DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 8(6), 833-846 (1982)

PREFORMULATION STUDIES III - VASICINONE - A BRONCHODILATORY ALKALOID FROM ADHATODA VASICA NEES (ABSORPTION, POTENCY AND TOXICITY STUDIES)

H.L. Bhalla and A.Y. Nimbkar
The Bombay College of Pharmacy
Kalina, Bombay-400 098

ABSTRACT

Bronchodilatory activity of pure samples of vasicinone hydrochloride is investigated and compared with isoprenaline and aminophylline. Acute toxicity in mice and absorption pattern in dogs of aqueous solution administered by different routes is studied. The studies confirm the activity and safety of vasicinone and its potentiating effect on bronchodilatory activity of aminophylline.

INTRODUCTION

Preliminary pharmacological screening tests[1-6] hitherto carried out on vasicinone, an alkaloid from Adhatoda vasica Nees, lack reproducibility, as physico-chemical characterization of a new compound forms a prerequisite for obtaining reproducible results. A meaningful pharmacological evalua-

tion also demands a high degree of purity of the compound under investigation and knowledge of potential absorption problems. With the aforesaid requirements in view, the present investigation was carried out on samples of vasicinone having a high degree of purity, defined physico-chemical characteristics,[7 & 8] and absorption studies preceded the pharmacological evaluation.

Since measurement of concentration of drug in plasma is assumed to provide an indirect measure of the concentration of drug at receptor site, the determination of drug levels in plasma as a function of time and route of administration was undertaken with the aim to study the time course of pharmacological activity and to collect information for product design. Comparison of plasma level profile obtained by administration of drug by different routes with that of intravenous could help to evaluate the suitability of these routes of administration as well as to separate the distribution, metabolism and excretion parameters from those of absorption.

Bronchodilatory activity was confirmed by *in vitro* and *in vivo* experiments. The doses required to initiate action and to produce a peak effect as well as ED50 value of vasicinone were determined and compared with aminophylline and isoprenaline. Its additive effect on bronchodilatory activity of aminophylline and isoprenaline was also studied.

Besides systematic gauging the activity of a new compound, toxicity testing, aimed at discerning the complications arising from the pharmacological action of the drug and also at discovering the unexpected side effects, forms an important parameter of preclinical and preformulation studies. Experimental evidence explaining the mode of toxic action or side effects in full biochemical or physiological

terms being beyond the scope of present study, the safety of the compound under investigation was studied by acute toxicity tests on mice. LD 50 value of drug by different routes of administration was determined and effect on gross behaviour of animals studied.

EXPERIMENTAL

A. Absorption of Vasicinone in Dogs

Healthy mongreal dogs (10-14 kg body weight) were fasted for 24 hours and anaesthetized by intravenous administration of pentobarbitone sodium (30 mgm/kg body weight).

Vasicinone hydrochloride (10 mg/kg) in normal saline solution was administered intravenously and intramusculary to anaesthetized dogs. Solution of vasicinone hydrochloride in 25 ml distilled water was administered orally to dogs through a stomach tube, followed by administration of 25 ml plain distilled water to flush the contents of the tube into stomach. The doses of 10 and 50 mgm/per kilogram body weight were given by this route. Similar doses in the form of suppository were administered to dogs through rectum.

10 ml of blood sample blank was withdrawn from anaesthetized dog in each case prior to administration of drug. Subsequent to drug administration, 6-8 ml blood samples were withdrawn at various intervals and an equal amount of lactated ringer solution injected into blood stream after every withdrawl. Blood samples were allowed to clot, centrifuged at 2000 r.p.m. for 10 minutes and serum was separated and drug content determined. Blank urine samples were collected before administration of drug. Ureters of dog were catheterized and urine was collected following administration of drug and stored at 4°C. Intact drug from blood serum and urine was effectively extracted and estimated.[7]

B. Determination of Bronchodilatory Activity of Vasicinone

(1) Effect on Isolated Guinea-pig Tracheal Chain

Adult healthy guinea-pigs of either sex weighing between 250-350 g were used for the study. The tracheal chain preparation was set up as described by Castillo and De Beer,[9] in an organ bath of 25 ml capacity, containing Kreb's solution at 37°C and gassed with Carbogen. Graded responses to vasicinone, isoprenaline and aminophylline were studied at normal and increased tone of tracheal muscle. The increase in muscle tone was brought about by addition of carbachol (25 mcg/ml) to the Kreb's solution. Minimum effective dose, ceiling dose and ED50 for the bronchodilator effect of vasicinone, aminophylline and isoprenaline were determined. In a separate set of experiments, graded responses to isoprenaline and aminophylline separately were obtained in presence of vasicinone concentration which alone did not elicit any response in the tissue.

(2) Effect on Anaesthetized Guinea-pig

A method recommended by 'Konzette Rossler'[10] was adopted to monitor the in-vivo bronchodilatory activity. Healthy guinea-pigs (350-400 g body weight) of either sex were selected and anaesthetized by intraperitoneal injection of urethene (250 mg/ml) and chloralose (15 mg/ml) in a dose of 4 ml/kg body weight and prepared for recording respiratory resistance. The animal was artificially ventilated with the help of a Sterling respiratory pump at 40 strokes per minute. The volume of air per stroke being 10-12 cc.

Bronchospasm was produced by administration of either histamine or 5-hydroxy tryptamine (5-HT). The bronchoconstrictor effect was expressed as percentage increase in intra tracheal pressure (I.T.P.). Initially, a dose response relationship to histamine or 5-HT was studied, and the succ-

essive doses of these were administered at a regular interval of 10 and 15 minutes respectively throughout the experiments.

Bronchodilatory activity of vasicinone was studied on a submaximal response to either histamine or 5-HT producing approximately 250 per cent increase in I.T.P. The bronchodilatory activity was expressed as per cent reduction of histamine or 5-HT induced response. The effect of intravenously and orally administered vasicinone on sensitivity of guinea-pig to histamine and 5-HT was studied and compared with that produced by isoprenaline (intravenous) and aminophylline (intravenous and oral).

C. Determination of Acute Toxicity of Vasicinone

LD 50 of vasicinone on its administration by oral, peritoneal and intravenous route to mice was determined. Adult healthy Albino mice of Haffkine strain of either sex (18-20 g weight) were selected and starved overnight (16-18 hours) prior to the administration of vasicinone. However, free supply of water was made available to the animals throughout the experiment. The animals were divided into several groups of 10 mice and each group (5 males and 5 females) was placed in a separate cage. All the animals of a particular group were then administered a predetermined dose of the drug by a route under study. The volume of drug solution in distilled water, administered to the animal by each route was 0.1 ml/ 10 g of body weight of the animal. Animals from the control group received plain distilled water. All the animals under consideration were closely and constantly observed over a period of 6 hours following drug administration. The changes in gross behaviour of animals were scored over an arbitrary rating scale from 0-8. The signs of severe intoxication and death if any were also recorded during this period. The number of dead animals from each group at the end of 96 hours

following drug administration was recorded. By plotting doses against corresponding mortality value on a log x 2 cycles probability paper, the LD 50 with 19/20 confidence limits was calculated as per method described by Litchfield and Wilcoxin.[11]

RESULTS AND DISCUSSION

Intravenous and intramuscular administration of vasicinone to dogs in a dose of 10 mg/kg body weight produced peak plasma levels of 33 mcg/ml and 9 mcg/ml respectively. Ten mg/kg oral dose of vasicinone could not be detected in the blood but 50 mg/kg p.o. dose produced peak plasma concentration of 15 mcg/ml. Following rectal administration of the

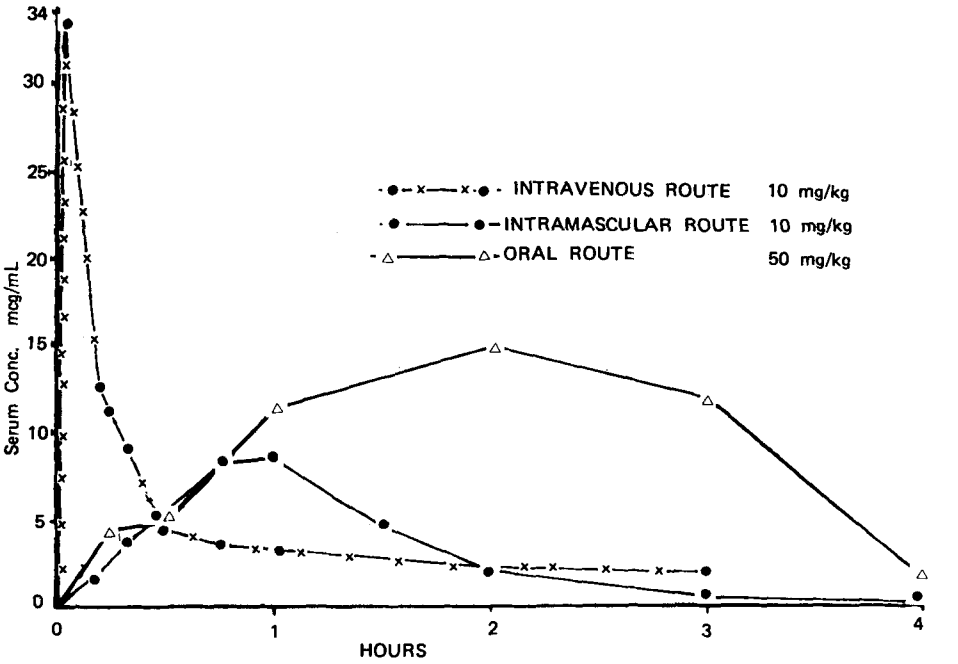

Figure 1 - SERUM LEVEL OF VASICINONE IN DOG.

Table 1

Dose-Response Relationship (*in-vitro*) of Vasicinone, Aminophylline and Isoprenaline in Isolated Guinea-Pig Tracheal-Chain Preparation

	ISOPRENALINE (ng/mL)		VASICINONE (mcg/mL)		AMINOPHYLLINE (mcg/mL)	
	Normal tone	Elevated tone	Normal tone	Elevated tone	Normal tone	Elevated tone
Threshold dose	10	5	50	25	50	25
Ceiling dose	320	140	1600	800	1600	1200
ED 50	52	62	800	245	620	230

The figures indicate the average of three determinations.

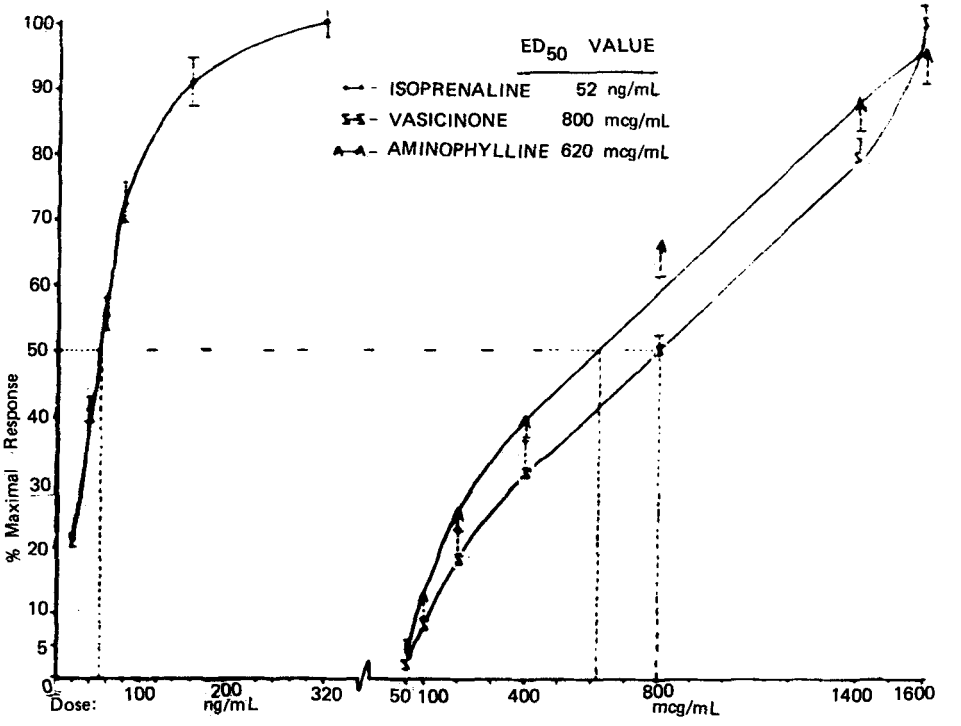

Figure 2 - DOSE-RESPONSE CURVES IN GUINEA-PIG ISOLATED TRACHEAL CHAIN PREPARATION (NORMAL TONE)

drug upto a dose of 50 mg/kg body weight no drug could be detected in the blood but absorption was indicated by presence of intact drug in the urine. These results reveal that the drug absorption takes place by all the routes tried. Drug serum level profile (Figure I) shows rapid elimination of drug from blood compartment. Intravenously administered drug peak level fell to its 1/7th within 30 minutes. This elimination of drug could be due to quick excretion, distribution to other tissues or degradation. It may also be due to its secretion into stomach as some of the basic drugs with low pKa value are reported[12] to do. Part of drug is eliminated

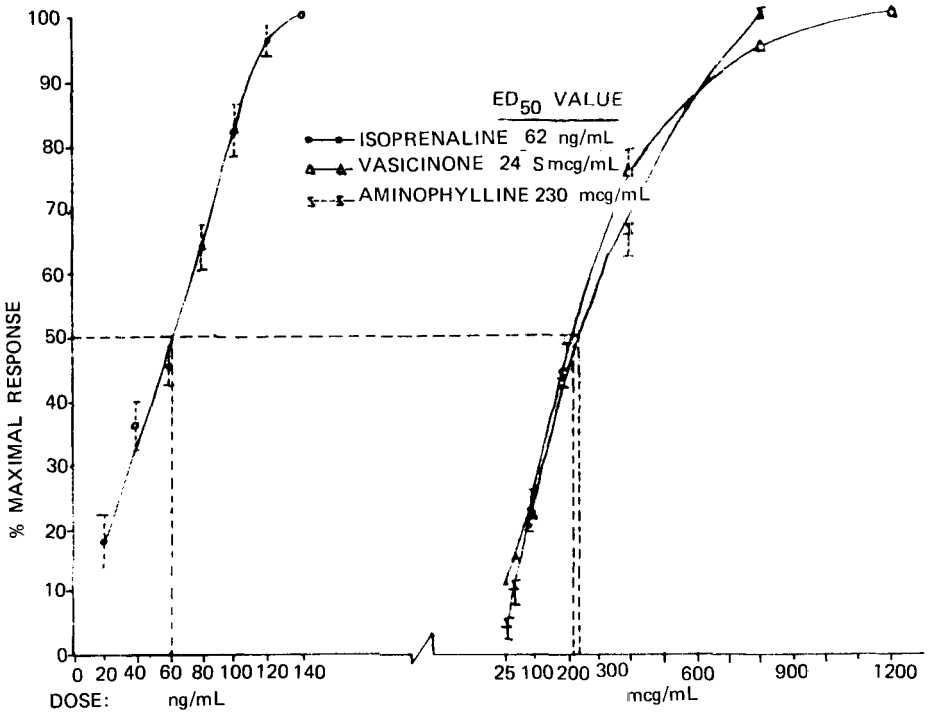

Figure 3 - DOSE-RESPONSE CURVES IN GUINEA-PIG ISOLATED TRACHEAL CHAIN PREPARATION (INCREASED TONE)

unchanged in the urine. Studies suggest the suitability of parenteral as well as oral route of administration for drug.

Vasicinone like isoprenaline and aminophylline produced dose dependent relaxation of smooth muscle in tracheal chain preparation (Table 1). Minimum effective dose (threshold concentration), ceiling dose and ED 50 of these drugs are represented graphically (Figure 2 and 3). Vasicinone in a sub-threshold concentration is found to potentiate the effect of aminophylline by 150-200 per cent and that of isoprenaline by 50-70 per cent (Table 2). The in vivo experimentation on anaesthetized guinea-pigs also confirmed the bronchodilatory

Table 2

Effect of Vasicinone on Graded Dose Responses of Isoprenaline and Aminophylline in Guinea-pig Isolated Tracheal Chain Preparation

Drug	Concentration		Response (in cm)		Percent increase in response
			Control	In the presence of vasicinone (10 mcg/ml)	
Isoprenaline	20	ng/ml	1	1.5	50
	40	"	1.6	2.5	56.25
	60	"	2.3	4.9	69.56
Aminophylline	25	mcg/ml	0.5	1.3	160
	50	"	0.7	2.1	200
	100	"	1	3	200
	150	"	1.5	4.3	186
	200	"	2.2	5.7	159

The figures indicate the average of three determinations.

Table 3

Dose-Response Relationship (in vivo) of Vasicinone, Aminophylline and Isoprenaline in Anaesthetized Guinea-Pig

Drug	Dose (i.v.)	Percent inhibition of bronchospasms induced by	
		Histamine (10 mcg/kg)	5-HT (20 mcg/kg)
Isoprenaline	2 (ng/kg)	25.2 ± 4	19.2 ± 0.8
	5 "	54.1 ± 3	48.4 ± 1.2
	10 "	94.5 ± 5	91.3 ± 1.0
	20 "	100	100 ± 2.1
Vasicinone	1 (mg/kg)	25 ± 4	30 ± 2
	2 "	42 ± 3.1	59.1 ± 3.2
	4 "	68 ± 4	75.4 ± 4.1
	6 "	96 ± 3	100
	8 "	100	-
Aminophylline	2 (mg/kg)	30 ± 2	28 ± 3
	4 "	64.5 ± 3.5	61.3 ± 3.5
	8 "	91.2 ± 4	89.2 ± 4.1
	16 "	100	100

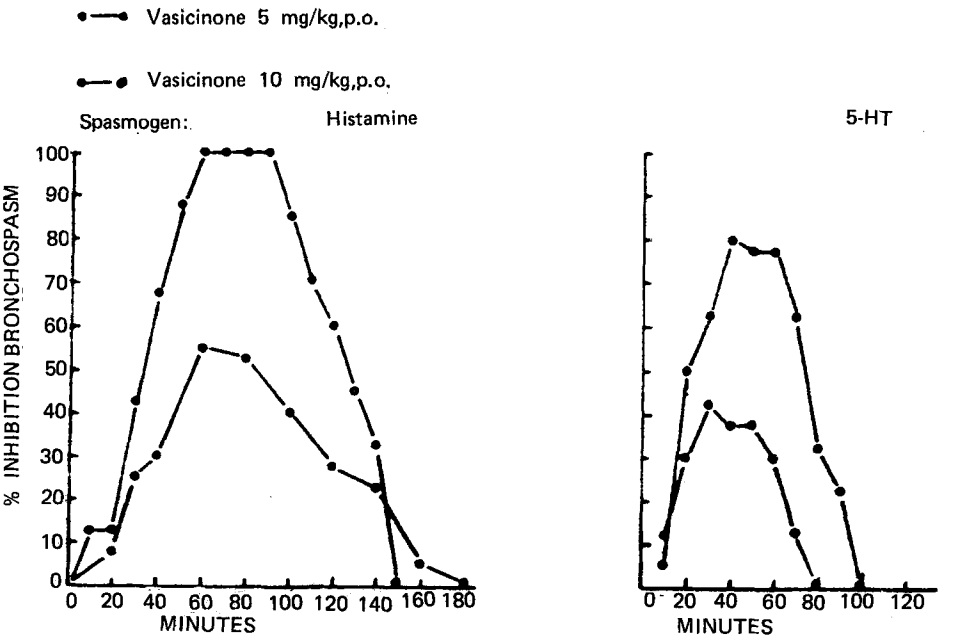

Figure 4 - EFFECT OF VASICINONE ON LUNG RESISTANCE OF GUINEA-PIG

effect of vasicinone. Like aminophylline and isoprenaline, vasicinone could effectively inhibit the bronchospasm induced by histamine and 5-HT (Table 3 and figure 4).

In vitro and in vivo findings substantially indicate the air-way smooth muscle relaxant property of the vasicinone. Since the experiments were carried out on a pure compound of established physico-chemical properties, the results are more reliable than of earlier investigations. Potentiating effect of vasicinone on bronchodilatory activity of isoprenaline and aminophylline is suggestive of combined therapy of these drugs, the admixing may increase therapeutic effect and reduce untowards effects by causing reduction in the dose of these potent drugs.

LD 50 values of vasicinone hydrochloride, administered orally, intraperitoneally and intravenously and their corresponding 19/20 confidence limits were found to be 1100.00 (1353.00-894.30), 520.00 (702.00-385.15), 440.00 (560.00-320.00) respectively.

Vasicinone in sub lethal doses did not elicit any change in the general pattern of behaviour of the animals. The administration of toxic doses of vasicinone reduced the alertness and caused a substantial decrease in spontaneous motor activity. Motor incoordination indicated by staggering gait and loss of muscle tone manifested by reduced grip strength was observed just prior to the death of the animals. Dilatation of pupil, fall in respiratory rate and gasping noted before death may be attributed to the effect of drug on autonomic nervous system. Studies indicate the safety of the drug as no untoward effect on the general behaviour was observed when administered in sub-lethal doses. Further a considerably large difference between effective and toxic dose is indicative of an appreciably large margin of safety on its administration by different routes. In therapeutic doses, the compound is expected to be free of untoward effects, as sub-lethal doses did not produce any neurotoxicity in experimental animals.

CONCLUSION

Investigations confirm the efficacy and safety of vasicinone as a bronchodilator for oral as well as parenteral administration. Combination of vasicinone with aminophylline may prove useful as indicated by its additive effect on bronchodilatory activity.

REFERENCES

1. Amin, A.H. and Mehta, D.R., Nature (London) 184, 1317 (1959)

2. Amin, A.H., Mehta, D.R. and Samarth, S.S., Proc. 1st Int. Pharmacological Meeting Stockholm, Pergamon Press Ltd., Oxford, 7, 377 (1963)

3. Bhide, M.B., Naik, P.Y., Mahajani, S., Chari, R.B. and Joshi, R.S., Bull. Haff. Instt., 2,6 (1974)

4. Bhide, M.B., Naik, P.Y. and Ghosh, R.B., Bull. Haff. Instt., 4 (2) 43, (1976)

5. Bhide, M.B. and Mahajani, S.S., Bull. Haff. Instt., 3 3 (1975)

6. Gupta, O.P., Sharma, M.L., Gholak, B.J.R. and Atal, C.K., J. Med. Res. 66 (4) 680 (1977)

7. Bhalla, H.L., Pharmaceutical Investigations of Anti-asthmatic Principles of Adhatoda vasica Nees. Thesis submitted to the University of Bombay for Ph.D. Pharmacy (Tech), April, 1980

8. Bhalla, H.L., Drug Dev. Ind. Pharm., 7, 6 (under print) (1981)

9. Castillo, J.C. and De Beer, E.J., J. Pharmacol. Exp. Ther., 90, 104 (1947)

10. Konzett, H. and Rossler, R., Arch. Exp. Path. Pharmak; 197, 27 (1940)

11. Litchfield, J.T. and Wilcoxon, F., J. Pharm. Exp. Therap., 96, 99 (1949)

12. Shore, F.A., Brodie, B.B. and Hogben, C.A.M., J. Pharm. Exp. Ther., 119, 361 (1957)