Antimicrobial potentiality of a phenothiazine group of antipsychotic drug-prochlorperazine

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Received 26 April 2001; revised 11 March 2002

The antipsychotic drug, prochlorperazine (Pep), was tested for its antimicrobial efficacy against 103 strains belonging to both gram positive and gram negative bacteria. The drug was found to possess maximum activity against Staphylococcus aureus, Vibrio cholerae and Shigella spp. Pep was moderately active against E. coli but most of the strains belonging to Bacillus spp, Klebsiella spp, Salmonella spp and Lactobacillus spp were found to be resistant to this drug. The drug was tested for its mode of antibacterial activity against Shigella dysenteriae 1 and it was found to be bacteriostatic in action. In in vivo studies, Pep offered significant protection to Swiss albino mice at concentrations of 0.75 μg/g (P<0.01) and 1.5 μg/g (P<0.001) body weight when challenged with 50 median lethal dose of Salmonella typhiurium NCTC 74. Thus the result depicts that prochlorperazine may emerge as a strong antimicrobial drug to replace the conventional antibiotics and to overcome the problem of drug resistance.

Systematic search among various pharmacological categories of drugs have shown that the antihistamines bromodiphenhydramine1, diphenhydramine1, methidilazine2, the tranquiliser promazine3, the antihypertensives propranolol3, the antipsychotics chlorpromazine5,6,7,5,7 trimeprazine7, and even local anesthetics lignocaine8 and procaine possess moderate to powerful antibacterial action. The present study was undertaken to determine the in vitro and in vivo antibacterial activity of the phenothiazine group of antipsychotic drug, prochlorperazine, with the basic objective of finding out ways and means to replace conventional antibiotics and thereby overcome the problem of drug resistance.

Drug—The antipsychotic drug prochlorperazine maleate was obtained as pure dry powder from Rallis India Limited, Mumbai, India and kept at 4°C.

Bacteria—One hundred and three strains of bacteria belonging to 12 genera were tested in this study. S. aureus AM 8/98, E.coli AM 8/98 and P. mirabilis AM 8/98, Klebsiella pneumoniae RM 8/98, Pseudomonas spp. were collected from S.C.B. Medical College, Cuttack; E.coli V C Sonawara 3:37C, S. typhi ATCC 6539, S. aureus NCTC 7447, S. pneumoniae NCTC 7465 were collected from Institute of Microbial Technology, Chandigarh. We have collected B. subtilis CD99/1, Lactobacillus arabinosus CD99/1, E.coli CD99/1, B. cereus var mucoides, S. aureus ATCC 29737 and Sarcina lutea CD99/1 from Central Drugs Laboratory, Kolkata. All the remaining strains were procured from Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

In vitro tests for detection of antimicrobial action—The drug Pep was suspended in sterile distilled water containing Tween 20 and Tween 80. These were then individually added at concentrations of 0, 10, 25, 50, 100, 150 and 200 μg/ml to molten nutrient agar (Oxoid), mixed thoroughly, adjusted to pH 7.2 to 7.4 and poured into sterile petridishes. Bacterial strains belonging to Gram positive genera were grown in nutrient broth (NB; Oxoid brand) and Gram negative types were grown in peptone water (PW; Oxoid brand bacteriological peptone 1.0% plus Amalar NaCl 0.5%) for 18 hr, and diluted so that 3 mm loopful of culture would contain 10⁶ colony forming units (CFU). These were then spot inoculated on the plates containing increasing amount of the drug, incubated at 37°C up to 72 hr for determination of minimum inhibitory concentration (MIC) of the drug against various strains by agar dilution technique9.

Mode of action of Pep—A highly Pep sensitive bacterial strain, Shigella dysenteriae 1 was grown in NB overnight, 2 ml from which were added to 4 ml of sterile NB and incubated for 2 hr at 37°C, so that the culture attained logarithmic phase of growth. After 2 hr
incubation prochlorperazine was added at higher concentration than its MIC value for that particular strain. The number of colony forming unit (CFU/ml) was determined by Miles and Mishra's method at an interval of 2 hr up to 6 hr and then after 18 hr starting from zero hour.

In vivo tests—The strain Salmonella typhimurium NCTC 74 was passed several times through Swiss strain of albino mice (maintained in our own animal house) to increase its virulence and 50 median lethal dose (MLD) of this strain corresponding to 0.95 X 10^6 CFU/ml suspended in 0.5 ml NB served as the challenge dose for all the groups of animals. Reproducibility of the challenge dose was ensured by standardization of its optical density in a Klett Summit Colorimeter at 640 nm and by determining the CFU count on nutrient agar.

Six batches of 20 male, Swiss albino inbred strain of mice (18-20 gm each) were kept in separate cages. The first group of mice consisting of two such batches were administered 15 μg of Pcp (by injecting, ip 0.1 ml from a stock solution of 150 μg/ml of the drug). The second group consisting of the next two batches were given 30 μg of the drug (0.1 ml from a solution containing 300 μg/ml of Pcp) and the third group consisting of the last two batches of mice received 60 μg of the agent (0.1 ml from 600 μg/ml solution). One batch of mice from each of the above mentioned three groups was challenged with 50 MLD of S. typhimurium NCTC 74 3 hr after the drug injection. A control group of 60 animals was also injected (ip) with S. typhimurium NCTC 74 and 0.1 ml sterile saline in place of the drug. The mortality of the animals in each group was recorded up to 100 hr to determine the protective effect of the drug (Table 2).

<table>
<thead>
<tr>
<th>Test groupa</th>
<th>Drug Mice died (μg/mouse)</th>
<th>Control groupb</th>
<th>Drug Mice died (μg/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10*</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>7**</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>60</td>
<td>20</td>
<td>60</td>
<td>12</td>
</tr>
</tbody>
</table>

*Received challenge dose of 0.95 X 10^6 CFU S. typhimurium NCTC 74 3 hr after administration of prochlorperazine
**Received only the drug but no challenge. In the control group that received challenge and saline (in place of drug), 50 out of 60 mice died.

* P < 0.01; ** P < 0.001

In vitro determination of antimicrobial action of prochlorperazine—Pcp was tested against a total of 103 bacteria (Table 1). Of the 6 tested strains of E.coli, 5 strains were inhibited between 10-15 μg/ml and the remaining was resistant even at 200 μg/ml of Pcp. 10 out of 12 strains of Shigella spp. were inhibited at 5-150 μg/ml of Pcp. Pcp showed MIC values within 150 μg/ml against both the tested strains of Pseudomonas spp. strains. 30 out of 40 strains of S. aureus and 24 out of 30 strains of Vibrio cholerae were inhibited by Pcp at 5-100 μg/ml. The only tested strain of Proteus mirabilis was inhibited at 100 μg/ml and all the remaining strains of Salmonella spp., Klebsiella spp., Bacillus spp., Streptococcus pneumoniae, Lactobacillus arabinosus and Sarcina lutea were found to be fairly resistant to the drug. Thus, the drug showed distinctly high inhibitory action against the strains of S. aureus, V. cholerae and Shigella spp. whereas the strains of E.coli, Pseudomonas spp. and Proteus mirabilis showed moderate sensitivity towards Pcp.
The MIC of Pcp against *Shigella dysenteriae* 1 was found to be 5 μg/ml. At the logarithmic growth phase of the culture, when CFU count of the strain was 10.6×10^5, 10 μg/ml of Pcp was added. Subsequently, the CFU of the culture was found to remain constant after 2, 4 and 6 hr and at the end of 18 hr. Thus it can be concluded that Pcp is bacteriostatic in nature.

In vivo experiments—Pcp offered significant protection to mice challenged with *S. typhimurium* NCTC 74 as depicted in Table 2. 15 μg/mouse and 30 μg/mouse doses of the drug reduce the number of death of mice and this difference was found to be significant using the \( \chi^2 \) test (\( P<0.01 - 0.001 \)). The dose 60 μg/mouse of Pcp was proved to be toxic.

The in vitro and in vivo studies involving prochlorperazone suggest that this drug has the potential for being developed into an antibacterial agent, another new non-antibiotic. Several strains of *Staphylococcus* spp., *Shigella* spp. and *Vibrio* spp. showed high sensitivity to the drug, while *E.coli*, *Pseudomonas* spp. and *Proteus mirabilis* responded moderately. The susceptibility of *Pseudomonas* spp. to prochlorperazone is noteworthy, as this organism is known to be resistant against many conventional antibiotics. Prochlorperazone has been found to be bacteriostatic against gram positive and gram negative bacteria. The antimicrobial property of this drug can be further enhanced by synthesizing its derivatives with suitable structural modifications or by appropriate synergistic combinations with other antimicrobials.