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Chlorcyclizine, an antihistamine, exhibiting antimetabolic activity and nonmutagenic behavior

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ABSTRACT

Direct evaluation of the carcinogenicity of compounds, employing animal and tissue culture models are often dilatory, costly and less predictable. Opportunities for application of bacterial prescreens for their detection are being successfully explored because of the basic similarities of many of biochemical processes between the microbial and malignant mammalian cells. Several chemotherapeutic agents were screened employing two such bacterial prescreens for detecting their antimetabolic activity and subsequently determining mutagenicity, following Ame's Test. From this study, Chlorcyclizine an antihistamine, structurally related to the phenothiazines emerged as a potential antimetabolite with nonmutagenic activity. Further invivo studies on malignant cell lines is suggested, with this promising drug chlorcyclizine to finally detect if it is endowed with anticarcinogenic property as well.

Key words: prescreens, antimetabolic activity, anticarcinogenecity, chlorcyclizine.

INTRODUCTION

The purpose for detecting antimetabolic activity in compounds, is for searching prospective anticancer agents (Goodman and Gilman,2006). There are several bacterial prescreens available to detect either antimetabolic property associated with anticarcinogenicity (Hanka *et al,*1978) or mutagenicity which involves carcinogenicity (Ames *et al.,* 1973,1982); prescreens which represent in vitro tests are preferred not only because they are simple, cheap and less time consuming, but also because these substantially correlate with the in vivo systems.

The anti-microbial property of many drugs, including several members of the phenothiazines have been reported from our laboratory (Saha *et al*,1976; Chattopadhyay *et al* 1988, Ray et al 1990). The present study was carried out with several chemotherapeutic agents for detecting their antimetabolic activity and then proceeding with determination of mutagenicity.

MATERIAL AND METHODS

Drugs

All the drugs screened are described in Table 1 below. These were all obtained from their respective manufacturers in the pure dry state.

Bacteria

For detection of antimetabolic property, *B.subtilis* UC 564 and *E. coli* UC51, were obtained from Dr. L.J. Hanka, Upjohn company, Michigan, USA. For determination of mutagenicity, *Salmonella typhimurium* TA 102 was received from Prof. B. Ames, University of California, Berkeley, USA (Hossain *et al* 1987)

Media

The composition of various media are given in Tables 2 and 3

Test for detection of antimetabolite activity

Sterile synthetic and nutrient agar plates were overlaid with 2 ml of the same media containing 1 ml of 18 hr old peptone water (1% Difco peptone + 0.5% Nacl, ph 7.4) cultures so as to produce a semiconfluent lawn of growth. The plates were dried and discs containing the drugs (100, 200, 400 ug/ disc) were placed at suitable distances apart . readings were taken after overnight incubation at 37c. The drug which failed to produce any inhibition on nutrient agar but showed a large zone of inhibition on synthetic agar was considered to contain a potential antimetabolite (Hanka,1978).

Table 1 : List of drugs screened for *in-vitro* antimetabolic property

Class of Drugs	No. Tested	List of Drugs screened
Antihistamines	12	Diphenhydramine, BromodyphenhydramineTripolidine, Cyclizine, Chlorcyclizine, Phenirmine,Chlorpheniramine, Halopyramine, Cyproheptadine,Promethazine, Trimeprazine, Methdilazine
Antihypertensives	7	Propranolol, Spirinolactone, Methyl-DOPA, Frusemide,Reserpine, Dihydrallazine, Chlorthalidone
Tranquiliser	1	Promazine
Antipsychotic drugs	2	Chlordiazepoxide, Chlorpromazine
Antibacterial Chemother -apeutic agents	3	Trimethoprim, Cotrimoxazole, Nalidixic acid
Anticancer agents	6	Mitomycin C,Bleomycin, Azaguanine, CytosineArabinoside, Thioguanine, Mercaptopurine

Table 2: Assay media for antimetabolic activity

1. Nutrient agar			
Peptone	0.5 %	Beef Extract	0.3%
Nacl	0,5 %	Agar	1.5%

2. Synthetic agar	***Bacillus subtilis* UC 564**	***Escherichia coli* UC51**
Na2HPO4	0.15%	0.22%
KH2PO4	0.43%	0.1%
(NH4)2SO4	0.1%	0.1%
MgSO4	0.01%	0.01%
GLUCOSE	0.2%	0.2%
Metallic ion :	Trace	Trace
Stock solution(Mo+3, Co+3, Cu+2, Mn+2		Ca+2, Fe+2)
AGAR	I.5%	1.5%
pH	6.2	6.7

Table 3: Media and buffer used for detection of mutagenicity

1. Vogel Bonner medium : K2HPO4 500 mg, $Na(NH_4)_2SO_4$ 175 mg, citric acid monohydrate 100 mg, $MgSO_4$ 1O mg, warm distilled water 100ml.
2. Soft agar : Difco agar 0.6% and Nacl 0.5% with a trace of histidine.
3. Minimal glucose agar : Difco agar 1.5% and glucose 2% in Vogel Bonner medium
4. 0.2 (M) Na-Phosphate buffer : 0.2 (M) NaH2PO4 (13.8 gm/500 ml) + 0.2 (M) Na_2HPO_4 (14.2 gm/500ml), Ph 7.4

Table 4 : Antimetabolic activity of known and unknown agents

Drugs	Strain	Conc. Of drug (ug/disc)	Zones of inhibition In mm		(S-N) mm
			Syn agar(S)	Nut agar(N)	
5 Fluouracil	*B. subtilis* UC564	25	20.5	0	20.5
	E.coli UC51	25	18.0	0	18.0
Nalidixic acid	*B. subtilis* UC 564	10	12.0	0	12.0
		25	17.0	0	17.0
		10	9.0	0	9.0
	E. coli UC 51	25	14.0	0	14.0
Chlorcyclizine	*E. coli* UC 51	100	7.0	0	7.0
		200	11.0	0	11.0
		400	12.0	0	12.0
Cotrimoxazole	*E. coli* UC 51	25	30.0	0	30.0
	B. subtilis UC 564	25	25.0	0	25.0

Mutagenicity test

Drug solutions were taken in sterile tubes kept on ice to which was added 0.5ml of 0.2 M sodium phosphate buffer, followed by 0.1ml of overnight peptone water culture of the test strain; incubation was at 37c for 20 mins with shaking. Soft agar(2ml) was added to the tubes and the contents vortexed and poured over minimal glucose agar (Vogel Bonner) plates. After setting, the plates were inverted and kept at 37c for 48hr, following which the revertant colonies were counted. Control plates containing bacteria, histidine and buffer was studied to give the number of colonies arising due to spontaneous mutation (Ames et al,1973).

Table 5: Effect of different drugs on *S. typhimurium* TA 102 to producehistidine revertant colonies

Drug	Amount (ug)	No. of Histidine revertant Colonies of TA 102
Spontaneous	Without any drug	268
Nalidixic acid	25	1100
Cotrimoxazole	25	1400
Chlorcyclizine	400	60

RESULTS

Table 4 describes the antimetaboliic activity of the chemotherapeutic agents. Of 32 compounds tested, nalidixic acid (20ug) and cotrimoxazole (20ug) discs produced inhibition zones 17.0 and 25.0 mm respectively against E.coli UC51, and 14.0 and 30.0 mm wide zones in case of B.subtilis UC564 on synthetic agar only. Similarly chlorcyclizine discs (100, 200 and 400ug) also produced inhibition reaction on synthetic agar and not nutrient agar. The known antimetabolite, 5-fluorouracil served as the control antimetabolic agent in this study.

Following Ame's test, it was noted that in case of Salmonella typhimurium TA 102, due to spontaneous mutation i.e when no drug is used, 268 colonies were formed mutation (Table 5). Nalidixic acid, and cotrimoxazole were found to be mutagenic since they produced significantly high number of revertant colonies. However , the antihistamine chlorcyclizine failed to score a detectable mutagenic effect as it scored a very low count of revertant colonies

DISCUSSION

Although, chlorcyclizine and chlorpromazine, differ with each other chemically, however, possess functional similarities, both of them being antihistaminic and antimicrobic. In addition, chlorcyclizine can act as an antimetabolite, whereas chlorpromazine is a powerful psychotropic drug. This functional difference between the two compounds may be explained in terms of their structural dissimilarities.

Thus, it was found that the phenothiazine exhibit a variety of activities which ranged from modulation of different components of the nervous system to antimicrobial and antiplasmid activity. All these functions appeared to depend on the type of attachment to the phenothiazine tricyclic ring structure. The structurally related yet distinctive chemical compound chlorcyclizine shows further variation of the range of functions in exhibiting a potentially antimetabolic and non-mutagenic effect. This shows the possibilities of detecting compounds with greater anticarcinogenic property.

REFERENCES

1. Goodman,L.S., Gilman, A. and Goodman Gilman, A., The Pharmacological basis of Therapeutics, Macmillan Publishing company, New York, 11th ed., 1140, (2006)
2. Hanka, L.J. 5TH International Congress Chemotherapy, (1978)
3. Hanka, L.J., Kuentzel, S.L., Martin, D.G., Wiley, P.F. and Neil G.L., *Recent Results Cancer Res.*, **63**: 69-74 (1978)
4. Ames, B.N., Lee, F.D. and Durston, W.E., Proc. Natl. Acad. Sci. USA, **7O**: 782 (1973).
5. Ames, B.N., Mutagens in our Environment, Sarsa,M., Vainio,H., and Liss, A.R.eds New York (1982)
6. Saha, P.K. and Dastidar, S.G. *Indian J. Med. Res.*, **64**: 1677 (1976)
7. Dastidar, S.G., Saha,P.K., Sanyamat,B. and Chakrabarty, A.N., *Journ. of Appl. Bact.,* **41**: 209 (1976).
8. Dash,S.K., Dastidar,S.G. and Chakrabarty, A.N., *Ind. J. Exp. Biol.,* **15**: 324 (1977).
9. Ray, S., Dastidar,S.G. and Chakrabarty, A.N., *Brit. J.Exp. Pathol.,* **61**: 465 (1980).
10. Chattopadhyay, D., Dastidar, S.G. and Chakrabarty, A.N., *Arzneim.Forsch.,* **38**: 869 (1988).
11. Ray, S., Chattopadhyay, D., Dastidar,S.G. and Chakrabarty, A.N., *Indian. J. Exp. Biol.*, **28**: 253 (1990).
12. Hossain, M.,Dastidar,S.G. and Chakrabarty, A.N., *Ind. J. Exp. Biol.,* **25**: 866 (1987).