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TRIFLUPROMAZINE: A MICROBICIDE NON-ANTIBIOTIC COMPOUND

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The antipsychotic phenothiazine trifluprom azine, possessing a methyl-thio substituent at position 10 and a fluorine moiety at position 2, exhibited significant antibacterial activity against 279 strains of Gram-positive and Gram-negative bacteria. The minimum inhibitory concentration (MIC) of the drug, according to the agar dilution method, was between 2 and 50 µg/ml for *Staphylococcus aureus*, and 5 and 100 µg/ml for shigellae and vibrios. Trifluprom azine, when injected intraperitoneally into Swiss albino mice at a concentration of 30 µg/mouse (20 g), manifested a significant protection to the mice ($p<0.001$) when they were challenged with 50 median lethal dose (MLD) of *Salmonella typhimurium* NCTC 74. Moreover, there was a statistically significant reduction in the number of viable bacteria in organ homogenates and blood of mice treated with this phenothiazine compound.

Keywords: trifluprom azine, phenothiazine, antimicrobial, microbicide, non-antibiotic

Introduction

Widespread use of antibiotics and antibacterial chemotherapeutic agents had caused a significant increase in the number of drug-resistant pathogenic bacteria during the last fifty years. This has necessitated a search for newer antimicrobials, even from non-conventional sources. Extensive studies by different groups of workers all over the world have shown that the antihistamines [1], tran-

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quilizers [2], antihypertensives [3, 4], antipsychotics [5–9] and antiinflammatory drugs [10, 11] possess moderate to powerful antimicrobial action. These non-conventional antimicrobial agents have been designated under a common title, "Non-antibiotics". The present study describes the antimicrobial potentiality of such a non-antibiotic – the antipsychotic drug triflupromazine [12].

Materials and methods

Drug

The drug triflupromazine was obtained as a pure dry powder from Sarabhai Chemicals, Gujarat, India.

Bacteria

A total of 279 strains of bacteria belonging to 8 genera comprising 79 Gram-positive and 200 Gram-negative types were tested (Tables I, II). These were human isolates, identified as described by Barrow and Feltham [13] and preserved in freeze-dried state.

Table I

Source of bacterial strains

Name	Source
Bacillus pumilus NCTC 8241	S. P. Lapage, London
Staphylococcus aureus NCTC 6571, 8530,8531, 8532	S. P. Lapage, London
Escherichia coli K12 Row	J. D. Abbott, U.K.
E. coli PBR 322	S. Palchaudhuri, USA
Salmonella typhimurium NCTC 11, 74, *S. viballerup*, *S. choleraesuis* 37, *S. uganda* 101, *S. paratyphi* 85, *S. typhi* 57, 59	J. Taylor, London
Shigella boydii, Sh. dysenteriae, Sh. sonnei	K. Patricia Carpenter, London
Vibrio cholerae ATCC 14033, 14035	S. Mukerjee, Calcutta
V. cholerae 80, 540, 546, 566, 590, 738, 764, 824, 838, 906, 1003, 1021, 1023.	National Institute of Cholera & Enteric Diseases, Calcutta
V. parahaemolyticus 4750, 9369, 72001, 72006	Y. Miyamoto, Japan

All the remaining organisms were available in the Department. They were clinical isolates collected from different hospitals in Calcutta and identified by the methods described by Barrow and Feltham [13].

Table II

Inhibitory spectrum of triflupromazine

Bacteria	No. tested	Minimum inhibitory concentration (μg/ml) 2	5	10	25	50	100	200	400	>400
Staphylococcus aureus	58	2	4	26	26					
Staphylococcus aureus	17[1]					16	1			
Bacillus spp.	4				2					2
Escherichia coli	7				6	1				
Escherichia coli	29[2]						1		2	26
Salmonella spp.	5		1	2	2					
Salmonella spp.	10[2]					3	2	2	2	1
Shigella spp.	8		4		4					
Shigella spp.	16[2]					4			3	9
Klebsiella spp.	5[2]									5
Vibrio cholerae	59	4	8	35	12					
Vibrio cholerae	33[3]					18	13			2
V. parahaemolyticus	14				14					
V. parahaemolyticus	6[3]					5	1			
Pseudomonas spp.	8				2		1			5
Total	279	6	17	63	68	47	19	2	7	50

The MIC of triflupromazine with respect to *S. typhimurium* NCTC 74 was 5 μg/ml.

[1] 16 strains were resistant to penicillin-G, ampicillin, amoxycillin, methicillin, cloxacillin and azithromycin,whereas the remaining one was simultaneously resistant to vancomycin as well.

[2] Resistant to tetracycline, fluoroquinolones, cotrimoxazole.

[3] Resistant to tetracycline, chloramphenicol and cotrimoxazole.

Pseudomonas strains were resistant to amikacin, mezlocillin and piperacillin.

Identification. The strains of *Staphylococcus aureus* were characterized on the basis of tube coagulase test and also bound coagulase test, performed on a microscope slide. Identification of *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp. were based on their differential biochemical reactions with respect to motility, gas from glucose, acid from lactose, urease activity, citrate utilization, hydrogen sulphide production and indole production. *Vibrio cholerae* and *V. parahaemolyticus* were identified with the help of various sugar fermentation reactions, oxidase, indole, Voges-Proskauer, Simmons' citrate, decarboxylase tests and NaCl tolerance. Serological tests were performed on all the test bacteria for confirmation of identification as required.

Antibiotic sensitivity pattern. The MIC of the antibiotics were determined both by tube dilution and agar dilution techniques, and the grade of sensitivity/resistance was as described by NCCLS [14]. Gram-positive bacteria (including *S. aureus*) were tested for their sensitivity/resistance to penicillin-G, ampicillin, amoxycillin, methicillin, cloxacillin, tetracycline, streptomycin, gentamicin,

cefotaxime and vancomycin. Gram-negative bacteria were screened for their sensitivity/resistance to ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, ofloxacin, tetracycline, cefotaxime, gentamicin, cotrimoxazole, and amikacin, mezlocillin and piperacillin, particularly for *Pseudomonas* spp.

In vitro tests for determination of MIC of triflupromazine

The Gram-negative bacteria were grown in peptone water (PW; 1.0% Oxoid brand bacteriological peptone plus 0.5% Analar NaCl) and Gram-positive ones in nutrient broth (NB, Oxoid Brand, UK) for 18 h to obtain optimum growth. An aqueous stock solution of triflupromazine (5 mg/ml) was sterilized by filtration through a sintered glass filter (G-5) and stored at 4ºC. This was added to molten nutrient agar (NA, Oxoid) to give final concentrations: 0 (control), 2, 5, 10, 25, 50, 100, 200 and 400 µg/ml at 50ºC, thoroughly mixed, final pH adjusted to 7.2 to 7.4 and poured into sterile Petri dishes. The inoculum consisted of a suitably diluted 18 h broth culture of the bacterium. The MIC of triflupromazine was determined by spotting one 2 mm (internal diameter) loopful of this inoculum, containing ca. 10^5 colony forming units (CFU), on the plates. The plates were incubated at 37ºC; growth was recorded at 18 h as well as upto 72 h.

Detection of bactericidal activity of triflupromazine

Strains of bacteria highly sensitive to triflupromazine were grown individually in NB for 18 h; 2 ml of this culture were added to 4 ml of fresh NB and incubated at 37ºC. After 2 h (at the logarithmic growth phase), the CFU count of the culture was determined; thereafter triflupromazine was added at a concentration exceeding the MIC value of the test strain and CFU counts were further determined after 2, 4, 6 and 18 h.

In vivo antibacterial tests

Determination of median lethal dose (MLD). Salmonella typhimurium NCTC 74 was passaged several times in Swiss white male mice (2 months old) to increase its virulence. A 50 MLD of this strain (corresponding to 0.95×10^9 CFU/mouse, suspended in 0.5 ml NB) was used as challenge [15]. The repro-

ducibility of this challenge test dose was checked with reference to fixed optical density at 640 nm in a Klett-Summerson colorimeter to give the predetermined number of CFU on NA.

Animal protection tests. Six batches of mice, 20 per batch, each 18–20 g in weight, were used, Group I (made of the first two batches) was given 15 µg triflupromazine, Group II (comprising the next two batches) was given 30 µg of the drug and Group III (representing the last two batches) received 60 µg triflupromazine; this was done by injecting intraperitoneally 0.1 ml sterile stock solution of the drug containing 150, 300 or 600 µg/ml. A control group of 54 mice was given 0.1 ml sterile saline each instead of the drug; 3 h later, one batch from each of the above three groups and the control group were challenged with 50 MLD of *S. typhimurium* 74. The number of mice dying upto 100 h in all the groups was recorded to assess the protective power of triflupromazine. 0.1 ml of heat-killed cells of *S. typhimurium* 74 (approximately 0.95×10^9 CFU/mouse) was given i.p. as a control to 20 animals to determine the efficacy of endotoxin.

Reduction in CFU counts in organs of triflupromazine treated vis-à-vis untreated mice. In a separate experiment, 10 mice were taken, 5 of which were injected with 30 µg/mouse of triflupromazine and the rest were administered only sterile saline. After 3 h, all the animals received a 50 MLD challenge dose of *S. typhimurium* 74. All the mice were autopsied after 18 h, their livers and spleens were removed aseptically, homogenized in tissue homogeniser; 0.2 to 0.4 ml of their heart blood was also collected aseptically at the same time. CFU counts of these organs were determined individually. Statistical analysis of the data was done by Student's t-test.

Results

Antimicrobial action of triflupromazine in vitro

Triflupromazine was found to possess remarkable antimicrobial action against 279 strains of bacteria tested. It can be seen from Table II that 86 bacterial strains were inhibited by the drug at 2–10 µg/ml and 201 strains at 50µg/ml concentration, thus proving its powerful antimicrobial nature. The staphylococci and vibrios were most sensitive to the drug; 58 of 75 strains of *S. aureus* and 59 of 92 strains of *V. cholerae* were inhibited at 25 µg/ml of this drug. Salmonellae and shigellae were also found moderately sensitive to triflupromazine. Resistant strains mostly belonged to *E. coli, Pseudomonas, Klebsiella* and *Bacillus* spp. The

MIC of triflupromazine against *S. aureus* 6571 was 10 µg/ml. When 20 µg/ml of the drug was added to a culture of *S. aureus* 6571 in the logarithmic phase (containing 5.1×10^8 CFU/ml), it was found that the viable count of this culture dropped to 7.8×10^6 after 2 h, to 1.1×10^6 after 4 h, to 8.3×10^5 after 6 h, and at the end of 18 h, the CFU count reached 0 level, proving the bactericidal nature of the drug (Figure 1). Other Gram-positive and Gram-negative strains also produced similar results.

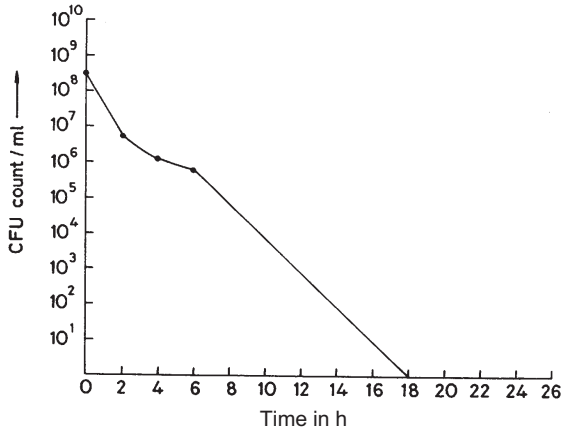

Figure 1. Bactericidal action of triflupromazine on *S. aureus* 6571 (MIC 10 µg/ml). Test carried out with 20 µg/ml of the drug

Animal experiments

The results in Table III showed that triflupromazine could protect mice challenged with *S. typhimurium* NCTC 74. There was reduction in the number of deaths in mice at 30-µg dose of the drug. The difference was shown to be significant using the χ^2 test ($p<0.001$). In the control group receiving only a lethal challenge, 41 of 54 mice died.

Triflupromazine also significantly reduced the CFU/ml of *S. typhimurium* NCTC 74 in the organ homogenates and heart blood samples of mice after challenge, compared with the control (Table IV).

Table III

Determination of protective capacity of triflupromazine

Test Batch		Control Batch	
Triflupromazime (μg) injected per mouse	Mouse died (out of 20)	Triflupromazine (μg) injected per mouse	Mice died (out of 20)
60	0[a]	60	4
30	4[a]	30	0
15	8[b]	15	0

Test Batch received the challenge dose of 0.95 × 109 CFU in 0.5 ml NB of *S. typhimurium* NCTC 74.
Control Batch received no challenge.
In the batch that received the challenge and 0.1 ml saline (in place of drug) 41 out of 54 animals died.
In the other batch of mice that received 0.1 ml of heat-killed *S. typhimurium* cells, none of the animals died.
[a] $p < 0.001$, [b] $p < 0.01$ according to Chi-square test.

Table IV

Reduction in CFU/ml of *S. typhimurium* NCTC 74 in organ homogenates and blood of triflupromazine treated and untreated mice

Drug	CFU/ml counts in		
	Liver[1]	Spleen[2]	Heart blood[3]
Triflupromazine (30 μg per mouse)	1.1×10^4 to 4.1×10^5	2.1×10^4 to 1.0×10^5	2.7×10^4 to 1.4×10^5
Saline	3.0×10^6 to 1.9×10^8	0.9×10^7 to 1.5×10^8	3.5×10^7 to 3.7×10^8

[1] $p < 0.05$, [2] $p < 0.01$, [3] $p < 0.01$ when compared by 't' test with control at 1% level of significance.

Discussion

The antipsychotic phenothiazine compound triflupromazine was seen to possess powerful antibacterial activity both *in vitro* and *in vivo* in mouse experiments. While sensitive bacterial strains occurred among *Staphylococcus, Shigella, Salmonella* and *Vibrio* spp., the drug was much less inhibitory relative to strains of *Bacillus, E. coli, Klebsiella* and *Pseudomonas* spp. It may be pointed out here that the strains of bacteria that were resistant to different antibiotics revealed higher MIC values with respect to triflupromazine (Table II). Further, the *in vitro* inhibitory action of triflupromazine was found to be bactericidal against both Gram-positive and Gram-negative bacteria. The protection offered by the drug to mice challenged with a virulent bacterium was found to be statistically highly significant.

Earlier studies by us and by others revealed that antihistaminic and/or antipsychotic compounds chemically similar to triflupromazine, e.g., methdilazine [1] and fluphenazine [7] were non-toxic to mice even at high doses. However, another antihistaminic drug trimeprazine could not be tolerated by the animals above 15 μg/mouse dose [16]. In this study therefore we applied several doses of the drug to batches of mice in order to determine its toxic effect. Triflupromazine was found to be totally non-toxic upto the 30 μg/ mouse level, and in the 60 μg/mouse dose, only 4 out of 20 animals died (Table III). Lower doses (15 μg/mouse) of the drug could offer considerable protection to the animals, but the protection offered by the 30 μg/ mouse dose turned out to be significant. Moreover, triflupromazine as an antipsychotic agent is administered to humans daily for several months, whereas the antibacterial protection that it offers to challenged mice was achieved after a single administration.

The phenothiazines have proved to be a unique class of compounds with potent antimicrobial activity. Investigations on the structure-activity relationships of phenothiazines show that their antimicrobial properties might be linked to the methyl-thio substituent in position 10 and a halogen moiety at position 2 of the basic phenothiazine ring [9]. Since this drug is in routine therapeutic usage satisfying human toxicity tests, triflupromazine may, in course of time, be developed as the second or even the first line antibacterial agent in many infections. Thus the present study suggests that triflupromazine has a potential for being developed into a powerful antibacterial agent, the efficacy of which may be enhanced further by various structural modifications and clinical or chemotherapeutic synergistic combinations of the drug.

References

1. Chattopadhyay, D., Dastidar, S. G., Chakrabarty, A. N.: Antimicrobial property of methdilazine and its synergism with antibiotics and some chemotherapeutic agents. Arzneimittel-Forschung/Drug Research (FRG) **38,** 869–872 (1988).
2. Dash, S. K., Dastidar, S. G., Chakrabarty, A. N.: Antimicrobial activity of promazine hydrochloride. Indian Journal of Experimental Biology **15,** 324–326 (1977).
3. Manna, K. K., Dastidar, S. G.: The antihypertensive drug propranolol hydrochloride (Carditap): its antibacterial property. Proceedings of the National Congress of IAMM (Image India, Calcutta) 1984, pp. 137–141.
4. Dastidar, S. G., Mondal, U., Niyogi, S., Chakrabarty, A. N.: Antibacterial property of methyl-DOPA and development of cross-resistance in m-DOPA mutants. Indian Journal of Medical Research **84,** 142-47 (1986).

5. Molnár, J., Mándi, Y., Király, J.: Antibacterial effect of some phenothiazine compounds and the R-factor elimination by chlorpromazine. Acta Microbiologica Acad. Sci. Hung. **23,** 45–54 (1976).
6. Kristiansen, J. E.: The antimicrobial activity of non-antibiotics. Acta Pathologica Microbiologica Scandinavia **100** (Suppl.), 7–19 (1992).
7. Dastidar, S. G., Chaudhuri, A., Annadurai, S., Ray, S., Mookerjee, M., Chakrabarty, A. N.: *In vitro* and *in vivo* antimicrobial action of fluphenazine. Journal of Chemotherapy **7,** 201–206 (1995).
8. Radhakrishnan, V., Ganguly, K., Ganguly, M., Dastidar, S. G., Chakrabarty, A. N.: Potentiality of tricyclic compound thioridazine as an effective antibacterial and antiplasmid agent. Indian Journal of Experimental Biology **37,** 671–675 (1999).
9. Bourlioux, P., Moreaux, J. M., Su, W. J., Boureau, H.: *In vitro* antimicrobial activity of 18 phenothiazine derivatives, structure-activity relationship. Acta Pathologica Microbiologica Immunologica Scandinavica **100** (Suppl.), 40–43 (1992).
10. Annadurai, S., Basu, S., Ray, S., Dastidar, S. G., Chakrabarty, A. N.: Antimicrobial activity of the antiinflammatory agent diclofenac sodium. Indian Journal of Experimental Biology **36,** 86–90 (1998).
11. Dastidar, S. G., Ganguly, K., Chaudhuri, K., Chakrabarty, A. N.: The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. International Journal of Antimicrobial Agents **14,** 249–251 (2000).
12. Dastidar, S. G., Debnath, S., Ganguly, M., Chakrabarty, A. N.: A new antibacterial agent: the non-antibiotic triflupromazine. Paper presented at the 3rd European Congress of Chemotherapy at Madrid, Spain, 7–10 May 2000 (Spanish Journal of Chemotherapy **13,** Suppl. 2, 125).
13. Barrow, G. I., Feltham, R. K. A.: Cowan and Steel's Manual for the Identification of Medical Bacteria. Cambridge University Press, Cambridge, U.K. (1993).
14. National Committee for Clinical Laboratory Standards. Methods for Dilution in Antimicrobial Susceptibility Tests. Approved Standard M2-A5. NCCLS, Villanova, PA (1993).
15. Reed, L. J., Muench, H.: A simple method of estimating fifty percent end points. American Journal of Hygiene **27,** 493–497 (1938).
16. Dastidar, S. G., Jairaj, J., Mookerjee, M., Chakrabarty, A. N.: Studies on antimicrobial effect of the antihistaminic phenothiazine trimeprazine tartrate. Acta Microbiologica et Immunologica Hungarica **44(3),** 241–247 (1997).