Antibacterial potential of an antispasmodic drug dicyclomine hydrochloride

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Background & objectives: Several compounds are known to possess antimicrobial activity in addition to their predesignated pharmacological actions. In the present study, dicyclomine hydrochloride, an antispasmodic drug, was tested for possible antimicrobial property in vitro and in vivo.

Methods: The minimum inhibitory concentration (MIC) of dicyclomine against the bacteria was determined by agar and broth dilution methods in vitro. The antibacterial activity of dicyclomine was confirmed by animal experiments. Toxicity and protective efficacy of the drug were tested in vivo.

Results: Dicyclomine inhibited most of the bacterial isolates tested at 25-100 µg/ml concentration, and a few were sensitive even at a lower concentration (10 µg/ml). Dicyclomine was found to be bacteriostatic in nature against Shigella dysenteriae 7, and bactericidal against S. aureus NCTC 6571, 8530, and 8531. When administered to Swiss white mice at doses of 30 and 60 µg/mouse, dicyclomine protected the animals challenged with 50 MLD of Salmonella typhimurium NCTC 74.

Interpretation & conclusion: Dicyclomine showed inhibitory action against several pathogenic bacteria. It also offered significant protection to mice against the bacterial challenge. As dicyclomine is in routine therapeutic use, it may be developed as a potent antimicrobial agent in many infections.

Key words: Anticholinergic - antimicrobial activity - antispasmodic - dicyclomine

Antibiotics are known to be the major protective agents against bacterial infections. However, the usage of antibiotics and antibacterial chemotherapeutics is becoming more and more restricted in the present age, despite the fact that there exists a large number of antibiotics. This is largely attributed to the emergence of drug-resistant bacteria, which render even some of the most broad spectrum antibiotics ineffective. In addition, most antibiotics have side effects. Thus, it becomes essential to investigate newer drugs with less resistance. 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 antihistamines, tranquilizers, antihypertensive, anti-psychotics and antiinflammatory agents. Such compounds, having antimicrobial properties in addition to their predesignated pharmacological actions, are termed as non-antibiotics.

Since many of these compounds possess two to three benzene rings, the present study was designed to determine antimicrobial action of an antispasmodic drug dicyclomine hydrochloride having two benzene rings.

Material & Methods

Drugs: Dicyclomine hydrochloride (Ameya Pharmaceuticals, Mumbai, India) obtained in pure dry powder form was dissolved in sterile distilled water, and kept at 4°C.
**Bacteria:** A total of 414 fully characterised bacterial isolates (obtained from different reference centres) belonging to 15 genera comprising 100 Gram positive and 314 Gram negative types were tested. Of these, 46 were National Collection of Type Culture (NCTC) and American Type Culture Collection (ATCC) strains. These were human isolates, identified as described by Barrow and Feltham and Collee et al and preserved in freeze-dried state.

**Media:** Peptone water (PW; Oxoid brand, UK), nutrient broth (NB; Oxoid), and Mueller Hinton broth (MHB; Difco, Detroit, USA) were obtained, and peptone agar (PA), nutrient agar (NA), and Mueller Hinton agar (MHA) were prepared by adding agar to the respective liquid media, according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines, and used for determining minimum inhibitory concentration (MIC) of dicyclomine. *Streptococcus pyogenes* was grown in MHB supplemented with blood.

**Determination of MIC of dicyclomine:** Both broth and agar dilution methods were used to determine the MIC of dicyclomine with respect to different test bacteria. For these methods, dicyclomine was added to each tube or plate at concentrations of 0 (control), 5, 10, 25, 50, 100 and 200 µg/ml. Since one solid agar medium containing the drug could be used for inoculation of a large number of bacteria at a time, this was done at least three times for every test bacterium and the results of agar dilution method only were presented.

**Determination of bacteriostatic/bactericidal action of dicyclomine:** Bacterial isolates sensitive to dicyclomine were chosen, viz., *Shigella dysenteriae* 7 and *Staphylococcus aureus* NCTC 6571, 8530, 8531. The drug was added at a concentration higher than the respective MIC level (50 µg/ml) at the logarithmic growth phase of the cultures and colony forming units/ml (cfu/ml) counts were determined at 2 hourly intervals up to 18 h.

**In vivo tests:** Swiss male white mice weighing 18-20g were maintained at the animal house at standard conditions of temperature (21±1°C) and relative humidity (50-60%) with a photoperiod of 14:10 h of light-darkness. Water and a dry pellet diet were given *ad libitum*. The virulence of the test strain *Salmonella typhimurium* NCTC 74 was exalted by repeated mouse passages 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 for the test groups of animals.

**Toxicity of the drug:** To determine the toxicity of dicyclomine, 40 mice were studied. Of these 20, were injected intraperitoneally 60 µg of the drug, and the rest received 30 µg of dicyclomine. The animals were kept under observation up to 100 h.

**Protective efficacy of the drug:** Of the two groups of 20 mice each (18-20g) group I was intraperitoneally administered 30 µg dicyclomine mouse (0.1 ml from 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, mice in both the groups were challenged with 50 MLD of *S. typhimurium* 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 up to 100 h.

In another experiment, 4 groups of five mice each were made. Animals in groups I and III received 60 µg of dicyclomine (i.p.), while animals in groups II and IV received 0.1 ml sterile saline. After 3 h, all groups were given a 50 MLD challenge of *S. typhimurium* 74. Two hours after the challenge, animals of groups I and II were sacrificed. The heart blood was collected aseptically; livers and spleens were removed aseptically and homogenised in tissue homogenisers. The cfu/ml homogenate counts of the individual organs were determined separately. The same procedure was applied on groups III and IV, 18 h after the challenge. 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, USA 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 agar. The drug concentrations in serum samples were determined by referring these values to a standard calibration curve prepared with known concentrations of the drugs.

**Statistical analysis:** The data were analysed using Student’s ‘t’-test and Chi square test.
Table II. Reduction in cfu/ml of S. typhimurium NCTC 74 in organ homogenates of mice treated with dicyclomine

<table>
<thead>
<tr>
<th>Time of sampling (h)</th>
<th>Group</th>
<th>Mouse No.</th>
<th>Drug/mouse</th>
<th>cfu/ml counts in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Heart blood</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>5</td>
<td>Dicyclomine</td>
<td>2.1x10^3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>60 µg</td>
<td>to</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3.1x10^4*</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>5</td>
<td>Saline</td>
<td>4.0x10^3</td>
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<td></td>
<td></td>
<td></td>
<td>(Control)</td>
<td>to</td>
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<td></td>
<td></td>
<td>7.8x10^6</td>
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<tr>
<td>18</td>
<td>III</td>
<td>5</td>
<td>Dicyclomine</td>
<td>1.1x10^3</td>
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<td></td>
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<td>60 µg</td>
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<td>4.5x10^4++</td>
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<td>18</td>
<td>IV</td>
<td>5</td>
<td>Saline</td>
<td>5.4x10^4</td>
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<td></td>
<td>(Control)</td>
<td>to</td>
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<td>7.2x10^6</td>
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*P*<0.05; **<0.01 compared to control group

**Results**

Bacterial inhibitory spectrum of dicyclomine: Of the 414 bacterial isolates tested, were inhibited by the drug at 5-25 µg/ml concentrations (Table I). The staphylococci, vibrios and some enterobacteria like *Arizona, Bordetella* and *Hafnia* were sensitive to this drug; 71 of 89 isolates of *S. aureus* and 112 of 133 isolates of *V. cholerae*
were inhibited within 100 µg/ml concentration. Bacilli and salmonellae were also found to be sensitive to dicyclomine. Resistant isolates mostly belonged to *Escherichia coli*, *Pseudomonas* and *Klebsiella* spp.

**Bacteriostatic and bactericidal action of dicyclomine:**

The MIC of dicyclomine against *S. dysenteriae* 7 was found to be 25 µg/ml. At the logarithmic growth phase of the culture, when the cfu/ml count of the strain was 5.5x10⁸, 50 µg/ml of dicyclomine was added. Subsequently, the cfu/ml counts of the culture were determined after 2, 4, 6 and at the end of 18 h. The counts were 1.0x10⁶, 2.0x10⁵, 4.0x10⁴ and 1.5x10⁴/ml respectively. The drug was bacteriostatic on some other Gram negative bacteria like *Salmonella typhimurium* 74 and *Shigella boydii* 8. However, dicyclomine proved to be highly bactericidal when tested against *S. aureus* NCTC 6571, 8530, 8531.

**In vivo toxicity of dicyclomine:**

No mortality was recorded in the two groups of mice injected with 30 and 70 µg dicyclomine during the observation period of 100 h.

**In vivo protection by dicyclomine:**

Of the 60 mice in the control group, 48 (80%) died within 100 h of the challenge while 65 and 20 per cent mortality was recorded in the two test groups of mice that received 30 and 60 µg of dicyclomine respectively. The difference in the mortality was found to be significant (*P*<0.001) between the drug treated and control groups. Dicyclomine significantly reduced the number of viable bacteria in heart blood, liver and spleen of mice in groups I and III both at 2 (*P*<0.05) and 18 h (*P*<0.01) after challenge, compared with the control (saline treated) mice (Table II).

**Discussion**

Dicyclomine is an anticholinergic and antispasmodic drug, having direct intestinal and other smooth muscle relaxant activity. It selectively inhibits the M1 receptor in the gastric mucosa and uterine smooth muscle and is thus used in the treatment of gastric and duodenal ulcer. The duration of action of dicyclomine in human body was found to be approximately 5-6 h. The adverse effects of the drug observed in humans included dryness of mouth, blurred vision, dry skin, tachycardia and difficulty in urination. Dicyclomine showed significant antibacterial potential against many Gram negative and Gram positive bacteria in the present study. The drug was found to be more active against generic pathogens. Though dicyclomine was bacteriostatic against Gram negative bacteria, it was bactericidal against Gram positive strains. The animal experiments were undertaken to determine its relevance to the human therapeutic application. The results of *in vivo* experiments showed that the drug was non toxic and significantly protected the mice against the bacterial challenge.

Of the various classes of pharmacological agents the phenothiazines, which contain tricyclic benzene rings, possess moderate to powerful antimicrobial action. In dicyclomine, the presence of two cyclic rings and nitrogen in the secondary or tertiary state may be playing a key role in conferring antimicrobial activity to this compound. Since this drug already is in routine therapeutic usage, it has the potential of being developed as a second or even first line antibacterial agent in many infections, especially those of the gastrointestinal tract. Structural modifications and clinical or chemotherapeutic synergistic combinations of the drug with conventional antimicrobial and/or non-antibiotics could enhance the antibacterial activity of this drug.

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**References**


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