ENHANCEMENT OF ANTIMICROBIAL ACTIVITY OF SULFA DRUGS BY METALLIC IONS

P. GUNDU RAO, P. RANGANATHAM, K. K. SRINIVASAN AND MISS N. R. SHARADA
College of Pharmacy and Kasturba Medical College, Manipal 576 119

ABSTRACT

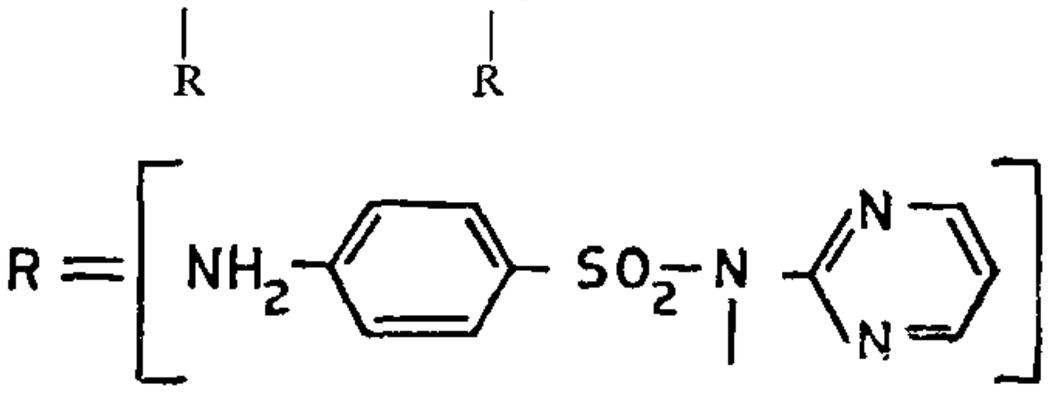
Various metallic compounds of sulfadiazine were prepared and their activity tested against *Ps. aeruginosa*, *E. coli*, *B. subtilis*, *S. aureus* and *Candida* sp. Some of the compounds especially mercuric salts were found to possess considerable activity against *Ps. aeruginosa*. Other metallic salts were found to possess weak activity.

THE combination of the bacteriostatic activity of sulfa drugs with the oligodynamic action of certain metallic ions by incorporating them in the structure of sulfa drugs, has led to the more efficaceous antimicrobial agents like silver sulfadiazine[1,2]. The superiority of silver sulfadiazine over sulfadiazine alone or silver nitrate alone in preventing or controlling the infection caused particularly by *Ps. aeruginosa* in burns is well established. It is reported that local application of a 1% ointment of silver sulfadiazine was not followed by electrolyte depletion, inhibition of carbonic anhydrase and metabolic acidosis[3]. As silver compounds are costly, zinc sulfadiazine, a cheaper substitute having similar activity has been reported[4,5] Some metallic derivatives of sulfa drugs, purporting to be complexes, have also been reported[6,7]. Encouraged by the results of these reports, other metallic compounds like mercuric, cadmium, cupric, ferric, nickel and manganese sulfadiazines were prepared and their *in vitro* activity against *Ps. aeruginosa*, *S. aureus*, *B. subtilis*, *E, coli* and *Candida* sp. was studied.

EXPERIMENTAL

Preparation of Metallic Compounds of Sulfadiazine

The sodium ion in sodium sulfadiazine was replaced by the required metallic ion by treating an aqueous solution of sodium sulfadiazine with the solution of a soluble salt of appropriate metal. The precipitated compound of sulfadiazine was washed free of the anions and dried at 80 °C. The compounds were analysed by assaying the sulfa drug part, volumetrically (diazotisation) and metallic part gravimetrically. Analytical data are in good agreement with the structures assigned to them as given below. The general formulae of these compounds can be represented as M_1–R, M_2–R, and R–M_3–R where



and M_1, M_2 and M_3 stand for monovalent, bivalent and trivalent metallic ions respectively.

In this connection it was noticed that the preparation of aluminium, manganese, calcium and bismuth compounds of sulfadiazine, according to the procedure adopted, was difficult. Melting points were determined in open capillary tubes and are uncorrected. Solubility of these compounds in water is given in Table I.

TABLE I

Analytical data of the title compounds

Sl. No.	Compound	% of sulfa		% of metallic part		Physical characters	Melting point °C	Solubility in water (100 ml)
		Calcd.	Found	Calcd.	Found			
1	2	3	4	5	6	7	8	9
1.	Silver sulfadiazine	68·02	68·89	31·98	30·97	White soft powder turning brown on exposure to light	290 (decomp.)	0·01234 g
2.	Zinc sulfadiazine	86·9	87·36	13·1	12·64	White soft powder	255 (decomp.)	0·07943 g

TABLE I (*Contd.*)

1	2	3	4	5	6	7	8	9
3.	Copper sulfadiazine	86·72	87·0	13·28	12·9	Dark grey powder	240 (decomp.)	0·01932 g
4.	Cadmium sulfadiazine	81·62	82·01	18·38	17·99	White soft powder	315 (decomp.)	0·01681 g
5.	Mercuric sulfadiazine	71·34	72·5	28·66	27·5	Heavy white powder	255	0·0601 g
6.	Ferric sulfadiazine	92·7	93·4	7·3	6·6	Light brown powder	227 (decomp.)	0·2579 g
7.	Nickel sulfadiazine	89·47	89·99	10·53	10·11	Light green powder	275 (decomp.)	0·2586 g
8.	Manganese sulfadiazine	90·1	89·7	9·9	10·3	Dark brown powder	255 (decomp.)	0·0381 g

The yields were found to be between 80% and 90%.

Microbial Screening

Five organisms were chosen for screening namely: *Ps. aeruginosa*, *S. aureus*, *B. subtilis*, *E. coli* and *Candida* sp. A cream in a water soluble base was formulated containing 0·004 molar concentration of the metallic compound of sulfadiazine. The cup plate method was followed for testing the activity. The organism was seeded in nutrient agar in a petri dish. With a sterile cork borer, suitable number of cups were made. These were filled with the cream and incubated for 48 hours. The diameters of zones of inhibition were measured. The data are summarised in Table II.

In the case of mercuric sulfadiazine a very clear zone of inhibition (20 mm) was obtained against *Ps. aeruginosa*, indicating its superior activity over other compounds. The compound was also found to be active against *E. coli* (25 mm) and *S. aureus* (20 mm) but not active against *Candida* sp. All the other compounds were inactive against *E. coli*, *S. aureus* and *Candida* sp. Quantitative comparisons were made using filter paper disc method, by dissolving mercuric sulfadiazine in dimethyl formamide (Fig. 1).

TABLE II

Antimicrobial activity of various metallic sulfadiazines and sulfadiazine

Sl. No.	Compound	Zones of inhibition in mm. with a cup of 6 mm against	
		Ps. aeruginosa	*B. subtilis*
1.	Sulfadiazine	8 (Diffused)	15
2.	Mercuric sulfadiazine	20	21
3.	Silver sulfadiazine	9	20
4.	Zinc sulfadiazine	..	27
5.	Copper sulfadiazine	..	26
6.	Cadmium sulfadiazine	14 (Diffused)	28
7.	Ferric sulfadiazine	..	23 (Diffused)
8.	Nickel sulfadiazine	24 (Diffused)	24 (Diffused)
9.	Manganese sulfadiazine	..	23

DISCUSSION

Even though *in vitro* studies of silver and zinc sulfadiazines against *Ps. aeruginosa* do not reveal appreciable activity[5], their clinical usefulness in the treatment of burns has been significant. From the data in Fig. 1, it would appear that about 40 mcg of mercuric sulfadiazine may equal 250 mcg of sulfadiazine. In view

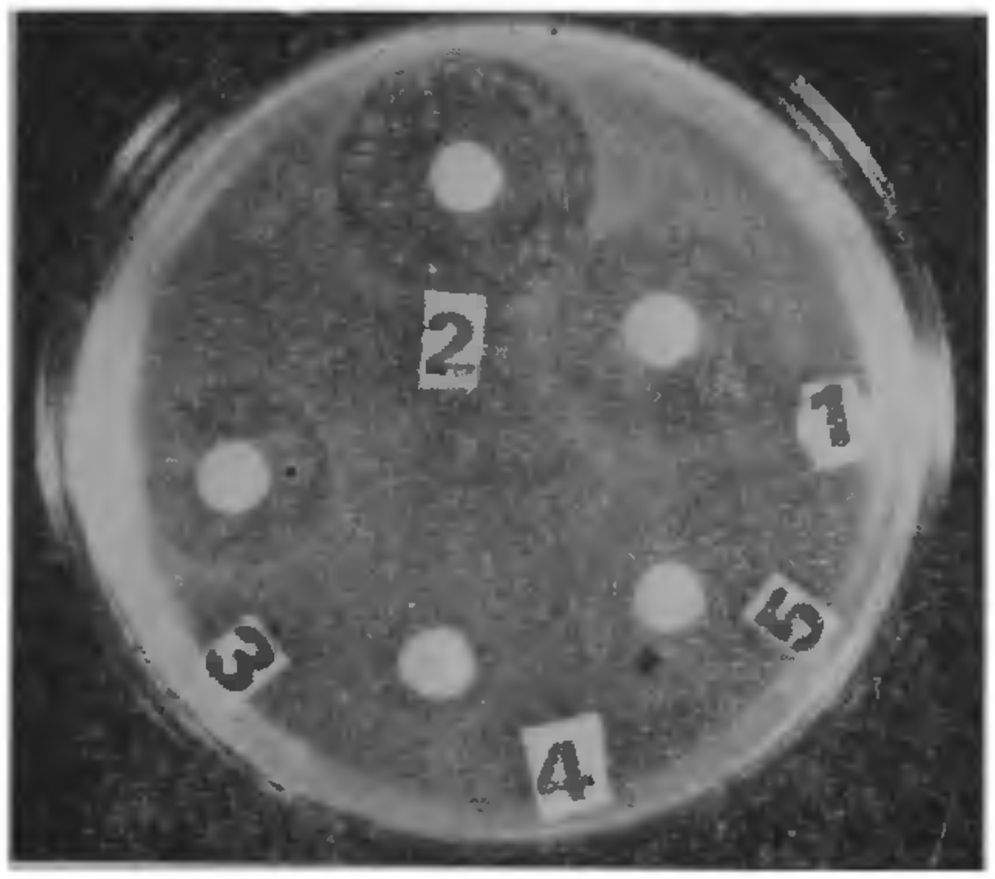


FIG. 1. Zones of inhibition effected by mercuric sulfadiazine against *Ps. aeruginosa.*

1. Sulfadiazine 250 mcg (20 mm); 2. Mercuric sulfadiazine 111 mcg (25 mm); 3. Mercuric sulfadiazine 11 mcg (18 mm); 4. Mercuric sulfadiazine 1·1 mcg (12 mm); 5. Solvent (dimethylformamide).

of this increased activity of mercuric sulfadiazine compared, the compound deserves clinical studies in the treatment of burns and other *Pseudomonas* infections. The official yellow mercuric oxide eye ointment[8] uses a 1% ointment giving a 0·046 molar concentration of mercury which is almost ten times the concentration found in the mercuric sulfadiazine cream tried by us. Hence further experimentation in its clinical applicability is warranted. Nickel sulfadiazine also shows promising activity and may be further investigated.

ACKNOWLEDGEMENTS

Our thanks are due to pharmacist, Mr. P. L. Suresh, for assisting in the preparation of various creams, to Lt. Col. Dr. K. N. Achyuta Rao for helpful advice and to the Dean, Kasturba Medical College, for providing facilities to conduct the work.

1. Fox, C. L. (Jr.), *Arch. Surg.*, 1968, 96, 184.
2. —, *Int. Surg.*, 1975, 60, 275.
3. *Extra Pharmacopoeia* (Martindale), 27th Ed., 1977, p. 1471, Pharmaceutical Press (London) (and references therein).
4. (*a*) Fox, C. L. (Jr.), Monafo, W. W. (Jr.), Ayvarzian, V. H., Skinner, A. M., Modak, S., Stanford, J. and Condict, R. N., *Surg. Gynaecol. Obstet.*, 1977, 144, 668.
   (*b*) Fox, C. L., Modak, S. and Stanford, J. W., *Ibid.*, 1976, 42, 553.
5. Nagesha, C. N. and Karuna Sagar, I., *Arch. Surg.*, 1978, 109 (in press).
6. Jain, N. K. and Kamal K. Chaturvedi, *Hindustan Antibiotics Bulletin*, 1975, 18, 40.
7. Chaturvedi, K. K., Agarwal, B. K., Siddiqui, S and Kaushal, R., *Indian J. Pharm.*, 1975, 37, 85.
8. *Indian Pharmacopoeia*, 2nd Edition, 1966, p. 444.

INTERPHASE SENSITIVITY AND SPECIFICITY OF SILIQUA MUTATIONS INDUCED BY E M S IN INDIAN MUSTARD

U. C. LAVANIA

Cytogenetics Laboratory, Department of Botany, University of Calcutta, Calcutta 700 019, *India*

ABSTRACT

Synchronised seeds of Indian mustard were treated with the mutagen E M S during G_1, earlier half S, later half S and G_2 phase of the mitotic cell cycle. Observations were recorded in M_2 generation on four different types of siliqua mutations. Mutation frequency on family basis was found to be higher when the mutagen was applied at S or G_2 as compared to G_1. While appressed pod mutants were observed in all the treatments, the other three types, *viz.*, curved pod, incurved pod and thickened pod mutants showed some specificity of mutagenesis with the mitotic cycle.

INTRODUCTION

CHANGING the spectrum of mutations in a predictable manner and thereby achieving directed mutagenesis is an important goal of current mutation research. Nilan[1] has reviewed the various reports of alteration in spectrum induced by specific mutagens and treatment conditions and has concluded that different mutagens and treatment procedures may induce some changes in the relative proportions of different types of mutations in higher plants. Differences in sensitivities to radiation and chemical induced