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Studies on the Antimicrobial Potential of the Cardiovascular Drug Lacidipine

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Abstract. *The cardiovascular drug lacidipine was screened in vitro for possible antibacterial activity with respect to 389 Gram-positive and Gram-negative bacterial strains. It was noticed that most bacteria (233) failed to grow at 50-200 μg/mL concentrations of the drug. Some strains were inhibited at even lower concentrations. The bacteria could be arranged according to their decreasing order of sensitivity as follows: Staphylococcus aureus, Vibrio cholerae, Salmonella spp., Shigellae, Escherichia coli, Bacillus spp., Klebsiellae and Pseudomonas spp. Lacidipine was found to be bacteriostatic in nature against S. aureus and V. cholerae. When administered to Swiss strain of white mice at doses of 30 and 60 μg/mouse, lacidipine significantly protected the animals challenged with 50 MLD of S. typhimurium NCTC 74. According to the chi-square test, the in vivo data were highly significant ($p<0.001$).*

The multifunctional nature of most medicinal agents has proved more to be the rule rather than the exception. Understanding this concept allowed scientists to investigate the antimicrobial properties of many drugs not pharmacologically classified as antimicrobial. Positive results were obtained for many drugs falling almost invariably under one of the following groups, namely psychotropics, neuroleptics, local anaesthetics, antihypertensives, antihistaminics, cardiovascular and antiinflammatory agents. Notable amongst them are the psychotropic chlorpromazine (1), the antihistamines bromodiphenhydramine and diphenhydramine (2), methdilazine (3), promethazine (4) and trimeprazine (5), the tranquilizer promazine (6), the antihypertensives propranolol (7) and methyl-DOPA (8), the local anesthetics procaine and lignocaine (9), the antiinflammatory agent diclofenac (10, 11), the neuroleptic phenothiazines trifluoperazine (12) and fluphenazine (13), the cardiovascular agent amlodipine and oxyfedrine (14, 15), and the antispasmodic compound dicyclomine (16). All these agents showing antimicrobial function were grouped together and termed as "non-antibiotics" (17). Here we screened the cardiovascular drug lacidipine for its antibacterial activity.

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Materials and Methods

Bacteria. A total of 389 bacterial isolates belonging to 16 genera comprising 115 Gram-positive and 274 Gram-negative types were tested. Several strains were obtained from the NCTC and ATCC. The rest were human isolates, identified by the method of Collee *et al.* (18) and were preserved in a freeze-dried state.

Drugs. The cardiovascular drug lacidipine (Figure 1) was obtained in pure dry powder form from Sun Pharmaceuticals, India and was preserved at 4˚C.

Media. Liquid media used for this study were peptone water (PW (Sigma, St. Louis, USA); Oxoid brand bacteriological peptone 1% (w/v) plus Analar NaCl 0.5% (w/v)(Oxoid, Basingstoke UK), nutrient broth (NB, Oxoid) and Mueller Hinton broth (MHB; Oxoid). Solid media were peptone agar (PA), nutrient agar (NA) and Mueller Hinton agar (MHA), obtained by solidifying the respective liquid media with 1.2% (w/v) agar (Oxoid No.3); another solid medium used was desoxycholate citrate agar (DCA, Oxoid). The pH was maintained at 7.2-7.4 for all the media. NA was used for tests with Gram-positive bacteria and PA and DCA were used for the remaining bacteria as needed.

Determination of minimum inhibitory concentration (MIC) of lacidipine. Lacidipine was added at concentrations of 0 (control), 10, 25, 50, 100 and 200 μg/mL in molten NA and poured into Petri-dishes according to NCCLS (19). The organisms were grown in NB or PW for 18 h and harvested during the stationary growth phase. A direct suspension of the organisms was prepared in 5 mL sterile distilled water. The turbidity of the suspension was adjusted to match a 0.5 McFarland's standard (20) with a spectrophotometer (Chemito UV

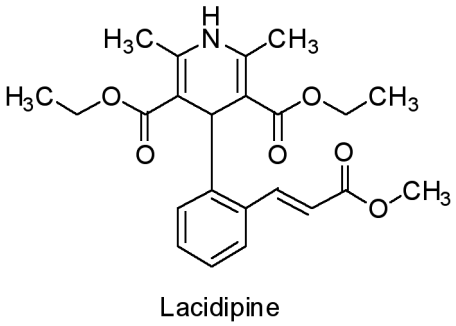

Figure 1. *Structure of lacidipine.*

2600 Double Beam UV-VIS Spectrophotometer (Mumbai, India)) at 625 nm, which corresponded to $2.4x10^8$ colony forming units (cfu)/mL. The inocula were prepared by further diluting the suspension 1:100 with sterile distilled water such that a 2 mm diameter loopful of a culture contained 10^5 cfu. These were spot-inoculated onto the NA plates containing increasing amounts of the drug, including a control. The plates were incubated at 37˚C, examined after 24 h and incubated further for 72 h, where necessary. The lowest concentration of the drug in a plate that failed to show any visible macroscopic growth was considered as its MIC. The MIC (MIC 50 and MIC 90) determination was performed in triplicate for each organism and the experiment was repeated where necessary.

Mechanism of antibacterial action of lacidipine. The MIC of lacidipine against *S. aureus* NCTC 6571 and *V. cholerae* 1347 were found to be 25 μg/mL. At the logarithmic growth phase of the cultures, the cfu counts of the strains were taken and twice the MIC of lacidipine (50 μg/mL) was added to each culture. Subsequently, the cfu counts of the cultures were determined after 2, 4, 6 and 18 h after adding the drug.

In vivo tests. Swiss strain of male white mice weighing 20 g each were used for the *in vivo* studies. Animals were maintained at standard conditions of 21±1˚C and 50-60% relative humidity with a photoperiod of 14:10 h light:dark. Water and a dry pellet diet were given *ad libitum*. The virulence of the test strain *S. typhimurium* NCTC 74 was determined by repeated mouse passage and the median lethal dose (MLD or LD_{50}) of the passaged strain corresponding to $1.85x10^9$ cfu/mouse suspended in 0.5 mL NB served as the challenge dose (21) for all the groups of animals. Reproducibility of the challenge dose was ensured by standardization of its optical density in a Klett-Summerson colorimeter (Lorton, VA, USA) at 640 nm. To determine the toxicity of lacidipine, 30 mice were taken, 10 of which were injected with 60 μg of the drug, 10 received 30 μg and the remaining mice received 15 μg of the drug. They were kept under observation up to 100 h. Three groups of mice, 20 animals/group, were kept in separate cages. Group I was intraperitoneally administered 15 μg lacidipine per mouse, Group II was given 30 μg and Group III received 60 μg of the drug per mouse. After 3 h, each group 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 lacidipine. The protective capacity of the drug was determined by recording the mortality of the mice in different groups up to 100 h of the treatment and statistically using the χ^2 test. In another experiment, 4 groups of mice, 5 animals/group,

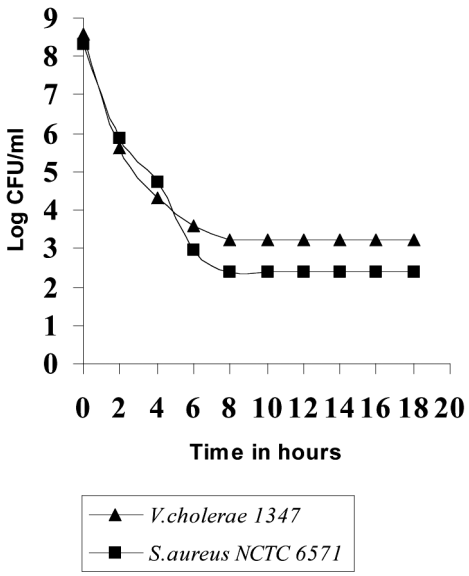

Figure 2. *Effect of lacidipine on Gram-positive and Gram-negative bacteria.*

were taken. Groups 1 and 3 were administered 60 μg of lacidipine, 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 and their livers and spleens were removed aseptically and homogenised in tissue homogenisers. The cfu counts of the individual organs were determined separately. The same procedure was applied to Groups 3 and 4, 18 h after the challenge. Statistical analysis of the *in vivo* data was performed using Student's *t*-test.

Results

Antibacterial activity of lacidipine by in vitro screening. Among 389 bacterial strains tested, it was found that Gram-positive organisms and vibrios were more sensitive to lacidipine than others used in this study. Interestingly, *Pseudomonas aeruginosa*, which is usually resistant to a large number of antibiotics and non-antibiotics, was sensitive towards this drug (Table I).

Bacteriostatic action of lacidipine. At the logarithmic growth phase of the culture of *S. aureus* NCTC 6571, the cfu count of the strain was $2x10^8$. Subsequently, the cfu was $8.0x10^5$ after 2 h, $5.2x10^4$ after 4 h, $9.6x10^2$ after 6 h and $4.0x10^2$ at the end of 18 h (Figure 2). A similar bacteriostatic action was recorded in *V. cholerae* 1347 (Figure 2).

In vivo tests. Table II shows that in the control group, 49 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 lacidipine alone, which was totally non-toxic. There was a significant protection in the drug-treated groups. In Table III, it can be seen that lacidipine significantly reduced the number of viable bacteria in heart blood, liver and spleen

Table I. *In vitro activity of lacidipine on Gram-positive and Gram-negative bacteria.*

Bacteria	No.	No. of strains inhibited by lacidipine (μg/mL)					
	Tested	10	25	50	100	200	>200
Bacillus spp.	10	4	1	2	1	1	1
Corynebacterium diphtheriae	1			1			
Staphylococcus aureus	105	18	34	34	17	2	
Escherichia coli	35			6	10	9	10
Salmonella spp.	17		3	4	4	6	
Shigella spp.	44	8		24	8	2	2
Klebsiella spp.	9			2	1		6
Hafnia spp.	1			1			
Proteus spp.	7					3	4
Providencia spp.	1				1		
Citrobacter spp.	1						1
Arizona spp.	1					1	
Pseudomonas aeruginosa	10		2	3	1	2	2
Pseudomonas putida	2			1	1		
Bordetella bronchiseptica	1			1			
Pasturella septica 136	1	1					
Vibrio cholerae	110	22	24	19	25	20	
V. parahaemolyticus	33	3	10	9	9	2	
Total	389	56	74	107	78	48	26

Table II. *Determination of the protective capacity of lacidipine in vivo.*

	Control group*	Test group*	
	Mouse deaths (out of 60)	Lacidipine (μg) injected per mouse	Mouse deaths (out of 20)
0.1 mL sterile saline	49	15	12
		30	8
		60	0

*Received a challenge dose of $1.85x10^9$ cfu of *S. typhimurium* NCTC 74 in 0.5 mL NB. None of the animals died when 15 μg, 30 μg or 60 μg lacidipine was injected into 3 separate groups of mice (20 mice in each), *i.e.* lacidipine was found to be totally non-toxic to mice. $p<0.001$, acccording to the Chi-square test.

Table III. *Reduction in cfu/mL of S. typhimurium NCTC 74 in organ homogenates of mice treated with lacidipine.*

Time of sampling	Group	Mouse No.	Lacidipine /mouse	Cfu/mL counts in		
				Heart blood	Liver	Spleen
2 h	I	1	60 μg	$2.1x10^3$	$1.1x10^3$	$4.3x10^3$
		2		$2.3x10^3$	$3.0x10^3$	$4.6x10^3$
		3		$2.5x10^3$	$6.5x10^4$	$1.2x10^3$
		4		$3.1x10^4$	$2.1x10^3$	$6.2x10^3$
		5		$5.6x10^3$	$1.2x10^4$	$2.5x10^4$
2 h	II	1	None	$5.7x10^6$	$2.8x10^6$	$8.4x10^6$
		2	saline	$4.0x10^5$	$4.6x10^6$	$1.2x10^5$
		3	(Control)	$5.8x10^5$	$6.0x10^6$	$5.4x10^6$
		4		$6.9x10^6$	$7.0x10^6$	$8.6x10^5$
		5		$7.8x10^6$	$8.5x10^6$	$8.8x10^6$
18 h	III	1	60 μg	$3.6x10^4$	$5.8x10^3$	$7.8x10^5$
		2		$2.6x10^3$	$7.3x10^4$	$3.5x10^5$
		3		$4.5x10^4$	$3.8x10^4$	$7.2x10^6$
		4		$1.1x10^3$	$2.3x10^4$	$4.0x10^4$
		5		$7.0x10^3$	$7.1x10^4$	$3.4x10^4$
18 h	IV	1	None	$4.7x10^8$	$5.8x10^8$	$5.0x10^8$
		2	saline	$5.4x10^8$	$5.2x10^8$	$5.4x10^9$
		3	(Control)	$6.8x10^7$	$2.7x10^7$	$8.2x10^8$
		4		$5.6x10^8$	$3.9x10^7$	$4.9x10^7$
		5		$7.2x10^9$	$8.0x10^8$	$1.8x10^7$

Viable counts between two groups significantly differ at: $p<0.05$ for 2 h samples and $p<0.01$ for 18 h samples, respectively.

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

Discussion

Lacidipine was found to possess powerful antibacterial activity both *in vitro* and *in vivo*. While sensitive bacterial strains occurred among *Staphylococcus, Bacillus, Vibrio* spp. and some enterobacteria, lacidipine was less active on strains of *Shigella*, *Salmonella*, *E. coli* and *Klebsiella*. It may be pointed out here that lacidipine demonstrated a pronounced inhibitory action against *Pseudomonas aeruginosa*, an organism which is known to be multidrug resistant. Lacidipine was bacteriostatic *in vitro* against both Gram-positive and Gram-negative bacteria. The protection offered by lacidipine in mice challenged with a virulent bacterium was found to be statistically highly

significant. Lacidipine is a widely used third-generation calcium channel blocker, which has both long-lasting antihypertensive activity and also antioxidant properties (22). This class of pharmaceutical agents relaxes smooth muscle and dilates coronary and peripheral arteries. Lacidipine has more influence on vessels and less on the myocardium and has no anti-arrhythmic activity. It rarely precipitates heart failure because any negative inotropic effect is often offset by a reduction in left ventricular work. The dose initially is 4 mg daily but may be increased to 6 mg, if necessary, after 3-4 weeks. In our study, we observed that successful protection of mice could be obtained when the amount of lacidipine was either 30 mg or 60 mg/animal. Looking at the low dose of lacidipine as applied to human beings for cardiovascular ailments, our dose as an antimicrobial drug may appear to be rather high. However, it may be mentioned here that the drug was totally non-toxic for the animals, even at the highest dose used, since all the mice survived not only for 100 h as presented here (Table II), but also up to 7 days. This again proves that lacidipine is a non-toxic agent. Moreover, in our present study, lacidipine was administered only once, whereas lacidipine is prescribed as a cardiovascular drug for a patient who may take lacidipine for a considerable time and even for their whole life. The present study indicates the potential of lacidipine as a noteworthy antimicrobial agent, because such properties are likely to improve its usage in humans. Furthermore, the antimicrobial efficiency of lacidipine may be enhanced by structural modifications or be augmented by suitably combining lacidipine with conventional antimicrobial agents to produce synergism.

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