Microbiol. Res. (2003) **158,** 259–264
http://www.urbanfischer.de/journals/microbiolres

Microbiological Research

Antimicrobial potentiality of a new non-antibiotic: the cardiovascular drug oxyfedrine hydrochloride

Kaushiki Mazumdar[1], Kumkum Ganguly[1], K. Asok Kumar[1], N. K. Dutta[1], A. N. Chakrabarty[2], Sujata G. Dastidar[1,*]

[1] Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University, Calcutta 700 032, India
[2] Department of Medical Microbiology and Parasitology, Calcutta University, Calcutta 700 020, India

Abstract

Ten cardiovascular drugs, having diverse pharmacological action, were screened for possible antimicrobial property against known eight sensitive bacteria, belonging to Gram positive and Gram negative types. Although five drugs failed to show antimicrobial activity and three had moderate antimicrobial action, oxyfedrine HCl and dobutamine were seen to possess pronounced antimicrobial property. Oxyfedrine was further tested *in vitro* against 471 strains of bacteria from two Gram positive and fourteen Gram negative genera. The minimum inhibitory concentration (MIC) of oxyfedrine was determined by agar dilution method, which ranged from 50–200 µg/ml in most of the strains, while some strains were inhibited at even lower concentrations. In animal experiments, this compound was capable of offering significant protection to Swiss strain of white mice, challenged with 50 median lethal dose (MLD) of a virulent strain of *Salmonella typhimurium* at concentrations of 15, 30 and 60 µg/mouse. The *in vivo* results were highly significant according to chi-square test.

Key words: Cardiovascular drug, Oxyfedrine, Antimicrobial activity, Antimicrobic, Non antibiotic.

Introduction

The usage of antibiotics and antibacterial chemotherapeutics is becoming more and more limited in the present age, despite the fact that they exist in large numbers. The reason behind such a rapid decline in the market of antibiotics is largely attributed to the emergence of drug-resistant bacteria, which render even some of the most broadspectrum antibiotics ineffective. Moreover, the toxic side effects produced by these antibiotics are also reducing their demand. Different studies on search of newer antimicrobials have revealed that moderate to remarkable antimicrobial action is present in several compounds, belonging to various pharmacological categories, such as the antihistamines (Dastidar *et al*. 1976; Chattopadhyay *et al.* 1988; Chakrabarty *et al.* 1989), the tranquilizers (Dash *et al.* 1977), the antihypertensives (Dastidar *et al.* 1986), the antipsychotics (Molnar *et al.* 1976; Kristiansen 1992; Dastidar *et al.* 1995; Radhakrishnan *et al.* 1999; Bourlioux *et al.* 1992) and the antiinflammatory agents (Annadurai *et al.* 1998; Dastidar *et al.* 2000). Such compounds, having antimicrobial properties in addition to their predesignated pharmacological actions, have been christened 'Non antibiotics' (Chakrabarty *et al.* 1998). Since many of these compounds possess two to three benzene rings, the present work was designed to determine antimicrobial action in cardiovascular drugs having two benzene rings.

Materials and methods

Bacteria. Four hundred and seventy one strains, belonging to 2 Gram positive and 14 Gram negative genera were tested. They were all human isolates and were identified by the method of Barrow and Feltham, 1993. They were preserved in freeze-dried state and at 4 °C in stab slant agar.

Drugs. The cardiovascular drugs were obtained in pure dry powder form from their respective manufacturers: clonidine, dipyridamole, oxyfedrine and xanthinol nicotinate from German Remedies, enalapril from Dr. Reddy's Labs., lacidipine from Glaxo, dobutamine from

Corresponding author: Sujata G. Dastidar
e-mails: jumicrobiol@yahoo.co.in; dastidar@cal2.vsnl.net.in
Fax: ++91-33-24 14-62 66;

Ranbaxy, digoxin from Novartis, nitrendipine from Concept and verapamil from Vulcan Pharmaceuticals. All the drugs were preserved at 4°C.

Media. The liquid media used were nutrient broth (NB; Difco, USA) and peptone water (PW; Difco bacteriological peptone 1.0% plus Analar NaCl 0.5%). The solid medium was nutrient agar (NA, Difco).

In vitro screening of drugs for detection of antibacterial activity. Gram positive bacteria were grown in NB and Gram negative ones in PW for 18 h.

The drugs were dissolved in sterile distilled water or dimethyl sulphoxide (DMSO), were individually added to molten nutrient agar at concentrations of 0 (Control), 25, 50, 100, 200 and 400 µg/ml, and poured into sterile Petridishes under aseptic conditions maintaining the pH at 7.2–7.4.

The inocula were prepared by diluting the 18 h broth cultures such that a 2 mm (internal diameter) loopful of a culture contained 10^5 colony forming units (CFU). These were spot inoculated on the NA plates containing increasing amounts of a drug, including a control. The plates were incubated at 37 °C, examined for appearance of growth after 24 h and extended upto 72 h where necessary.

Determination of bacteriostatic/bactericidal action of oxyfedrine. Two bacterial strains sensitive to oxyfedrine were chosen, viz., S. *aureus* NCTC 6571 and *V. cholerae* 865. Each strain was grown in 4 ml NB for 18 h; 2 ml of this culture was then added to 4 ml of fresh NB and incubated at 37 °C for 2 h to help the strain attain logarithmic growth phase. At this stage, the CFU count was determined, and oxyfedrine was added at a concentration higher than the respective MIC level. CFU counts from the cultures were individually taken after 2, 4, 6 and 18 h of adding the drug.

In vivo tests. Swiss strain of albino male mice maintained in our animal house were given commercial feed and water *ad libitum.*

The animals were intraperitoneally (i.p.) challenged with *Salmonella typhimurium* NCTC 74

This organism was passaged several times through mice to enhance its virulence. A 50 MLD of this strain, corresponding to 1.85×10^9 CFU/mouse, suspended in 0.5 ml NB, was used as the challenge dose (Reed and Muench 1938), whose reproducibility was confirmed by standardising its optical density at 640 nm in a Klett Summerson colorimeter, and also by determining the CFU count on NA.

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

Three groups of mice, 20 animals per group (each mouse weighing 20 g) were kept in separate cages. Group I was intraperitoneally administered 15 µg oxyfedrine per mouse (0.1ml from 150 µg/ml solution of oxyfedrine); group II received 30 µg oxyfedrine per mouse (0.1 ml from 300 µg/ml solution of oxyfedrine) and group III was given 60 µg of the drug per mouse (0.1 ml from 600 µg/ml solution of oxyfedrine). After 3 h, each of the groups I, II and III 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 0.1 ml sterile saline instead of oxyfedrine. The protective capacity of the drug was determined by recording the mortality of the mice in different groups upto 100 h of the treatment, and statistically by χ^2 test.

In another experiment, 4 groups of mice, 5 animals per group, were taken. Groups 1 and 3 were administered 60 µg of oxyfedrine, while groups 2 and 4 were given 0.1 ml sterile saline. After 3 h, all the groups were given a 50 MLD challenge of *S. typhimurium* NCTC 74. After 2 h, groups 1 and 2 were sacrificed. Their heart blood was collected aseptically; their livers and spleens were removed aseptically and homogenized in tissue homogenizers. CFU counts of the individual organs were determined separately. The same procedure was applied on groups 3 and 4 18 h after the challenge. Statistical analysis of the *in vivo* data was done by Student's t-test. The concentration of oxyfedrine in mouse blood was assayed by measuring the diameter of the inhibition zones by serum-soaked filter paper discs (6 mm diameter, 3 mm thick, Millipore, absorbing 0.03 ml volume) on a lawn flooded with 10^6 bacteria from an 18 h broth culture of *S. typhimurium* 74 on peptone water 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 (Cruickshank 1975).

Results

Determination of antibacterial activity of the cardiovascular drugs by in vitro screening

Ten cardiovascular drugs were preliminarily screened for antibacterial activity, by testing them *in vitro* against eight sensitive bacteria (Table 1). All the bacteria were resistant to clonidine, digoxin, dipyridamole, nitrendipine and xanthinol nicotinate, while enalapril, lacidipine and verapamil produced inhibitory action against few bacteria at high concentrations (400–800 µg/ml level) only. Dobutamine and oxyfedrine showed significant antibacterial action against most of the test bacteria. Subsequently, oxyfedrine was screened against 471 strains of bacteria (Table 2).

Table 1. Primary screening of cardiovascular drugs *in vitro* for presence of antibacterial action.

Bacteria	Minimum inhibitory concentration (μg/ml) of the drugs		
	Clonidine, dipyridamole, xanthinol, digoxin, nitrendipine	enalapril lacidipine verapamil	dobutamine, oxyfedrine
Bacillus leicheniformis 10341	R	> 800	200
Staphylococcus aureus NCTC 6571	E	> 800	100
S. aureus NCTC 8530	S	> 800	100
Escherichia coli K12Row	I	> 800	> 800
Salmonella typhimurium NCTC 74	S	100–200	50–200
Shigellae	T	100–200	> 200
Klebsiella pneumoniae 14	A	> 800	> 200
Vibrio cholerae 865, ATCC 14033, ATCC 14035	N T	100–200	50–200
Pseudomonas aeruginosa APC		> 800	> 800

Table 2. Antibacterial spectrum of oxyfedrine *in vitro.*

Bacteria	No. Tested	No. of strains inhibited by oxyfedrine (μg/ml)						
		25	50	100	200	400	800	>800
Bacillus spp.	10	1		4	5			
Corynebacterium diphtheriae	1			1				
Staphylococcus aureus	154	7	38	62	46	1		
Escherichia coli	35			1	1	4	3	26
Salmonella spp.	15		2	4		4	5	
S. typhimurium NCTC 11	1			1				
S. typhimurium NCTC 74	1		1					
Shigella spp.	44	2	2	3	5	10	12	10
Klebsiella spp.	9			2	1			6
Hafnia spp.	1			1				
Proteus spp.	9							9
Providencia spp.	1							1
Citrobacter spp.	1							1
Arizona spp.	1						1	
Pseudomonas spp.	12				1			11
Bordetella bronchiseptica	1							1
Pasturella septica 136	1	1						
Vibrio cholerae	149	22	31	38	32	26		
V. parahaemolyticus	25			7	8	10		
Total	471	33	74	124	99	55	21	65

Out of 10 strains of *Bacillus* spp., 5 were inhibited by oxyfedrine within 100 μg/ml; among 154 strains of staphylococci tested, 7 were inhibited at 25 μg/ml, 38 at 50 μg/ml, 62 strains at 100 μg/ml, and the rest at 200 μg/ml. With respect to strains of *Salmonella* spp., 6 were inhibited at 50–100 μg/ml of oxyfedrine. *Salmonella typhimurium* 11 stopped growing at 100 μg/ml, while *S. typhimurium* 74 at 50 μg/ml. For shigellae, MIC ranged from 50–200 μg/ml for 10 strains out of 44 tested. Out of 149 *Vibrio cholerae,* 22 could not grow at 25 μg/ml of the drug, 69 were inhibited at 50–100 μg/ml, 32 at 200 μg/ml and the rest at 400 μg/ml. Oxyfedrine was also equally inhibitory for *V. parahaemolyticus* strains. The drug had insignificant antimicrobial action on *E. coli* and *Klebsiella* spp. Strains of *Pseudomonas* and *Proteus* were resistant to the drug. 7 different strains of enterobacteria were also studied, most of which showed significant sensitivity to oxyfedrine (MIC at 25–100 μg/ml).

Bacteriostatic action of oxyfedrine

The MIC of oxyfedrine against *S. aureus* NCTC 6571 and *V. cholerae* 865 were found to be 100 μg/ml. At the

logarithmic growth phase of the cultures, when the CFU counts of the strains were 2.5×10^8 and 3.0×10^8 respectively, 200 µg/ml of oxyfedrine was added to each. Subsequently, the CFU of the cultures were determined. For *S. aureus* NCTC 6571, the CFU was 2.0×10^5 after 2 h, 3.0×10^4 after 4 h, 2.5×10^4 after 6 h and 1.2×10^4 at the end of 18 h (Fig. 1). Similar bacteriostatic action was recorded in *V. cholerae* 865 (Fig. 2).

In vivo tests

Table 3 shows that in the control group, 48 out of 60 animals died within 100 h of the challenge and no mortality was recorded in those groups of mice that received different doses of oxyfedrine only. There was a significant protection in the drug-treated groups by oxyfedrine. In Table 4, it is seen that oxyfedrine significantly reduced the number of viable bacteria in heart

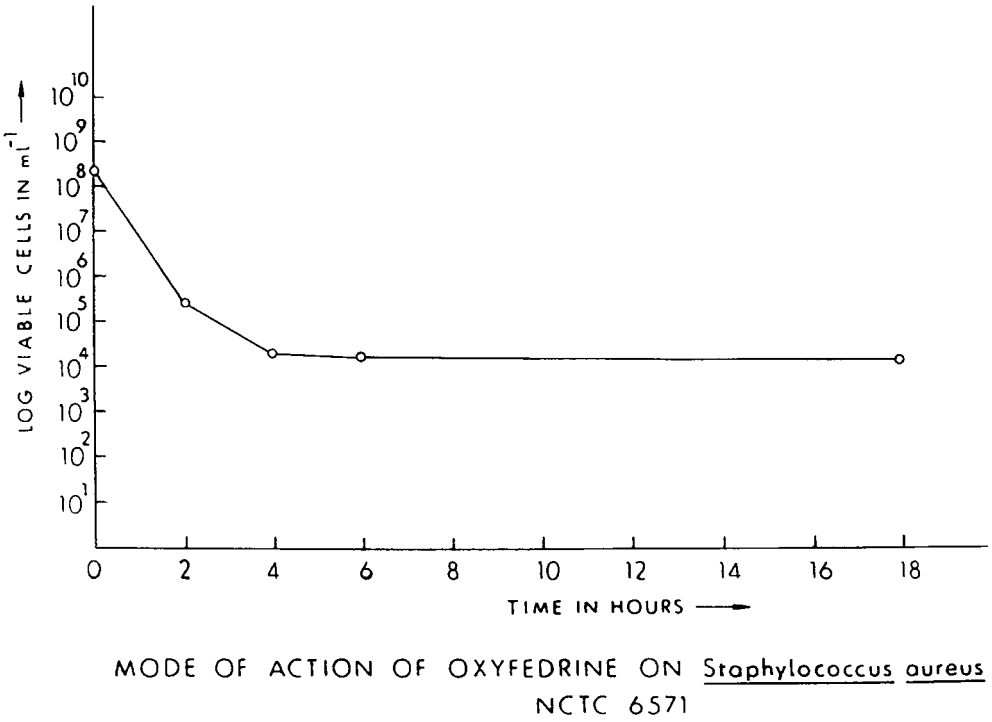

Fig. 1. The action of oxyfedrine on *S. aureus* NCTC 6571 (MIC 100 µg/ml)

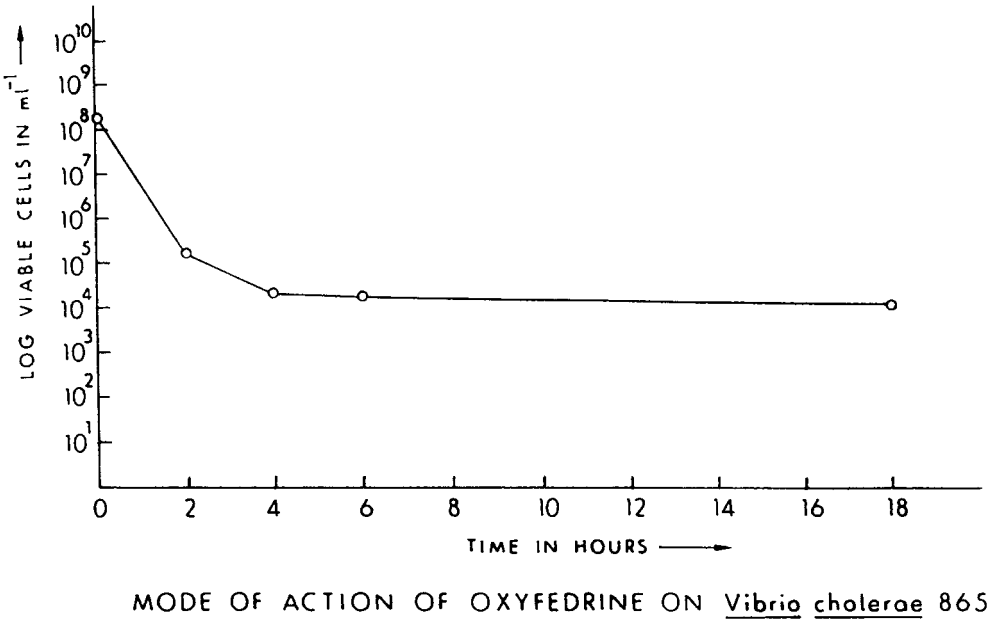

Fig. 2. Effect of oxyfedrine on *V. cholerae* 865 (MIC 100 µg/ml)

blood, liver and spleen of mice, both at 2 h and 18 h after challenge compared with the control (saline treated) mice. Statistical analysis showed $p < 0.05$ for 2 h samples and $p < 0.01$ for 18 h samples.

Discussion

The antianginal and coronary vasodilator cardiovascular drug oxyfedrine hydrochloride was seen to possess powerful antibacterial activity both *in vitro* and *in vivo* in mouse experiments. While sensitive bacterial strains occurred among *Staphylococcus, Bacillus, Vibrio* spp. and some enterobacteria, the drug was much less powerful with respect to strains of *Shigella, Salmonella, E. coli, Klebsiella* and *Pseudomonas* spp. Oxyfedrine was found to be bacteriostatic *in vitro* against both Gram positive and Gram negative bacteria.

The protection offered by the drug to mice challenged with a virulent bacterium was found to be statistically highly significant. Oxyfedrine can be administered to a human being at the dose of 25–100 mg per day orally. After administration, action of the drug takes place at the end of 30 minutes. Duration of action is close to 2 hours. Calculating the dose given to human beings for a 20 g mouse, oxyfedrine can be administered up to 33 µg/day. On this basis, 30 µg of the drug was administered to each mouse in order to find out whether this dose could protect the challenged animal. It was found that there was considerable protection at this dose. Since 30 µg of oxyfedrine was nontoxic to the animals, a higher dose of the drug (60 µg/mouse) was subsequently injected to the challenged animals to check for better protection. It was observed that this dose was also totally nontoxic to the mice, and the protection offered by this dose was statistically more significant than that offered by the 30 µg/mouse dose. Moreover, oxyfedrine as a cardiovascular drug is administered to humans daily for several months, whereas the antibacterial protection

Table 3. Determination of protective capacity of oxyfedrine *in vivo*

Control group*		Test group*	
Drug injected per mouse	Mice died (out of 60)	Drug (µg) injected per mouse	Mice died (out of 20)
0.1 ml sterile saline	48	15	12
		30	13
		60	4

* Received a challenge dose of 1.85×10^9 CFU in 0.5 ml NB of *S. typhimurium* NCTC 74.
None of the animals died when 30 µg or 60 µg oxyfedrine was injected to 2 separate groups of mice (20 mice in each), i.e., oxyfedrine was found to be non-toxic to mice.
$p < 0.001$, according to Chi-square test.

Table 4. Reduction in CFU/ml of *S. typhimurium* NCTC 74 in organ homogenates of mice treated with oxyfedrine.

Time of sampling	Group	Mouse nos.	Drug/Mouse	CFU/ml counts in		
				Heart blood	Liver	Spleen
2 h	1	1 2 3 4 5	Oxyfedrine 60 µg	1.5×10^4 to 7.2×10^5	1.6×10^5 to 3.5×10^5	2.2×10^5 to 5×10^6
	2	1 2 3 4 5	Control 0.1 ml saline	3.4×10^6 to 8.3×10^6	2.8×10^6 to 9.5×10^5	0.6×10^5 to 8.2×10^6
18 h	3	1 2 3 4 5	Oxyfedrine 60 µg	4.8×10^3 to 2.2×10^4	3.1×10^3 to 6.5×10^4	2.4×10^3 to 3.2×10^4
	4	1 2 3 4 5	Control 0.1 ml saline	2×10^8 to 6.3×10^8	5.8×10^8 to 9.5×10^7	4.5×10^8 to 9.2×10^7

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

that it offers to challenged mice was achieved after a single administration.

Since this drug is in routine therapeutic usage satisfying human toxicity tests, oxyfedrine may, in course of time, be developed as the second or even the first line antimicrobial agent in many infections; such properties would further enhance its applicability in humans. Thus, the present study suggests that oxyfedrine has a potential for being developed into a powerful antimicrobial agent, the efficacy of which may be enhanced further by various structural modifications and clinical or chemotherapeutic synergistic combinations of the drug with conventional antimicrobics and/or non-antibiotics.

References

Annadurai, S., Basu, S., Ray, S., Dastidar, S. G., Chakrabarty, A. N. (1998): Antimicrobial activity of the antiinflammatory agent diclofenac sodium. Indian J. Exp. Biol. **36,** 86–90.

Barrow, G. I., Feltham, R. K. A. (1993): In: Cowan and Steel's Manual for the identification of medical bacteria. Cambridge University Press, Cambridge, U. K.

Bourlioux, P., Moreaux, J. M., Su, W. J., Boureau, H. (1992): *In vitro* antimicrobial activity of 18 phenothiazine derivatives, structure-activity relationship. Acta Pathol. Microbiol. Immun. Scand. **100 (Suppl.),** 40–43.

Chakrabarty, A. N., Acharya, D. P., Niyogi, D. K., Dastidar, S. G. (1989): Drug interaction of some non-conventional antimicrobial chemotherapeutic agents with special reference to promethazine. Indian J. Med. Res. **89,** 233–237.

Chakrabarty, A. N., Molnár, J., Dastidar, S. G., Motohashi, N. (1998): In: Non-antibiotics: A new class of unrecognised antimicrobics. NISCOM, India.

Chattopadhyay, D., Dastidar, S. G., Chakrabarty, A. N. (1988): Antimicrobial property of methdilazine and its synergism with antibiotics and some chemotherapeutic agents. Arzneim-Forsch./Drug Res. (FRG). **38,** 869–872.

Cruickshank, R., Duguid, J. P., Marmion, B. P., Swain, R. H. A. (1975): In: Medical Microbiology. Churchill Livingstone, London, 201–208.

Dash, S. K., Dastidar, S. G., Chakrabarty, A. N. (1977): Antimicrobial activity of promazine hydrochloride. Indian J. Exp. Biol. **15,** 324–326.

Dastidar, S. G., Chaudhuri, A., Annadurai, S., Ray, S., Mookerjee, M., Chakrabarty, A. N. (1995): *In vitro* and *in vivo* antimicrobial action of fluphenazine. J. Chemother. **7,** 201–206.

Dastidar, S. G., Ganguly, K., Chaudhuri, K., Chakrabarty, A. N. (2000): The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. Int. J. Antimicrob. Agents. **14,** 249–251.

Dastidar, S. G., Mondal, U., Niyogi, S., Chakrabarty, A. N. (1986): Antibacterial property of methyl-DOPA and development of cross-resistance in m-DOPA mutants. Indian J. Med. Res. **84,** 142–147.

Dastidar, S. G., Saha, P. K., Sanyamat, B., Chakrabarty, A. N. (1976): Antibacterial activities of ambodryl and benadryl. J. Appl. Bact. **41,** 209–214.

Kristiansen, J. E. (1992): The antimicrobial activity of non-antibiotics. Acta Path. Microbiol. Scand. **100 (Suppl.),** 7–19.

Molnár, J., Mandi, Y., Király, J. (1976): Antibacterial effect of some phenothiazine compounds and the R-factor elimination by chlorpromazine. Acta Microbiol. Acad. Sci. Hung. **23,** 45–54.

Radhakrishnan, V., Ganguly, K., Ganguly, M., Dastidar, S. G., Chakrabarty, A. N. (1999): Potentiality of tricyclic compound thioridazine as an effective antibacterial and antiplasmid agent. Indian J. Exp. Biol. **37,** 671–675.

Reed, L. J., Muench, H. (1938): A simple method of estimating fifty percent end points. American J. Hygiene. **27,** 493–497