Experimental Analysis of Antimicrobial Action of Dicyclomine Hydrochloride

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Dicyclomine hydrochloride is an antispasmodic agent. The MIC of dicyclomine against standard strains of Gram positive and Gram negative bacteria were performed by NCCLS broth dilution technique. These drugs showed a rapid killing action on Gram positive bacteria, *Staphylococcus aureus* NCTC 6571, 8530 and several other reference strains. The killing effect against Gram negative bacteria, *Shigella boydii* 8 NCTC 254/66 and *Salmonella typhimurium* NCTC 74 showed that the drug was bacteriostatic with respect to these strains. High rate of killing was achieved for most strains of Gram positive bacteria within 2 h. When administered to Swiss strain of white mice at doses of 30 and 60 μg/g of mouse, the drug could significantly protect the animals challenged with 50 MLD of *Salmonella typhimurium* NCTC 74. According to χ^2 test, the *in vivo* data were highly significant ($p < 0.001$). Since dicyclomine showed a remarkable inhibitory action against several pathogenic bacteria, in the course of time, it may be developed as a potent antimicrobial agent for many bacterial infections.

Key words antispasmodic; antimicrobial; non-antibiotic; dicyclomine; bacteriostatic; bactericidal

From the history of the development of pharmaceutical compounds it is evident that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine. Different studies in the search for newer antimicrobials have revealed that moderate to remarkable antimicrobial action is present in several compounds belonging to various pharmacological categories, such as the antihistamines,[1—3] tranquilizers,[4] antihypertensives,[5] antipsychotics[6—10] and antiinflammatory agents.[11,12] Such chemotherapeutics have been grouped together under one common title, "non-antibiotics".[13] Some of these compounds have been recognized for their antitubercular function as well.[14] The present paper describes the antimicrobial action of the antispasmodic agent dicyclomine.

MATERIALS AND METHODS

Bacterial Strains The strains used in this study were *Staphylococcus aureus, Bacillus* spp., *Shigella boydii, Salmonella typhimurium, Escherichia coli* and *Vibrio cholerae*. All were received from either the National Collection of Typed Culture (NCTC), London or the American Type Culture Collection (ATCC), U.S.A. (Table 1). These strains were identified as described by Barrow and Feltham[15] and Collee *et al*.[16] and preserved in a freeze-dried state.

Media Bacteria were cultivated in nutrient broth (NB; Oxoid), nutrient agar (NA, Oxoid) and desoxycholate citrate agar (DCA; Oxoid) according to the National Committee for Clinical Laboratory Standards (NCCLS).[17] The pH in all the media was maintained at 7.2 to 7.4.

Chemical Compound Dicyclomine hydrochloride was obtained as pure dry powder from Ameya Pharmaceuticals, India and was dissolved in sterile distilled water at 10 mg/ml as the stock solution and kept at 4 °C.

Determination of Minimum Inhibitory Concentration (MIC) of Dicyclomine Broth dilution method was used to determine the MIC of dicyclomine with respect to different test bacteria. For this method, dicyclomine was added to each tube at a concentration of 0 (control), 5, 10, 25, 50, 100 and 200 μg/ml.[18] This was repeated at least three times for each test bacterium (Table 1).

Determination of Bactericidal/Bacteriostatic Activity[19] For this purpose *S. aureus* NCTC 6571, 8530, *Sh. boydii* 8 NCTC 254/66, and *S. typhimurium* NCTC 74 were grown in NB overnight at 37 °C. Two milliliters from each of these was added to 4 ml of fresh NB and incubated for 2 h so that the cultures could attain the logarithmic growth phase. The number of colony forming units (CFU) was determined with respect to all the organisms and dicyclomine was added at this stage at a concentration higher than the MIC values of the test bacteria. The CFU counts were recorded at intervals of 0.5 h from each sample up to 3 h in case of Gram positive bacteria; however, in Gram negative bacteria CFU counts were recorded at an interval of 3 h from each sample up to 12 h and then after 18 h.

***In Vivo* Tests** The Swiss strain of male white mice weighing 18—20 g was used for the animal experiments. Animals were maintained at 21±1 °C and 50—60% relative humidity with a photoperiod of 14 : 10 h of light–darkness. Water and a dry pellet diet were supplied *ad libitum*.

The virulence of the test strain *S. typhimurium* NCTC 74 was enhanced by repeated mouse passages and the median lethal dose (MLD or LD_{50})[20] of the passaged strain corresponding to 1.85×10^9 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 at 640 nm and determination of the CFU count on NA.

To determine the toxicity of dicyclomine, 40 mice were taken, 20 were injected with 60 μg of the drug and the rest received 30 μg. Since the permissible human dosage of the drug is 3 mg/kg body weight, 210 mg can be administered to

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Table 1 Determination of *in Vitro* Activity of Dicyclomine on Gram Positive and Gram Negative Bacteria by NCCLS Broth Dilution Technique

Organism	Total No.	Minimum inhibitory concentration of dicyclomine (μg/ml)					
		5	10	25	50	100	200
Staphylococcus aureus	12	1	4	3	2	2	
Bacillus spp.	7	2	1	2	2		
Escherichia coli	7		2	1	4		
Salmonella spp.	22	1	14	3	4		
Shigella spp.	28	1	9	2	1	15	
Vibrio cholerae	41	1	6	20	4	8	2
Total	117	6	36	31	17	25	2

a 70 kg human. Based on this data a mouse weighing 20 g can be given 28 μg (or approximately 30 μg) of the drug. Hence, 30 μg and its double amount (60 μg) were used in this study. The animals were kept under observation for 100 h.

In a separate experiment 2 groups of mice, 20 animals per group, were kept in separate cages. Each mouse of group I was intraperitoneally (i.p.) administered 30 μg dicyclomine (0.1 ml from a 300 μg/ml solution of dicyclomine), and group II was given 60 μg of the drug per mouse (0.1 ml from 600 μg/ml solution of dicyclomine). After 3 h, animals in both groups were 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 dicyclomine. The protective capacity of the drug was determined by recording the mortality of the mice in different groups following 100 h of the treatment, and statistically by χ^2 test.

Another experiment involved 2 batches of mice, with 5 animals per batch. Batch I was administered 60 μg of dicyclomine, while batch II was given 0.1 ml sterile saline. After 3 h, all the animals were given a 50 MLD challenge of *S. typhimurium* NCTC 74, and after 18 h all the mice in both batches I and II were sacrificed. Their heart blood was collected aseptically; their livers and spleens were removed aseptically and homogenised in a tissue homogeniser. CFU counts of the individual organs were determined separately. Statistical analysis of the *in vivo* data was carried out by Student's *t*-test. The concentration of dicyclomine 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 a 0.03 ml volume) on a lawn flooded with 10^6 bacteria from an 18 h broth culture of *S. typhimurium* 74 on NA 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.[22)]

RESULTS AND DISCUSSION

MIC Determination Out of 12 strains of *S. aureus*, 1 was inhibited by dicyclomine at 5 μg/ml level, 4 at 10 μg/ml, 3 at 25 μg/ml and 2 each at 50 and 100 μg/ml; with respect to strains of *Bacillus* spp., 2 were inhibited at 5 μg/ml, 1 at 10 μg/ml and 2 each at 25 and 50 μg/ml levels. A similar pattern of inhibition was noted among *Salmonella* spp., *Escherichia coli* and *Vibrio cholerae* by NCCLS broth dilution technique. However, the strains of shigellae were less sensitive (Table 1).

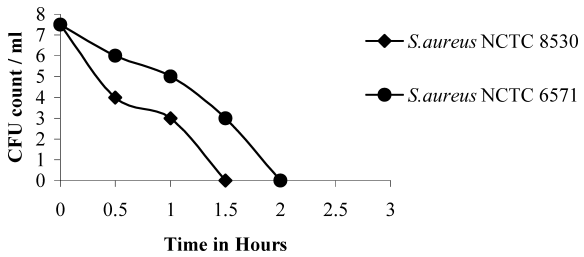

Fig. 1. Mode of Action of Dicyclomine on Two Different Gram Positive Bacteria

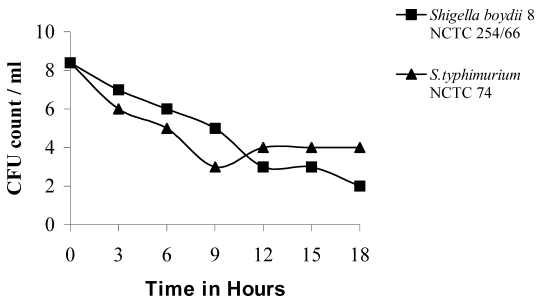

Fig. 2 Mode of Action of Dicyclomine on Two Different Gram Negative Bacteria

Bactericidal and Bacteriostatic Determination The MIC of dicyclomine with respect to *S. aureus* NCTC 6571 and 8530 was 10 μg/ml; when 20 μg/ml of the drug was added to both the cultures in their logarithmic growth phase, the CFU counts were found to be 1.5×10^8 and 1.8×10^8, respectively. This was considered as the zero (0) h. Subsequently, the CFU counts decreased sharply and in *S. aureus* 8530 there were no viable cells after 1.5 h; however, by 2 h all the cells in *S. aureus* 6571 were dead (Fig. 1). Hence, the drug was bactericidal for these organisms.

The MIC of *Sh. boydii* 8 NCTC 254/66 and *S. typhimurium* NCTC 74 was 25 μg/ml; in the logarithmic growth phase their CFU counts were 5.5×10^8 and 6.1×10^8, respectively. At this zero (0) h. 50 μg/ml of dicyclomine was administered to each of the culture tubes. Dicyclomine was found to be bacteriostatic to both strains (Fig. 2). It should be noted that although there was a decrease in the number of CFU during the first 6 h the cells survived in the presence of the drug for up to 18 h.

***In Vivo* Protection by Dicyclomine**[1,2)] The *in vivo* antibacterial effect was determined by administering 50 MLD

dosages (1.85×10^9 CFU in 0.5 ml of NB) of *S. typhimurium* NCTC 74 to different groups of mice with or without administration of dicyclomine (Table 2). In the group that received the challenge and saline (in place of the drug), 49 of 60 mice died within 100 h (contend). In the other groups, which were administered different doses of dicyclomine only, none of the animals died, proving that the drug was totally non-toxic. The protection test turned out to be highly significant ($p<0.001$ in Chi-square test) at both 30 μg/mouse and 60 μg/mouse dosages of dicyclomine compared to the control (without drug).

The results of the experiment for determination of the effect of dicyclomine on CFU/ml in blood and other organs of mice showed significantly reduced counts 18 h after challenge (Table 3, $p<0.001$). The heart blood samples also produced significant reduction in cell counts in treated animals. The free drug concentrations in the sera of the challenged animals at 0 h varied from 0.5 to 1.5 μg/ml and those at 18 h varied from 0.2 to 0.6 μg/ml.

The present study has shown that dicyclomine possesses a powerful inhibitory action against a large number of bacteria *in vitro* and against virulent salmonellae *in vivo* in mice. Many strains of *Staphylococcus aureus*, *Bacillus* spp., *V. cholerae, E. coli* and even a few strains of shigellae were sensitive to this compound, while others were moderately sensitive. The drug was found to be bactericidal against *S. aureus* but bacteriostatic with respect to *Sh. boydii, S. typhimurium* and other Gram negative organisms.

The drug dicyclomine is a tertiary amine, and although structurally related to the antimuscarinics, it has little antimuscarinic activity at low doses. It appears to act directly as a non-selective smooth muscle relaxant[23] and is indicated for the relief of intestinal, biliary and genitourinary spasm.[24]

Table 2. Determination of Protective Capacity of Dicyclomine *in Vivo*

Test group[a]		Control group[a]	
Drug (μg) injected per mouse	Dead mice (out of 20)	Drug (μg) injected per mouse	Dead mice (out of 60)
30	13	0.1 ml sterile saline	49
60	4*		

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

The duration of action in the human body for dicyclomine was noted to be approximately 5—6 h. In our study we have seen that this agent is non-toxic at the highest dose (*i.e.*, 60 μg for 20 g mouse) used in our *in vivo* experiment. Hence, this dose of dicyclomine was used in the mice infected with *S. typhimurium*. The protection offered by the drug in these mice was found to be statistically significant.

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

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Table 3. Reduction in CFU/ml of *S. typhimurium* NCTC 74 in Blood and Organ Homogenates of Mice Treated with Dicyclomine

Period of sampling	Batch	Mouse No.	Drug/mouse	CFU/ml counts in		
				Heart blood	Liver	Spleen
18 h	I	1	Dicyclomine	3.6×10^4	5.8×10^3	7.8×10^5
		2	60 μg	2.6×10^3	7.3×10^4	3.5×10^3
		3		4.5×10^4	3.8×10^4	7.2×10^3
		4		1.1×10^3	2.3×10^4	4.0×10^4
		5		7.0×10^3	7.1×10^4	3.4×10^4
18 h	II	1	Saline	4.7×10^9	5.8×10^8	5.0×10^8
		2	(Control)	5.4×10^8	5.2×10^9	5.4×10^9
		3		6.8×10^8	2.7×10^9	8.2×10^9
		4		5.6×10^9	3.9×10^9	4.9×10^8
		5		7.2×10^9	8.0×10^3	1.8×10^8

CFU counts between two batches showed $p<0.01$ in 18 h samples by Student's *t* test.

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