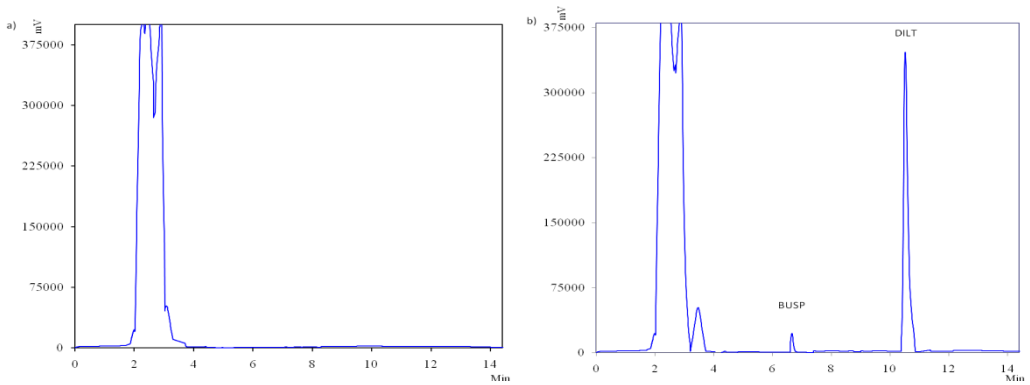

Fig: 2 HPLC chromatograms of (a) blank rabbit serum (b) serum spiked with 1 ng of buspirone and 150 ng of diltiazem hydrochloride. The retention times of buspirone and diltiazem hydrochloride were 6.67 and 10.53 min respectively.

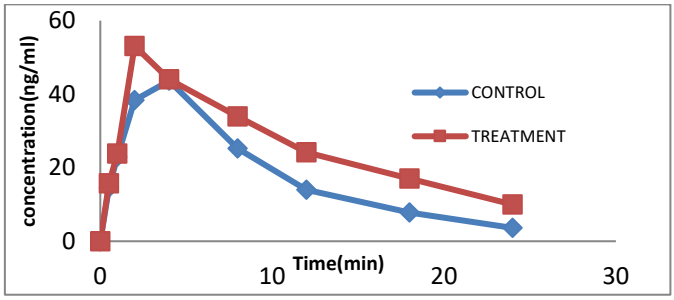

Figure 3. Effect of Resveratrol pre-treatment on bioavailability of buspirone.

Table 1. Individual serum concentration (ng/mL) of Buspirone in Rabbits before administration of Resveratrol during control phase.

Time (h)	Serum concentration (ng/mL)							
	Rabbit1	Rabbit2	Rabbit3	Rabbit4	Rabbit5	Rabbit6	Mean	SD
0	0	0	0	0	0	0	0	0
0.5	12.7	16.23	14.5	15.315	16.85	12.15	14.62	1.80
1	21.35	24.18	23.27	21.18	26.16	21.64	22.96	1.87
2	31.8	42.5	37.50	38.34	41.25	38.93	38.39	3.56
4	37.59	45.57	42.09	44.03	49.05	43.48	43.63	3.62
8	24.37	28.12	24.34	23.33	27.26	23.91	25.22	1.88
12	12.31	14.25	14.76	16.12	16.25	10.28	13.99	2.21
18	7.13	7.67	8.26	9.38	7.36	7.22	7.84	0.82
24	3.35	4.87	3.26	2.36	4.86	2.82	3.59	1.00

Table 2. Individual serum concentration (ng/mL) of Buspirone in Rabbits after administration of Resveratrol during during treatment phase.

Time (h)	Serum concentration (ng/mL)							
	Rabbit1	Rabbit2	Rabbit3	Rabbit4	Rabbit5	Rabbit6	Mean	SD
0	0	0	0	0	0	0	0	0
0.5	16.8	15.3	14.8	13.6	17.1	16.3	15.7	1.3
1	26.3	20.7	19.2	19.5	29.2	27.9	23.8	4.3
2	56.3	53.3	52.9	48.2	58.7	49.4	53.1	3.8
4	43.4	43.0	42.4	38.3	48.7	48.5	44.0	3.8
8	34.2	32.5	33.9	32.0	35.9	35.0	33.9	1.4
12	24.1	23.8	23.8	21.7	25.5	26.7	24.2	1.6
18	16.4	16.7	13.6	13.3	21.3	20.8	17.0	3.3
24	10.9	11.8	9.7	7.8	10.7	9.3	10.0	1.4

Table 3. Pharmacokinetic parameters of Buspirone in rabbits before pretreatment with Resveratrol.

	C_{max} (ng/mL)	T_{max} (ng/mL)	Auc_{0-n} (ng-hr / mL)	$Auc_{0-\infty}$ (ng -hr / mL)	$T_{1/2}$ (hr)	Cl (ml/h)
Rabbit 1	39.59	4	388.31	416.16	5.76	0.023
Rabbit 2	47.57	4	468.5	523.59	7.74	0.019
Rabbit 3	42.09	4	431.58	460.23	5.63	0.021
Rabbit 4	44.03	4	437.2	458.6	5.05	0.021
Rabbit 5	49.05	4	483.55	527.95	6.5	0.018
Rabbit 6	43.48	4	392.09	413.23	5.39	0.024
Mean	44.302	4.000	433.538	466.627	6.012	0.021
STD	3.495	0.000	38.741	50.018	0.974	0.002

Table 4. Pharmacokinetic parameters of Buspirone in rabbits after pretreatment with Resveratrol.

	$C_{max\ (ng/mL)}$	$T_{max\ (ng/mL)}$	Auc_{0-n} (ng-hr / mL)	$Auc_{0-\infty}$ (ng -hr / mL)	$T_{1/2\ (hr)}$	Cl (ml/h)
Rabbit 1	56.3	2	626.112	791.611	10.48	0.0126
Rabbit 2	53.3	2	612.37	813.966	11.85	0.0122
Rabbit 3	52.9	2	587.94	706.019	8.85	0.014
Rabbit 4	48.2	2	542.73	632.07	7.93	0.0156
Rabbit 5	58.7	2	688.8	849.02	9.57	0.011
Rabbit 6	49.4	2	667.34	805.38	8.99	0.161
Mean	53.133	2	620.88	766.34	9.61	0.04
STD	3.98	0.00	53.04	81.16	1.38	0.06

DISCUSSION

The most versatile enzyme system involved in the metabolism of xenobiotics is cytochrome 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 [12]. A congener of CYP family is CYP3A4, is the most abundant form [13]. This CYP3A4 enzyme is present primarily in the hepatocytes and enterocytes [14, 15]. 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.

The results from previous studies [16-18] 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 [18, 19]. These observations strongly suggest that CYP3A isoforms play an important role in the metabolism of buspirone in humans. From the present study, it appears that pretreatment with silymarin and pomegranate juice had effect on the intestinal transport of buspirone. Preliminary data suggested that silymarin might influence the metabolic activity of CYP3A4, a CYP450 iso-enzyme responsible for hepatic and intestinal metabolism of many important classes of drugs [20]. Silymarin also may 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 was proved [21-24]. Silymarin pretreatment appear to have a significant influence on CYP3A4 mediated intestinal metabolism of buspirone.

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-dose grapefruit juice” has been shown to raise the AUC of buspirone between 3 and 20-fold and the maximum concentration between 2 and 16-fold [25]. Resveratrol pretreatment appear to have a significant influence on CYP3A4 mediated metabolism of buspirone. However, it is difficult to extrapolate our results, which were obtained in rabbits to humans. Evaluation of Resveratrol pretreatment on buspirone interaction in humans needs to be verified.

Dietary supplements and foods, including fruits, vegetables, herbs, spices and teas, that contain complex mixtures of phytochemicals have the greatest potential to induce or inhibit the expression and activity of drug-metabolizing enzymes. CYPs may be particularly vulnerable to modulation by the multiple active constituents of foods, including dietary supplements [26]

CYP3A4 is known to be involved in the most common food–drug interactions, as demonstrated by reports of potentially clinically important interactions involving orally administered drugs that are substrates of this enzyme [27, 28]. CYP3A4 (equivalent to CYP3A1 in rats) is the most common CYP and is known to metabolize drugs such as diazepam, erythromycin, lidocaine, nifedipine and taxol in humans. CYP3A1 was inhibited by Aloe Juice and Aloe supplements. *Aloe* supplement classes that modulated at least one CYP [29]. Ashwagandha and Aloe vera juice pretreatment appear to have a significant influence on CYP3A4 mediated intestinal metabolism of buspirone.

Resveratrol (*trans*-3,4’,5-trihydroxystilbene) is a naturally occurring polyphenol found in grape skin and red wine that is often used as a food supplement. Many positive health effects, including cardio

protection, tumor suppression, and immune modulation, are associated with the intake of resveratrol. Resveratrol is well tolerated in healthy subjects without any comedication. It is believed to contribute to the so-called "French paradox," which is based on the observation that the French population has a low incidence of cardiovascular diseases while consuming a diet that is relatively high in fat. Next to its potential cardioprotective effects, additional health benefits have been ascribed to resveratrol [30]. Most recently, for instance, it has been demonstrated that resveratrol mimics calorie-restriction effects in obese humans [31]. With respect to the cancer-preventive activity of resveratrol, a reduction in the exposure of cells to carcinogens was proposed, resulting from its inhibition of various cytochrome P450 metabolic enzymes (CYPs). In addition, resveratrol was proposed to block the transcription of various CYPs through antagonism of the nuclear aryl hydrocarbon receptor (AHR). These mechanisms are expected to reduce the cellular load of chemically reactive— and therefore potentially toxic—drug metabolites. On the other hand, inhibition of CYP activity by resveratrol could lead to safety problems by altering the pharmacokinetics (i.e., absorption and disposition) of co-administered drugs.

Resveratrol has been reported to inhibit the activity of CYP3A4 *in vitro* and *in vivo*. High intakes of resveratrol could theoretically increase bioavailability and the risk of toxicity of drugs that undergo extensive first-pass metabolism by CYP3A4. *In vitro* resveratrol showed inhibition of human CYP3A4-dependent transformation of cyclosporine and also inhibition of 6β-hydroxylation of testosterone by CYP3A4 [32]. In a clinical trial performed by Chow *et al*., an increased area under the plasma-concentration versus time curve (AUC) buspirone was observed after administrating resveratrol for 4 weeks [33]. For the CYP3A4 substrate nicardipine, which has a low bioavailability, it was shown, in male rats, that resveratrol increased the AUC and maximum concentration (C_{max}) by a factor of 2.3 and 2.2, respectively [34]. Human microsomes appear to be more sensitive to inhibition, because the IC50 value for CYP3A-mediated testosterone 6β-hydroxylation in rat microsomes was observed to be much higher (20 versus 4 µM) [35].

A broad range of therapeutic drugs, as well as herbal and dietary constituents, have been reported to undergo metabolic activation by metabolizing enzymes. Such reactive metabolites may bind covalently to various target proteins, such as the reactive site of metabolizing enzymes. Drug-protein adducts may cause toxicity either through immune-mediated mechanisms or mechanism-based inhibition (i.e., irreversible inactivation) of CYPs [36]. It has been proposed that metabolism of resveratrol may yield chemically reactive metabolites, although the overall rate of metabolism of resveratrol by phase I enzymes seems to be very low [37]. Resveratrol has the potential to inactivate CYP3A4, and possibly CYP1A2, by acting as a mechanism-based inhibitor. Bioactivation of chemicals is a common phenomenon.

It has been reported that resveratrol enhanced the oral bioavailability of diltiazem by inhibiting P-gp mediated drug efflux and CYP3A mediated drug metabolism in rat liver and intestine [38]. In addition, resveratrol and its metabolite resveratrol-3-sulfate have been reported as inhibitors of CYP3A. Previously it has been reported that resveratrol exerted inhibitory effect on metabolism of carbamazepine and nicardipine [39].

In the study, we assessed the effect of resveratrol on pharmacokinetics of buspirone, a substrate of CYP3A4, in human volunteers and found that resveratrol affected the pharmacokinetic of buspirone and increased its oral bioavailability. Resveratrol pretreatment significantly enhanced the mean C_{max} and AUC of diltiazem compared to control phase. There is no significant change in T_{max} of diltiazem was observed upon resveratrol treatment compared to control. These results confirmed the possibility that the increased bioavailability of buspirone in the presence of resveratrol might be associated with the inhibition of CYP3A4 activity. As a result, resveratrol may become an inhibitor of CYP 3A4 therefore, resveratrol resulted in significantly enhancing the bioavailability of buspirone.

CONCLUSIONS

This study reveals that resveratrol increases the bioavailability of buspirone due to the inhibition of CYP 3A4. Thus, there is a potential pharmacokinetic interaction between Resveratrol and buspirone has been observed. Accordingly, caution should be taken when Resveratrol supplements are used in combination with therapeutic drugs which are the substrates of P-gp and/or CYP3A4. Dose adjustment of these substrates is necessary, while taking concomitant therapy with Resveratrol supplements. From the results it can be concluded that Resveratrol 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.

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