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Original Article

EVALUATION OF *IN VITRO* AND *IN VIVO* ANTIBACTERIAL ACTIVITY OF DOBUTAMINE HYDROCHLORIDE

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Abstract

Purpose: To determine the *in vitro* and *in vivo* antibacterial activity of a cardiovascular drug dobutamine hydrochloride. **Methods:** The minimum inhibitory concentration (MIC) of dobutamine was determined both by agar and broth dilution methods against 331 strains of bacteria from three gram positive and 13 gram negative genera. The antibacterial action of dobutamine was further tested in animal models. **Results:** Dobutamine was seen to possess powerful inhibitory action (5-200 g/mL) against most test bacteria in *in vitro* studies. It was bacteriostatic in nature. *In vivo* studies showed that the drug offered significant protection ($p<0.001$) to mice challenged with a virulent bacterium. **Conclusion:** Dobutamine showed remarkable antibacterial property against several pathogenic bacteria. Its potential as an antibacterial agent may be confirmed after further pharmacological studies.

Key words: *Dobutamine, cardiovascular drug, antimicrobial*

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine. Drugs belonging to different pharmacological classes such as antihistamines like diphenhydramine and bromodiphenhydramine,[1] methdilazine[2] and promethazine,[3] psychotropics, e.g., promazine,[4] chlorpromazine,[5] fluphenazine,[6] and trifluoperazine,[7] antihypertensives, such as methyl-DOPA,[8] local anaesthetics like procaine[9] and antiinflammatory drugs, e.g., diclofenac[10] possess powerful antibacterial activity. Such chemotherapeutic agents have been grouped together and are now entitled as "Non- antibiotics".[11,12] The present paper describes the detailed *in vitro* and *in vivo* activity of such a non antibiotic-the cardiovascular drug dobutamine.

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Materials and Methods

Drugs

The cardiovascular drug dobutamine was obtained from Ranbaxy, clonidine and dipryridamole from German Remedies, enalapril from Nicholas Piramal, lacidipine was obtained from Glaxo Pharma, nimodipine from Torrent, nitrendipine from Concept, felodipine was procured from Cipla, digoxin from Cadila Pharma and benidipine from Stancare. All these drugs were obtained in pure dry powder form and dissolved in either distilled water or dimethyl sulfoxide (DMSO) depending on their solubility, and kept at 4°C.

Bacteria

A total of 331 strains of bacteria belonging to 16 genera comprising 111 gram positive and 220 gram negative types was tested (Table 1). These were of human origin, identified as described by Barrow and Feltham[13] and preserved in freeze dried state.

Media

Liquid media used for this study were peptone water [PW; Oxoid brand bacteriological peptone 1%(w/v) plus Analar NaCl 0.5%(w/v)], nutrient broth (NB; Oxoid), Mueller Hinton broth (MHB; Difco). Solid media were peptone agar (PA), bromothymol blue lactose agar media (BLA), nutrient agar (NA) and Mueller Hinton agar (MHA), obtained by solidifying the liquid media with 1.2%(w/v) agar (Oxoid No.3). In case of BLA,

Table 1: Source of bacterial strain

Name	Source
Bacillus pumilus NCTC 8241	S. P. Lapage, London
Staphylococcus aureus NCTC 6571, 8530,8531, 8532	S. P. Lapage, London
Escherichia coli K12 Row, *E. coli* C 600	J. D. Abbott, U.K.
E. coli pBR 322, *E.coli* ATCC 25922	S. Palchaudhuri, USA
Salmonella typhimurium NCTC 11, NCTC 74, S. *viballerup*, *S. choleraesuis* 6, *S. choleraesuis* NCTC 37, S. *uganda* 101, *S. paratyphi* 85, *S. london* NCTC 76, *S. typhi* 57, 59	J. Taylor, London
Shigella boydii 5 NCTC 541/60,*Sh. boydii* 8 NCTC254/66, *Sh. boydii* 9 NCTC 304/67, *Sh. dysenteriae* 3 NCTC 102/65, *Sh. dysenteriae* 7 NCTC 519/66, *Sh. dysenteriae* 8 NCTC 599/52, *Sh. sonnei* NCTC 5/59, *Sh. flexneri* 4a 24	K. Patricia Carpenter, London
Vibrio cholerae ATCC 14033, 14035	S. Mukerjee, Calcutta
V. *cholerae* 80, 540, 546, 566, 569 B, 590, 738, 764, 824, 838, 906, 1003, 1021, 1023.	National Institute of Cholera & Enteric Diseases, Calcutta.
V. parahaemolyticus 4750, 9369, 72001, 72006	Y. Miyamoto, Japan
Klebsiella pneumoniae 14, ATCC 10031	A.N.Chakrabarty, Calcutta
K. oxytoca ATCC 130988	M.K. Lalitha, Christian Medical College, Vellore

All the remaining organisms were available in the department. They were clinical isolates collected from different hospitals in Kolkata and identified by the methods described by Barrow and Feltham.

bromothymol blue indicator 1.2%(w/v) and lactose 1%(w/v) were added. The pH was maintained at 7.2-7.4 for all the media. NA agar was used for tests with gram positive bacteria and PA and BLA were used for the rest of the bacteria as needed.

Determination of minimum inhibitory concentration (MIC) of different drugs

The MIC of clonidine, dipyridamole, enalapril, digoxin, benidipine, nitrendipine, nimodipine, lacidipine, felodipine and dobutamine with respect to different test bacteria was determined both by broth and agar dilution methods. For broth dilution,[14] 0.1mL of standardized suspension of a strain (10^6 CFU/mL) was added to each tube containing dobutamine at concentrations of 0(control), 2, 5, 10, 25, 50, 100 and 200 g/mL in MHB. The tubes were incubated at 37°C for 24 hours, and looked for visible growth after vortexing the tubes gently. For agar dilution the drug was added at concentrations of 0(control), 2, 5, 10, 25, 50, 100 and 200 g/mL in molten NA and poured in petridishes.[15] The organisms were grown in PW, and the overnight culture was spot-inoculated on the NA plates such that each inoculum contained 2 x 10^6 CFU. The plates were incubated at 37°C, examined after 24 hours and incubated further for 72 hours, if necessary. Since one solid agar medium containing dobutamine could be used for inoculation of a large number of bacteria at a time, the results of this method are being presented here, as the total number of test bacteria was 331. The lowest concentration of dobutamine in a tube or plate that failed to show any visible macroscopic growth was considered as its MIC. The MIC determination was performed in duplicate for each organism, and the experiment was repeated where necessary. The MIC values for a given isolate were either identical, or within one dilution.

Determination of the mode of action of dobutamine on Shigella boydii 8

For this purpose, *Sh. boydii* 8 was grown in NB overnight at 37°C. Two millilitre from this culture was added to 4mL of fresh NB and incubated for 2 hours so that the culture could attain the logarithmic growth phase. The number of viable cells (CFU) was then determined and dobutamine was added at a concentration higher than the MIC value (20 g/mL) of the test bacterium. The CFU counts were determined upto 6 hours at intervals of 2 hours and then after 18 hours.[16]

In vivo tests

Swiss strain of male white mice weighing 18-20g were used for the *in vivo* studies. Animals were maintained at standard conditions at 21° ± 1°C and 50-60% relative humidity with a photoperiod of 14:10hours of semidarkness. Water and a dry pellet diet were given ad libitum. The virulence of the test strain *S. typhimurium* NCTC 74 was exalted by repeated mouse passage and the median lethal dose (MLD or LD_{50}) of the passaged strain corresponding to $0.95x10^9$ CFU/ mouse suspended in 0.5mL NB served as the challenge dose[17] for all the groups of animals. Reproducibility of the challenge dose was ensured by standardization of its optical density in a Klett-Summerson colorimeter at 640nm and determination of the CFU count in NA.

To determine the toxicity of dobutamine, 40 mice were taken, 20 of which were injected 60µg of the drug, and the rest 20 received 30µg of dobutamine. They were kept under observation upto 100 hours.

Two groups of mice, 20 animals per group (each mouse weighing about 20g) were kept in separate cages. Group I was intraperitoneally administered 30µg dobutamine per mouse (0.1mL from 300µg/mL solution of dobutamine), and group II was given 60µg of the drug per mouse (0.1mL from 600µg/mL solution of dobutamine). After 3 hours, each group I and II was challenged with 50 MLD of *S.typhimurium* NCTC 74. A control group of 60 mice was also injected similarly with the same bacterial strain, and 0.1mL sterile saline instead of dobutamine. The protective capacity of the drug was determined by recording the mortality of the mice in different groups upto 100 hours of the treatment, and statistically by x^2 test.

In another experiment, 4 groups of mice, 5 animals per group, were taken. Groups 1 and 3 were administered 60 µg of dobutamine, while groups 2 and 4 were given 0.1mL sterile saline. After 3 hours, all the groups were given a 50 MLD challenge of *S.typhimurium* NCTC 74. After 2 hours, groups 1 and 2 were sacrificed. Their heart blood was collected aseptically; their livers and spleens were removed aseptically and homogenised in tissue homogenisers. CFU counts of the individual organs were determined separately. The same procedure was applied on groups 3 and 4, 18 hours after the challenge. Statistical analysis of the *in vivo* data was done by Student's t-test. The concentration of dobutamine in mouse blood was assayed by measuring the diameter of the inhibition zones by serum-soaked filter paper discs (6mm diameter, 3mm thick, Millipore, absorbing 0.03mL volume) on a lawn flooded with 10^6 bacteria from an 18 hours broth culture of *S. typhimurium* 74 on peptone agar. The drug concentrations in the sera were determined by referring these values to a standard calibration curve prepared with known concentrations of the drugs.[18]

Results

In vitro determination of antimicrobial action of cardiovascular drugs

All the bacterial strains tested were found to be resistant to clonidine, dipyridamole, digoxin, enalapril and nitrendipine, while felodipine, lacidipine, benidipine and nimodipine produced moderate inhibitory action. However, Dobutamine showed powerful antimicrobial action against all the bacteria (Table 2).

Table 2: Primary screening of cardiovascular drugs *in vitro* for presence of antibacterial action

Bacteria	Minimum inhibitory concentration (µg/mL) of the drugs		
	Clonidine, dipyridamole, digoxin, enalapril, nitrendipine	Felodipine, lacidipine, benidipine, nimodipine	Dobutamine
Bacillus pumilus 8241	R	200	25
Staphylococcus aureus NCTC 6571	R	200 - 400	10
S. aureus NCTC 8530	R	200 - 400	25
Escherichia coli K12Row	R	200 - 400	>200
Salmonella typhimurium NCTC 74	R	200 - 400	50
Salmonella typhi 59	R	100 - 200	50
Shigella dysenteriae 7	R	25 - 200	10
Shigella sonnei / NCTC 5/59	R	200	50
Shigella flexneri 4a 24	R	100 - 200	25
Shigella boydii 8	R	200	10
Klebsiella pneumoniae 14	R	200 - 400	>200
Vibrio cholerae 569B, 14033, 14035	R	100 - 200	25
Pseudomonas aeruginosa APC	R	>800	>200

R - resistant

Bacterial inhibitory spectrum of dobutamine

Ninety-nine strains of *Staphylococcus aureus* were tested, of which 8 were inhibited at 5 µg/mL of dobutamine, 14 at 10 µg/mL, 36 at 25 µg/mL, 23 at 50 µg/mL, 12 at 100 µg/mL and 6 by 200 µg/mL of the drug. Of 9 strains of *Bacillus* spp., 2 strains were inhibited at 10 µg/mL, 4 at 25 µg/mL, 2 at 50 µg/mL and the remaining 1 at 100 µg/mL of the drug. *Streptococcus pyogenes* (1 strain) was unable to grow at 25 µg/mL while, 2 strains of *Streptococcus faecalis* were inhibited at 100 µg/mL level of the drug.

In case of gram negative bacteria tested, of 32 strains of *Shigella* spp., 3 were inhibited at 5 µg/mL, 3 at 10 µg/mL, 22 within 50 µg/mL and 4 could not grow at 100 µg/mL of dobutamine. Of 23 *Salmonella* spp., 2 were inhibited at 25 µg/mL, 2 by 100 µg/mL, 3 at 200 µg/mL and 16 were inhibited at concentrations above 200 µg/mL of dobutamine. Most strains of *E. coli* and *Klebsiella* spp. were inhibited between 25-200 µg/mL level. *Pseudomonas aeruginosa* were not inhibited upto 200 µg/mL. The MIC of 36 out of 93 strains of *V. cholerae* were found to be between 10-25 µg/mL, 10 had MIC at 50 µg/mL, 17 at 100 µg/mL, 23 at 200 µg/mL and 7 above 200 µg/mL. Similarly, of 14 strains of *V. parahemolyticus*, 2 were inhibited at 10 µg/mL, 2 strains at 25 µg/mL and 10 could not grow at 100g/mL of the drug.

The drug also showed good inhibitory action (50-100 µg/mL) against strains of *Arizona*, *Bordetella bronchiseptica*, *Citrobacter* and *Providencia*, while dobutamine was less inhibitory to *Proteus* spp. and *Enterobacter cloacae* (Table 3).

Table 3: *In vitro* activity of dobutamine on gram positive and gram negative bacteria

Bacteria	No. tested	No. of strains inhibited by dobutamine (µg/mL)						
		5	10	25	50	100	200	>200
Bacillus spp.	9		2	4	2	1		
Staphylococcus aureus	99	8	14	36	23	12	6	
Streptococcus spp.	3			1		2		
Escherichia coli	31			2	3	5	10	11
Salmonella spp.	23			2	1	1	3	16
Shigella spp.	32	3	3	17	5	4		
Klebsiella spp.	6				1	3		2
Proteus spp.	7					2	4	1
Providencia spp.	1					1		
Citrobacter spp.	1				1			
Arizona spp.	1				1			
Pseudomonas spp.	9							9
Bordetella bronchiseptica	1					1		
Enterobacter cloacae	1							1
Vibrio cholerae	93		18	18	10	17	23	7
Vibrio parahaemolyticus	14		2	2		10		
Total	**331**	**11**	**39**	**82**	**47**	**59**	**46**	**47**

Kinetic studies on the action of dobutamine

The MIC of dobutamine against *Sh. boydii* 8 was found to be 10 µg/mL. At the logarithmic growth phase of the culture, when the CFU count of the strain was 3.0×10^8, 20µg/mL of dobutamine was added. Subsequently, the CFU count of the culture was determined. For *Sh. boydii* 8, it was 4.0×10^6 after 2 hours, 3.0×10^5 after 4 hours, 2.0×10^4 after 6 hours and 2.0×10^4 at the end of 18 hours (Fig.).

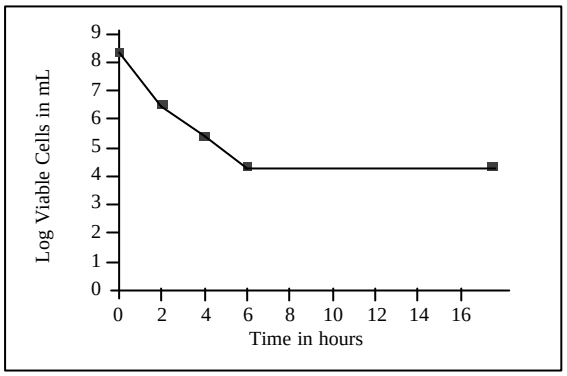

Figure : Action of dobutamine on the changing kinetics of the growth of *Sh. boydii* 8

Determination of protective capacity of dobutamine in vivo

None of the animals in the two batches of mice (20 in each) receiving 30 µg or 60 µg of dobutamine died, proving thereby that the compound was non-toxic for the animals. Subsequently, two more groups of mice (20 per group) were given 30 µg or 60 µg of dobutamine, both of which were challenged with 50 MLD of *S.typhimurium* NCTC 74 after 3 hours. In the first group (30 µg/mouse), 13 out of 20 animals died, while in the other group (60 µg/mouse) only 4 animals died. In the last batch (control) of 60 mice challenged with the same strain, 49 animals died within 100 hours. In table 4, it is seen that dobutamine significantly reduced the number of viable bacteria in heart blood, liver and spleen of mice, both at 2 hours and 18 hours after challenge, compared with the control (saline treated) mice. Statistical analysis showed $p<0.05$ for 2 hours samples and $p<0.01$ for 18 hours samples. The free drug concentrations in the sera of the challenged animals at 0 hours and 2 hours varied from 0.5- 1.5 µg/mL and those at 18 hours varied from 0.2-0.6 µg/mL.

Table 4: Reduction in CFU/mL of *S. typhimurium* NCTC 74 in organ homogenates of mice treated with dobutamine

Time of sampling (hours)	Mouse No.	Drug /mouse	CFU/mL counts in		
			Heart blood	Liver	Spleen
2	1 2 3 4 5	Dobutamine 60 µg	$2.1x\ 10^3$ to $3.1x10^4$	$1.1x10^3$ to $6.5x10^4$	$4.6x10^2$ to $2.5x10^4$
2	1 2 3 4 5	Saline (Control)	$4.0x10^5$ to $7.8x10^6$	$2.8x10^6$ to $8.5x10^6$	$1.2x10^5$ to $8.8x10^6$
18	1 2 3 4 5	Dobutamine 60 µg	$1.1x10^3$ to $4.5x10^4$	$5.8x10^3$ to $7.3x10^4$	$3.5x10^3$ to $7.8x10^5$
18	1 2 3 4 5	Saline (Control)	$5.4x10^8$ to $7.2x10^9$	$8.0x10^3$ to $5.2x10^9$	$1.8x10^8$ to $8.2x10^9$

Viable counts between two groups were significant; $p < 0.05$ in 2 hours samples and $p < 0.01$ in 18 hours samples.

Discussion

The inotropic sympathomimetic drug dobutamine, which is regularly used to treat congestive heart failure, has shown significant action against many bacteria *in vitro* and against *Salmonella typhimurium in vivo* in mice. Many strains of *Staphylococcus* spp., *Shigella* spp., *Salmonella* spp. and *Vibrio* spp. were sensitive to

this compound, although others were only moderately sensitive. Moreover, dobutamine was found to be lethal for *Sh. boydii* 8 upto 6 hours without any increase in CFU upto 18 hours. It is known that the biological half-life of dobutamine is very short. As long as dobutamine was actively available in the medium, the bacteria were killed. After 6 hours, the activity of dobutamine was possibly lost; hence, there was no more inhibitory action of the drug. The animal experiments were undertaken to determine the relevance of dobutamine to human therapeutic application and find the equivalent of mouse doses to possible human doses.

Search among various classes of pharmacological agents have revealed that the tricyclic phenothiazines in general possess moderate to powerful antimicrobial action. They are either antihistamines (methdilazine[2] and trimeprazine[19]) or neuroleptics (fluphenazine[6] and trifluoperazine[7]). The drug dobutamine, in having a benzene ring attached to another one, may be conceived to mimic a phenothiazine structure, thereby explaining its antibacterial property.[20] Further pharmacological studies are necessary to confirm our findings on the possible use of this drug to treat bacterial infections. With suitable structural modifications, it may be possible to obtain compounds with greater antimicrobial actions, thereby, creating a new generation of potential non-antibiotic drugs.

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