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THE THERAPEUTIC ACTIVITY OF SOME SULPHONES AND SULPHOXIDES IN EXPERIMENTAL TUBERCULOSIS OF GUINEA-PIGS

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It has been suggested by Anand, Vyas, and Dhar (1953) that a lipophilic drug would have a good chance of reaching lipid-rich mycobacteria and the harbouring tissues. Based on this assumption, a series of unsymmetric sulphides, sulphoxides, and sulphones carrying an alkylamino-group at one end and a free or potentially free amino-group at the other end of the molecule have been synthesized (Anand *et al.*, 1953 ; Anand, Wadia, and Dhar, 1954 ; Khosla, Anand, and Dhar, 1954 ; Vyas, Anand, and Dhar, 1954).

Some of these compounds have been tested for their therapeutic value in experimental tuberculosis of guinea-pigs and the results are presented in this paper.

MATERIALS AND METHODS

The drugs used were: SN 44 (*p*-ethylamino-*p'*-aminodiphenyl sulphone) ; SN 45 (*p*-propylamino-*p'*-aminodiphenyl sulphone) ; SN 47 (*p-iso*butylamino-*p'*-aminodiphenyl sulphone) ; SN 50 (*p-iso*amylamino-*p'*-aminodiphenyl sulphone) ; SN 89 (*p*-methylamino-*p'*-aminodiphenyl sulphoxide) ; SN 105 (Na salt) (*p*-methylamino-*p'*-sodium formaldehyde sulphoxylate aminodiphenyl sulphone) ; SN 153 (*p*-(ω-iodoethylamino)-*p'*-aminodiphenyl sulphone) ; SN 199 (*p*-ethylamino-*p'*-amino-*o*-hydoxyphenyl sulphone).

In the first experiment the therapeutic activity of SN 44, SN 45, SN 50, and SN 89 was compared with dihydrostreptomycin sulphate (DHS). Thirty-six tuberculin-negative guinea-pigs of about 300 g. in weight were inoculated subcutaneously in the right groin with 2 mg. (moist weight) of an 11-day culture of *Mycobacterium tuberculosis var. hominis* strain H 37 Rv, in Lowenstein-Jensen medium, suspended in 0.5 ml. of basal Dubos medium containing Tween 80. The animals were divided into groups of six each. The first group served as the untreated control, while groups two to six were treated with DHS, SN 44, SN 45, SN 50, and SN 89 respectively. DHS was administered subcutaneously, whereas the other drugs were suspended in water with 1% tragacanth and fed orally through a syringe. The treatment was begun on the tenth day and was continued daily for a period of 54 days. The average daily dose of the drugs in g./kg. of body weight is given in Table I. All the surviving animals were sacrificed at the end and examined post-mortem ; the animals which died during the experiment were also examined. A few guinea-pigs which died of causes other than tuberculosis were left out of consideration.

In a second experiment the activity of drugs SN 44, SN 47, SN 153, SN 199, SN 105 (Na salt), and SN 89 was compared with that of isonicotinic acid hydrazide (isoniazid, INH) and *p-p'*-diaminodiphenyl sulphone (dapsone, DDS). Fifty tuberculin-negative guinea-pigs were infected in the manner described above, using the same strain. The infecting dose was reduced to 0.5 mg. (moist weight) in order to produce a less severe disease. All the animals were seen to react to old tuberculin (Burroughs Wellcome) on the 20th day. On the 22nd day five animals were necropsied, and macroscopic lesions were seen at the site of inoculation, inguinal glands, spleen, and liver. The remaining animals were then divided into 9 groups of 5 animals each. The first group was kept as untreated control, while the rest were fed with drugs as before. Treatment was commenced on the 22nd day and was continued for 55 days. The average daily dose of the drugs in g./kg. of body weight is given in Table III.

Necropsy Score.—The following scores were given on post-mortem examination: 0, no lesions; 1, slight lesions (miliary lesions with no necrosis) ; 2, moderate lesions (nodular tubercles with slight necrosis) ; 3 to 4, severe lesions (moderate to severe necrosis).

Histopathology.—Sections of the lung, liver, spleen, and local glands, after fixing and staining, were studied for the presence of "active" lesions (necrotic foci) or "inactive" lesions (fibrosis, calcification, or epithelioid cell collection with no necrosis). The percentage of "active" lesions was calculated on the basis of the number of tissues showing these lesions. Since only four tissues (lung, liver, spleen, and gland) were examined, the total number of tissues was obtained by multiplying the number of animals by four. The percentage of "inactive" and of "no"

lesions were calculated in a similar manner (Karlson and Feldman, 1953).

Tuberculin tests and spleen cultures were made on all animals employed in the second experiment at the conclusion of the test.

RESULTS

None of the drugs employed in this investigation produced any toxic symptoms in the animals, and the tissues of the lung, liver, spleen, and kidney did not show any damage attributable to the drug. In the first experiment SN 44 and DHS were seen to be therapeutically more active than SN 45, SN 50, and SN 89, judged from the percentage of infectivity (Table I). "Active" lesions were least in animals treated with these two drugs (Table II) and were confined to the local glands. There was a corresponding preponderance of animals with "no" lesions. Active lesions were maximal in the untreated animals, whereas animals treated with SN 45, SN 50, and SN 89 occupied an intermediate position.

In the second experiment SN 44 and SN 47 were as good as INH. SN 153 and DDS came next in the order of therapeutic activity. SN 199 and SN 89 were less effective than the rest, while SN 105 (Na salt) was ineffective. The percentage infectivity data is presented in Table III. Histopathological findings agreed in general with the necropsy findings. "Active" lesions were least in animals treated with SN 44 and SN 47, while a high percentage of "no" lesions was seen in animals treated with SN 44 and INH (Table IV). Spleen cultures from animals treated with SN 44, SN 47, SN 153, DDS and INH proved negative for *M. tuberculosis*. On the contrary *M. tuberculosis* could be recovered from the spleens of animals treated with SN 199, SN 89, and SN 105 (Na salt).

Tuberculin tests carried out after 55 days of treatment showed a diminution in the intensity of reaction in animals treated with INH, SN 44, SN 47, and DDS as compared with the control animals. There was no necrosis and the oedema was appreciably less. Two animals of the SN 44 group and one animal of the SN 47 group gave a doubtful reaction of erythema without oedema.

TABLE I

THERAPEUTIC ACTIVITY OF SULPHONES AND SULPHOXIDES COMPARED WITH DIHYDROSTREPTOMYCIN SULPHATE IN EXPERIMENTAL TUBERCULOSIS

Drug	No. of Animals	Average Daily Dose g./kg.	Average Post-mortem Scores of Infectivity*					%† Infection
			Lung	Liver	Spleen	Local Gland	Total (Max. =12)	
Control (no drug)	6	Nil	3·0	2·5	2·8	3·0	11·3	94·2
DHS ..	4	0·03	0	0	0·7	1·5	2·2	18·7
SN 44 ..	5	0·045	0	0	0	2·3	2·3	19·4
SN 45 ..	6	0·03	0·8	0·8	1·5	3·0	6·2	51·3
SN 50 ..	6	0·03	0	2·0	1·5	3·0	6·5	54·1
SN 89 ..	6	0·03	0·2	2·4	1·6	2·6	6·8	56·7

* Score of infectivity: 0 (normal) to 3 (severe).

† % infection = $\frac{\text{Actual total score}}{\text{Maximum total score}} \times 100$.

TABLE II

EFFECT OF SULPHONES AND SULPHOXIDES COMPARED WITH DIHYDROSTREPTOMYCIN SULPHATE (DHS) ON TISSUES OF INFECTED GUINEA-PIGS AS REVEALED BY MICROSCOPIC EXAMINATION

Drug	No. of Animals	Total No. of Tissues	Active Lesions		Inactive Lesions		No Lesions	
			No.	%	No.	%	No.	%
Control (no drug)	6	24	21	87·5	2	8·3	1	4·1
DHS ..	4	16	4	25·0	1	6·2	11	68·7
SN 44 ..	5	20	5	25·0	3	15·0	12	60·0
SN 45 ..	6	24	10	41·7	11	45·8	3	12·5
SN 50 ..	6	24	12	50·0	8	33·3	4	16·7
SN 89 ..	6	24	17	70·8	6	25·0	1	4·2

TABLE III

ACTIVITY OF SULPHONES AND SULPHOXIDES COMPARED WITH ISONIAZID (INH) AND DAPSONE (DDS) IN EXPERIMENTAL TUBERCULOSIS OF GUINEA-PIGS

Drug	No. of Animals	Average Daily Dose g./kg.	Average Post-mortem Scores of Infectivity*					%† Infection
			Lung	Liver	Spleen	Local Gland	Total (Max. =16)	
Control (no drug)	5	Nil	3·5	3·5	3·1	3·5	13·6	85·0
DDS ..	5	0·1	0·3	1·7	1·0	2·7	5·7	35·6
INH ..	5	0·015	0	0	2·0	2·5	4·5	28·1
SN 44 ..	5	0·1	0·2	0·2	0·7	2·0	3·2	20·3
SN 47 ..	5	0·1	0	0·5	1·3	2·0	3·8	23·7
SN 153	5	0·1	0·6	1·6	1·6	2·4	6·2	38·7
SN 199	5	0·1	1·0	2·6	2·0	3·0	8·6	53·7
SN 89 ..	5	0·1	1·0	2·5	1·5	3·5	8·5	53·1
SN 105 (Na salt)	5	0·1	3·0	2·8	3·3	3·5	12·6	78·7

* Score of infectivity: 0 (normal) to 4 (severe).

† % infection = $\frac{\text{Actual total score}}{\text{Maximum total score}} \times 100$.

TABLE IV

EFFECT OF SULPHONES AND SULPHOXIDES COMPARED WITH ISONIAZID (INH) AND DAPSONE (DDS) ON TISSUES OF INFECTED GUINEA-PIGS AS REVEALED BY MICROSCOPIC EXAMINATION

Drug	No. of Animals	Total No. of Tissues	Active Lesions		Inactive Lesions		No Lesions	
			No.	%	No.	%	No.	%
Control (no drug)	5	20	18	90·0	2	10·0	0	0
INH ..	5	20	7	35·0	1	5·0	12	60·0
DDS ..	5	20	8	40·0	9	45·0	3	15·0
SN 44 ..	5	20	4	20·0	4	20·0	12	60·0
SN 47 ..	5	20	3	15·0	8	40·0	9	45·0
SN 153	5	20	6	30·0	10	50·0	4	20·0

Based on these two experiments it seems safe to conclude that SN 44 and SN 47 were as good as INH and DHS in the treatment of experimental tuberculosis in guinea-pigs. Promin, diasone, promizole, solapsone and other derivatives of diaminodiphenyl sulphone are known to act synergistically or additively with streptomycin in the treatment of experimental as well as human tuberculosis (references in Kolmer, 1951). It may therefore be of interest to study the combined action of these lipophilic sulphones, SN 44 and SN 47, with streptomycin or isoniazid. The sulphones now in the therapeutic field are less lipophilic than those employed in this investigation.

Summary

Of some sulphones and sulphoxides tested for therapeutic activity in experimental tuberculosis of guinea-pigs, SN 44 (*p*-ethylamino-*p'*-aminodiphenyl sulphone) and SN 47 (*p-iso*butylamino-*p'*-aminodiphenyl sulphone) were found to be active and to be comparable with dihydrostreptomycin sulphate and isonicotinic acid hydrazide.

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