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.

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) antipsychotics6—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 (ATCC), U.S.A. (Table 1). These strains were identified as described by Barrow and Feltham15) 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.19) This was repeated at least three times for each test bacterium (Table 1).

Determination of Bactericidal/Bacteriostatic Activity19) 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 dose21) 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 © 2004 Pharmaceutical Society of Japan
a 70 kg human. Based on this data a mouse weighing 20 g can be given 28 \( \mu \text{g} \) (or approximately 30 \( \mu \text{g} \)) of the drug. Hence, 30 \( \mu \text{g} \) and its double amount (60 \( \mu \text{g} \)) 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 \( \mu \text{g} \) dicyclomine (0.1 ml from a 300 \( \mu \text{g}/\text{ml} \) solution of dicyclomine), and group II was given 60 \( \mu \text{g} \) of the drug per mouse (0.1 ml from 600 \( \mu \text{g}/\text{ml} \) 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 \( \chi^2 \) test.

Another experiment involved 2 batches of mice, with 5 animals per batch. Batch I was administered 60 \( \mu \text{g} \) 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 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 \( \mu \text{g}/\text{ml} \) level, 4 at 10 \( \mu \text{g}/\text{ml} \), 3 at 25 \( \mu \text{g}/\text{ml} \) and 2 each at 50 and 100 \( \mu \text{g}/\text{ml} \); with respect to strains of \( B. \) subtilis spp., 2 were inhibited at 5 \( \mu \text{g}/\text{ml} \), 1 at 10 \( \mu \text{g}/\text{ml} \) and 2 each at 25 and 50 \( \mu \text{g}/\text{ml} \) levels. A similar pattern of inhibition was noted among \( S. \) spp., \( E. \) coli and \( V. \) cholerae by NCCLS broth dilution technique. However, the strains of shigellae were less sensitive (Table 1).

**Bactericidal and Bacteriostatic Determination** The MIC of dicyclomine with respect to \( S. \) aureus NCTC 6571 and 8530 was 10 \( \mu \text{g}/\text{ml} \); when 20 \( \mu \text{g}/\text{ml} \) of the drug was added to both the cultures in their logarithmic growth phase, the CFU counts were found to be \( 1.5 \times 10^8 \) and \( 1.8 \times 10^8 \), 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 \( S. \) boydii 8 NCTC 254/66 and \( S. \) typhimurium NCTC 74 was 25 \( \mu \text{g}/\text{ml} \); in the logarithmic growth phase their CFU counts were \( 5.5 \times 10^8 \) and \( 6.1 \times 10^8 \), respectively. At this zero (0) h 50 \( \mu \text{g}/\text{ml} \) 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 \(1.85 \times 10^9\) 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 \(\mu\)g/mouse and 60 \(\mu\)g/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 \(\mu\)g/ml and those at 18 h varied from 0.2 to 0.6 \(\mu\)g/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 and other Gram negative organisms.

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 \(\mu\)g 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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**REFERENCES**

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**Table 2. Determination of Protective Capacity of Dicyclomine \(in\ Vivo\)**

<table>
<thead>
<tr>
<th>Test group(^a)</th>
<th>Control group(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug ((\mu)g) injected per mouse</td>
<td>Deadly mice (out of 20)</td>
</tr>
<tr>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>60</td>
<td>4(^*)</td>
</tr>
</tbody>
</table>

\(^a\) Received a challenge dose of \(1.85 \times 10^9\) 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 \(\mu\)g or 60 \(\mu\)g dicyclomine was injected. \(^*p<0.001\), according to Chi-square test.

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

<table>
<thead>
<tr>
<th>Period of sampling</th>
<th>Batch</th>
<th>Mouse No.</th>
<th>Drug/mouse</th>
<th>CFU/ml counts in</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 h</td>
<td>I</td>
<td>1</td>
<td>Dicyclomine</td>
<td>Heart blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60 (\mu)g</td>
<td>3.6 (\times)10^6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>2.6 (\times)10^6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>4.5 (\times)10^4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1</td>
<td>Saline</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>(Control)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
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<td>4</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
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</tbody>
</table>

CFU counts between two batches showed \(p<0.01\) in 18 h samples by Student’s \(t\) test.
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