



Research Article

Pharmacokinetic Interaction of Salbutamol Co-administered with Vasicine Isolated from *Adhathoda vasica* on Rabbit

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ABSTRACT

Background: The study aims at the establishment of pharmacokinetic interaction between vasicine and salbutamol in low and high dose combinations on rabbits.

Methods: Previously developed *in vitro* simultaneous estimation method of vasicine and salbutamol was further validated by recovery study in the spiked plasma sample. Pharmacokinetic interaction study was performed on the rabbit at 30 and 60 mg/kg vasicine administered with 2 and 4 mg/kg salbutamol orally based on literature reports. Vasicine and salbutamol were extracted from plasma up to 12 hr post drug administration, analyzed by HPLC and pharmacokinetic parameters were calculated.

Results: HPLC co-analysis of vasicine and salbutamol in the spiked plasma samples showed recovery in the range of 92.44 to 99.14% and RSD less than 1%. Vasicine showed the limit of quantification 136 ng/ml with interday and intraday variation less than 1% indicating reproducibility. Co-administration of vasicine and salbutamol significantly ($p < 0.001$) elevates elimination rate constant, decreases clearance, biological half-life and volume of distribution of salbutamol compared to administration alone. Salbutamol showed high ($p < 0.001$) clearance and AUC value, whereas vasicine showed significantly high ($p < 0.01-0.001$) elimination rate constant, clearance, volume of distribution and AUC when co-administered.

Conclusions: Combined administration of vasicine and salbutamol has drastically increased the bioavailability of salbutamol though vasicine bioavailability was practically unchanged. This study signifies that concurrently administered salbutamol with vasicine can induce occurrence of serious life threatening adverse event may be due to additive vasodilatory effect.

Introduction

Vasicine is the major bioactive component present in vasaka, *Adhathoda vasica* (L.) Nees. (Family Acanthaceae) leaves having bronchodilator property.¹ Pyrroquinazoline alkaloid, vasicine occurs on average of 1.3% concentration in vasaka, that is reported to possess oxytocic, abortifacient, uterine stimulant, bronchodilatory, antioxidant, anti-inflammatory, hypotensive and bradycardic effects.²⁻⁷ In India patients commonly consume both Ayurvedic and allopathic medicines simultaneously without consent of the physician.^{8,9} Though in general herbal medicines are considered to possess less or almost no side effects still there are many examples of drug interaction between herbal active constituents and allopathic medicines are available. Herb-drug interaction can occur due to pharmacokinetic or pharmacodynamic actions resulting in an additive or synergistic response.

Our previous survey reported high extent of self medication practices (58.6% of participants) among the asthmatic patients with concomitant use of Ayurvedic medicines and allopathic drugs in 45% participants for

quick relief. Vasaka was the most commonly found herb in Ayurvedic medicines used for chronic asthmatic conditions. Frequently observed side effects were sweating, restlessness and irregular heartbeat in patients using both allopathic and Ayurvedic concurrently, may be due to additive bronchodilatory and vasodilatory response.¹⁰ Studies on drug interaction between vasaka, the very commonly used herbal drug in asthma with other allopathic bronchodilators have not been reported. Encouraged by the survey outcome we have decided to explore *in vivo* pharmacokinetic interaction between widely used antiasthmatic bronchodilator salbutamol when concurrently administered with vasicine the bioactive phytoconstituents of vasaka.

Study aimed at isolation, identification, and development of a validated simultaneous estimation method for co-estimation of vasicine and salbutamol *in vitro* we have developed a sensitive, accurate and validated reverse phase HPLC analytical method for determination of vasicine and salbutamol in combination. This method was further implemented for determination of concentration in spiked plasma to evaluate pharmacokinetic drug

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interaction between vasicine and salbutamol. The assay and validation results confirmed that the developed method is sensitive, precise and reproducible for co-estimation of vasicine and salbutamol. The method was further applied successfully for co-estimation of vasicine and salbutamol from *in vitro* blood plasma sample suggesting its suitability and sufficiency for use in pharmacokinetic studies.¹¹ The current study aims at validation of the HPLC method for vasicine and salbutamol simultaneous estimation from *in vivo* rabbit plasma followed by pharmacokinetic interaction study in low dose and high dose combinations of vasicine and salbutamol.

Materials and Methods

Drugs

Herb was collected from, Jabalpur (M.P) in the month of August-September 2013 and authenticated by Dr. Ziaul Hasan assistant Professor, Department of Botany Saifia Science College, Bhopal (specimen voucher no. 265/Bot/Saifia/13). Isolation of vasicine was done following the method of Francis et al.¹² Standard salbutamol was provided from Sun Pharmaceutical Industries Ltd., Vadodara, Gujarat, as a gift sample. Vasicine (> 97% pure by HPLC) was purchased from Natural Remedies Pvt. Ltd., Bangalore, India.

Authentication of vasicine and salbutamol

Authentication of isolated vasicine was done by performing thin layer chromatography (TLC),¹³ Fourier Transform Infrared spectroscopy (FT-IR; Perkin Elmer, 883),¹⁴ High Performance Liquid Chromatography (HPLC; Shimadzu, Japan) following the reported method and compared with reported spectra.^{15,16} Authentication of salbutamol was done by melting point, FT-IR spectroscopy, Ultra Violet spectroscopy (UV 1800, Shimadzu, Japan) and HPLC analysis.¹⁷

In vitro HPLC recovery study of vasicine and salbutamol from plasma

As reported previously, sensitive and accurate reverse phase HPLC method for determination of vasicine and salbutamol in combination and *in vitro* whole blood was developed. The validated method showed low LOQ, over 98% recovery, and less than 2% relative standard deviation.¹¹ Following this method, *in vitro* recovery of vasicine and salbutamol from plasma was estimated collecting blood from a healthy adult rabbit. The recovery and accuracy were expressed as the percentage of the drug recovered and percentage relative standard deviation (% RSD).

In vivo HPLC co-estimation of vasicine and salbutamol in rabbit

Animals

Albino rabbits (Newzeland white) of either sex, 4-6 month, weighing 1.5-2.0 kg were used for the study. The animals were housed at 22 ± 2°C temperature and 65 ± 5% relative humidity, in a 12:12 light: day cycle. The

experimental procedures and protocols were reviewed by Institutional Animal Ethics Committee of Radharaman College of pharmacy proposal number IAEC/RCP/2013/09 and were in accordance with the guidelines of the CPCSEA, New Delhi.

Dose selection

In accordance with the study rationale oral route of administration was chosen for this study as herbal preparation are mostly administered by this route. Very low plasma concentration of vasicine was observed after oral administration as it is metabolized to vasicinone and other metabolites in the liver which contributes to the first pass effect and was found to be an important way of elimination of vasicine.¹⁸ Gupta et al. attempted a detailed pharmacological study of vasicine and vasicinone and reported cardio-depressant effect of vasicine at 5.0, 7.5 and 10 mg/kg i.v. dose. Vasicine had shown marked respiratory stimulant effects, both on i.v. (10 mg/kg) and i.m. (30 mg/kg) administration on rabbits.¹⁹

Amin and Mehta confirmed bronchodilatory activity of vasicine and bronchoconstriction by vasicinone.²⁰ They suggested that, due to the similarity of their structure, vasicine and vasicinone act through the same receptors and also being metabolized by the same enzyme. Enhancement of bronchodilatory response by vasicine *in vivo* may be due to not only by the occupation of the receptor sites by vasicine molecules and letting the bio-transformed vasicinone molecules remain unattached but also perhaps due to the bio-transformed vasicinone serving as a substrate for the metabolizing enzyme and therefore protecting vasicine against metabolic breakdown thus enhancing its activity.

Pharmacokinetic study of vasicine was conducted by Atal (1980) at 20 mg/kg body wt. given intramuscularly which gets well absorbed in rats.²¹ Oral administration of a mixture of vasicine and vasicinone 25 mg/kg three times a day showed good bronchodilator activity on asthma patients.²² Based on these literature reports 30 and 60 mg/kg oral dose of vasicine were selected for pharmacokinetic interaction study. Salbutamol was administered in the therapeutic dose range of 2 and 4 mg/kg, oral.

Methodology

Isolated vasicine was dissolved in saline with hydrochloride as pH regulator (pH 6) and salbutamol was suspended in 2 % carboxymethyl cellulose prepared on distilled water administered orally using oral cannula. One overnight fasted rabbit was administered with vasicine (30 mg/kg) and salbutamol (2 mg/kg) orally at an interval of 5 minutes. After 45 min blood samples were collected from marginal ear vein, centrifuged to separate plasma. Vasicine and salbutamol were extracted from 100 µl of plasma and analyzed by HPLC as described earlier to record the Rt in the chromatogram.¹¹ Analysis was performed on Shimadzu (Japan) liquid chromatography system having prominence (LC-20AD) pump, SPD-M20A photodiode array UV-Visible detector (PDA-100),

a quaternary solvent delivery system, degasser (DGU-20A5), stainless steel Luna column (150 mm × 4.6 mm) packed with octadecylsilane bonded to porous silica (5 µm, C-18, 100A) and data analysis system LC solution.

Pharmacokinetic study

Study protocol

Rabbits were fasted for 18 hr before experiment but had free access to water. Rabbits were divided into 4 groups each containing 5 animals. Group I dosed orally with isolated test compound i.e. vasicine (60 mg/kg), Group II given salbutamol (4 mg/kg), Group III co-administered with vasicine (30 mg/kg) and salbutamol (2 mg/kg) at an interval of 5 minutes and Group IV co-administered with vasicine (60 mg/kg) and salbutamol (4 mg/kg) at an interval of 5 minutes.

Blood collection

Blood samples were periodically withdrawn from marginal ear vein by using 22 gauge sterilized needle at 0, 0.5, 1, 1.5, 2, 4, 6 and 12 hr post drug administration. The collected blood was kept in heparinized microcentrifuge tubes and plasma was immediately separated by centrifugation at 2000 g for 10 minutes at 4°C and stored in a deep freezer at -20°C in separate tubes until analysis.

Calculation of pharmacokinetic parameters

Vasicine and salbutamol were extracted from 100 µl of plasma and analyzed by HPLC as described earlier. The mean plasma concentration vs time curve was plotted. Vasicine and salbutamol concentration in plasma samples were calculated from the standard plot.²³

Area % = normalized peak area

Response Factor = Standard peak (Area %)/
Concentration of sample

Concentration of sample = Sample peak (Area %)/
Response factor.

Peak plasma concentration (C_{max}) and time of peak plasma concentration (T_{max}) were observed from the plasma concentration vs time plot. The area under the concentration-time curve (AUC) was calculated by the trapezoidal method with extrapolation to infinity by addition of last observed concentration divided by the terminal elimination rate constant. Absorption rate constant (K_a), elimination rate constant (K_e), volume of distribution (V_d), area under first momentum curve (AUMC), biological half-life ($t_{1/2}$), clearance (Cl), mean residence time (MRT) was calculated using the standard

formula.^{24,25}

Statistical Analysis

The individual pharmacokinetic parameters were computed with PK Solutions 2.0 Version-Windows 2.0.6 (10) using curve stripping method. Raw data had been presented in the form of Mean ± SEM (standard error mean). One-way ANOVA was applied to determine the difference in bioavailability parameters and extent of significance which was considered

Result

Authentication of vasicine and salbutamol

TLC showed Rf value 0.67 for isolated vasicine compared to 0.68 for reference standard.¹² FT-IR spectra were comparable to the reported literature.²⁶ HPLC showed Rt at 3.734 and 3.692 min for isolated and standard vasicine respectively.¹⁵ Salbutamol sulphate showed the melting point at 157-158°C, UV_{max} at 276.5 nm and FT-IR spectra showed identical spectra to standard salbutamol.¹³ Salbutamol sulphate showed Rt at 2.85 min compared to standard Rt 2.76 min.¹⁷

In vitro HPLC recovery study of vasicine and salbutamol from plasma

Previously developed and optimized HPLC method¹¹ was followed for co-analysis of vasicine and salbutamol in the spiked plasma samples. The method was used for extraction of salbutamol and vasicine after spiking with different concentrations of drugs. Vasicine showed recovery in the range between 94.89 to 99.04% in the concentration range between 25 to 150 µg/ml whereas salbutamol showed 92.44 to 99.14% recovery in the concentration range from 100 to 200 ng/ml. Table 1 also showed the relation between the amount of drug added, % recovered and % RSD which is found to be less than 1%.

In vivo HPLC co-estimation of vasicine and salbutamol in rabbit

Methanolic extract of drug plasma was injected for HPLC estimation. Sharp peaks were found at RT: 2.350 (plasma), RT: 2.892 (Salbutamol) and RT:3.684 (Vasicine). The limit of quantification of vasicine with this method was 136 ng/ml with a coefficient of variation less than 1% for interday and for intraday and the mean percent drug recovered for the above concentration range in plasma was 106% indicating the method is reproducible.

Table 1. Recovery studies for salbutamol and vasicine from spiked rabbit blood plasma.

Vasicine			Salbutamol		
Spiked concentration (µg/ml)	% Recovered*	% RSD	Spiked concentration (ng/ml)	% Recovered*	% RSD
1	88.34	0.876	25	87.56	0.453
10	89.04	0.376	50	89.78	0.682
25	94.89	0.936	75	89.65	0.530
50	96.35	0.243	100	92.44	0.361
75	96.22	0.572	125	97.68	0.860
100	98.35	0.845	150	99.34	0.740
125	98.52	0.283	175	99.17	0.421
150	99.04	0.651	200	99.14	0.840

*Mean of three estimates. % RSD = percentage relative standard deviation.

Table 2. Pharmacokinetic parameters of vasicine and salbutamol co-administration in rabbit plasma.

Parameters	Group I	Group II	Group III	Group IV		
	Vasicine (60 mg/kg)	Salbutamol (4 mg/kg)	Vasicine (30 mg/kg)	Salbutamol (2 mg/kg)	Vasicine (60 mg/kg) Salbutamol (4 mg/kg)	
C_{max}	59.10 ± 3.07 (µg/ml)	68.84 ± 4.98 (ng/ml)	47.83 ± 2.60 ^a (µg/ml)	239.36 ± 8.09 ^z (ng/ml)	53.26 ± 2.38 ^{ns,ns} (µg/ml)	320.12 ± 10.88 ^{z,1} (ng/ml)
T_{max} (min)	90 ± 1.42	120 ± 2.08	90 ± 1.93 ^{ns}	120 ± 3.24 ^{ns}	90 ± 2.86 ^{ns,ns}	120 ± 4.87 ^{ns,ns}
K_a (hr ⁻¹)	0.52 ± 0.004	0.48 ± 0.002	0.48 ± 0.005 ^c	1.46 ± 0.12 ^x	0.70 ± 0.004 ^{c,3}	2.21 ± 0.35 ^{z,ns}
K_e (hr ⁻¹)	0.16 ± 0.002	0.27 ± 0.003	0.16 ± 0.009 ^{ns}	0.57 ± 0.006 ^z	0.41 ± 0.002 ^{c,3}	0.53 ± 0.008 ^{z,ns}
$T_{1/2a}$ (hr)	1.33 ± 0.03	1.44 ± 0.02	1.44 ± 0.05 ^{ns}	0.47 ± 0.005 ^z	1.39 ± 0.19 ^{ns,ns}	0.32 ± 0.008 ^{z,3}
$T_{1/2e}$ (hr)	4.34 ± 0.12	2.56 ± 0.08	4.33 ± 0.22 ^{ns}	1.22 ± 0.06 ^z	2.09 ± 0.25 ^{c,3}	1.31 ± 0.08 ^{z,ns}
V_d (L)	1.02 ± 0.04	58.11 ± 2.93	0.63 ± 0.002 ^b	8.35 ± 0.91 ^z	1.13 ± 0.09 ^{ns,2}	12.49 ± 1.53 ^{z,ns}
Cl (ml/hr)	162.43 ± 7.23	13946.40 ± 23.04	100.35 ± 5.06 ^c	4762.70 ± 11.17 ^z	463.30 ± 8.19 ^{c,3}	6622.52 ± 20.42 ^{z,3}
AUC_{0-24} (µg.min/ml)	7892.65 ± 22.06	276.03 ± 8.44	7851.66 ± 21.51 ^{ns}	994.07 ± 13.94 ^z	7985.16 ± 16.12 ^{a,2}	2141.34 ± 11.69 ^{z,3}
$AUMC_{0-24}$ (µg.hr ² /L)	119827.12 ± 153.62	786.51 ± 12.93	127162.02 ± 113.09 ^c	2107.20 ± 14.19 ^z	120303.15 ± 102.06 ^{a,3}	6538.22 ± 13.63 ^{z,3}
MRT (hr)	15.18 ± 1.26	2.84 ± 0.69	16.19 ± 1.08 ^{ns}	2.11 ± 0.49 ^{ns}	15.06 ± 2.02 ^{ns,ns}	3.05 ± 0.81 ^{ns,ns}
Relative bioavailability	--	--	99.48%	763.40%	101.17%	775.72%

All the values are in M ± SEM of five animals in each group. When compared to vasicine alone: a = p < 0.05, b = p < 0.01 and c = p < 0.001. When compared to Salbutamol alone: x = p < 0.05 and z = p < 0.001. When compared to vasicine (30 mg/kg) + Salbutamol (2 mg/kg) group: 1 = p < 0.05, 2 = p < 0.01, 3 = p < 0.001 and ns = non-significant.

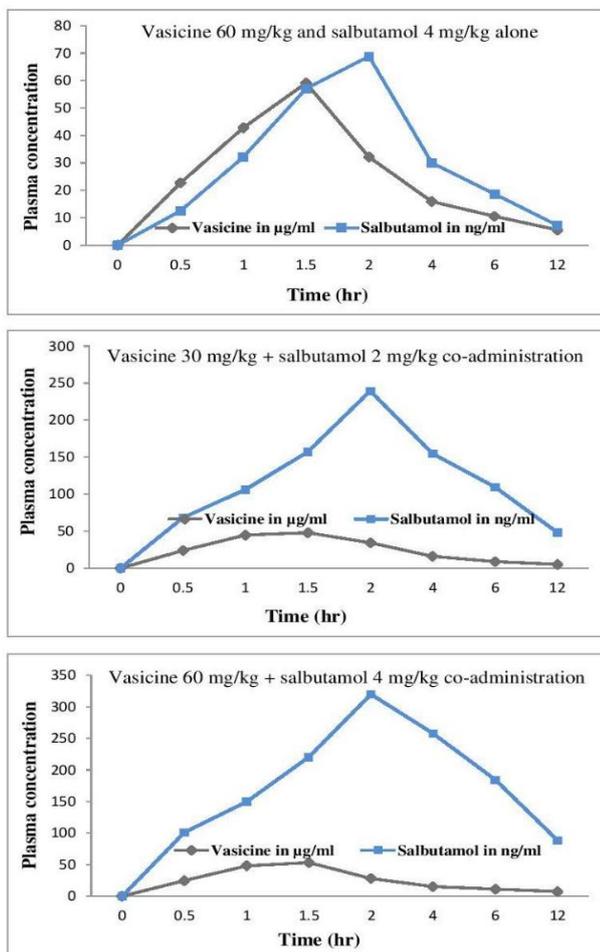


Figure 1. Mean plasma concentration vs time curve of vasicine and salbutamol administered in different combinations on rabbit

Pharmacokinetic study

Vasicine 60 mg/kg alone treated group showed T_{max} 90 ± 1.42 min when C_{max} was 59.10 ± 3.07 µg/ml. Salbutamol 4 mg/kg only administration had T_{max} 120 ± 2.08 min and

C_{max} 68.84 ± 4.98 ng/ml. Vasicine and salbutamol co-administered at the low dose (30 and 2 mg/kg) respectively were found to have significantly (p < 0.001) higher C_{max} of 239.36 ± 8.09 ng/ml for salbutamol whereas C_{max} of vasicine was significantly (p < 0.05) reduced to 47.83 ± 2.60 µg/ml. Co-administration significantly (p < 0.001) elevates elimination rate constant and decreases clearance, biological half life and volume of distribution of salbutamol compared to administration alone. Vasicine and salbutamol co-administration at the normal dose (60 and 4 mg/kg) has a similar effect on elimination rate constant, clearance, biological half life and volume of distribution of salbutamol. C_{max} 320.12 ± 10.88 ng/ml for salbutamol was found to be significantly (p < 0.05-0.001) higher from alone and low dose co-administration but C_{max} of vasicine was on a similar level as of the alone treatment group. Compared to low dose co-administration in the normal dose combination salbutamol showed high (p < 0.001) clearance and AUC value, whereas vasicine showed significantly high (p < 0.01-0.001) elimination rate constant, clearance, volume of distribution and AUC. Co-administration at both the doses has a non-significant effect on T_{max} and mean residence time (Table 2 and Figure 1).

Discussion

This study explores the interaction of vasicine with salbutamol on coadministration. Combination of vasicine and salbutamol has a drastic effect on the bioavailability of salbutamol both at low and normal dose with an average 750% increase, whereas vasicine bioavailability is practically unchanged. Salbutamol absorption rate was very high with nearly 5 times increase, though T_{max} was not affected significantly may be due to the simultaneous doubling of elimination rate with resulting decrease in biological half life. Altogether reduction in the volume of distribution and clearance rate gives high salbutamol bioavailability. Vasicine and salbutamol concurrent administration have significantly increased the

bioavailability of salbutamol compared to administration alone. Vasicine not only affects the extent of salbutamol absorption but also the total amount absorbed.

The study outcome signifies effect of vasicine primarily on volume of distribution and clearance of salbutamol. Previous reports have found that salbutamol peak plasma concentrations occurs approximately 2-2.5 hours after ingestion. Salbutamol gets readily absorbed from the intestine, binds to plasma protein and is released as required. Salbutamol is metabolized in the liver, mainly by conjugation to inactive salbutamol-4'-O-sulphate. Salbutamol's plasma half life is reportedly to be 2.7-5 hours after oral administration. Nearly 72% unchanged drug and metabolites are excreted in the urine within the first 24 hours.²⁷

Ram et al. assumed that the site of vasicine absorption is the intestine.¹⁵ Vasicine is metabolized to vasicinone and other metabolites in the liver which contributes to the first pass effect and was found to be an important way of elimination.²⁸ A total of 72 vasicine metabolites were found in rat plasma and out of these key metabolites isolated from rat urine is elucidated as vasicinone, vasicinol, vasicinolone. The metabolic pathway of vasicine *in vivo* and *in vitro* mainly involved monohydroxylation, dihydroxylation, trihydroxylation, oxidation, desaturation, sulfation, and glucuronidation.²⁹ It is reported that vasicine and its metabolites are mainly excreted in the urine. Following vasicine oral administration, about 18% of the excreted product was vasicine itself during the first 24 hr.³

Vasicine, a quinazoline alkaloid, though extensively metabolized in liver, interaction with any particular class of cytochrome P450 enzyme is not reported. Farrar et al. reported potent inhibitors of CYP2E1 by three synthesized quinazoline compounds following Baculosome assay using purified CYP450 isozymes.³⁰ Salbutamol is metabolized mainly by sulphate conjugation also having no reported interaction with any specific category of cytochrome P450. High plasma concentration of salbutamol is associated with stimulation of β -adrenergic receptors leading to a variety of metabolic effects, including increase in fatty acids and plasma glucose concentrations, glycogenolysis, gluconeogenesis, lipolysis, and hypokalaemia.³¹ Our previous survey reported sweating, restlessness and irregular heartbeat as most commonly observed side effects in patients using both allopathic and Ayurvedic combination, may be due to an additive vasodilation effect induced by both vasicine and salbutamol.⁸

Conclusion

This study explores pharmacokinetic interaction potential of vasicine with salbutamol. Vasicine interactively decreases the volume of distribution and clearance of salbutamol with a simultaneous increase in bioavailability. Chances of pharmacokinetic interaction that may delay excretion of salbutamol in presence of vasicine can be possible as both the drugs and their metabolites are reported to get excreted primarily in the

urine. This study signifies that salbutamol should not be taken concurrently with vasicine or vasaka containing Ayurvedic medicines to avoid any chances of serious and life threatening adverse event occurrence.

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Conflict of interests

The authors claim that there is no conflict of interest.

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