COMPARATIVE STUDIES ON THE *IN VITRO* ANTITUBERCULOUS ACTION OF LONG-ACTING SULFONAMIDES

MICHIO TSUKAMURA

The Obuso National Sanatorium, Obu, Aichi-Pref.

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Previously the author and his associates[1,2] reported that, among sulfonamides, sulfathiazole and sulfisoxazole have the most active *in vitro* antituberculous action and most effectively retard the *in vitro* emergence of streptomycin resistance or isoniazid resistance when used in combination with streptomycin or isoniazid. NAITO[3,4] reported that the combined chemotherapy with isoniazid and sulfisoxazole exhibits a significant therapeutic effect in tuberculous patients. This finding was supported by USHIBA, SUNOHARA, SHIMAMURA, *et al.*[5] nd many other investigators. It was also stated by KATSUNUMA, KIMINO and ABO[6] that the combined use of isoniazid and sulfathiazole was very effective for tuberculous patients, and it was reported by KIMINO, *et al.*[7] that sulfathiazole is effective in delaying the emergence of isoniazid resistance in tuberculous patients. The combined use of isoniazid and sulfisoxazole appeared to be better than that of isoniazid and sulfathiazole, since sulfathiazole is more toxic to human body than sulfisoxazole. In addition, it was reported by NISHIMURA, *et al.*[8] that the combined use of isoniazid and sulfisoxazole is more effective for tuberculosis in mice than that of isoniazid and sulfathiazole. The results suggested that the *in vivo* therapeutic effect is not always parallel with the *in vitro* antituberculous action. TSUKAMURA, *et al.*[9] observed that the antagonistic ratio of sulfisoxazole : PABA (*p*-aminobenzoic acid) is smaller than that of sulfathiazole : PABA. The results partially explained the *in vivo* results obtained by NISHIMURA, *et al.* and suggested that the antagonistic ratio of sulfa drug · PABA may be useful for detecting *in vivo* effectve sulfonamides.

Recently, a number of new long-acting sulfonamides have been introduced for clinical use. It appears to be desirable to make comparative studies on the antituberculous action of the long-acting sulfonamides and especially to compare with the antituberculous action of sulfisoxazole.

Materials and Methods

New long-acting sulfonamides tested in the present study were as follows

(1) 5-Methyl-3-sulfanilamido-isoxazole = Sulfisomezole (Sinomin, Shionogi, Co.).

(2) 6-Sulfanilamido-2,4-dimethoxy-pyrimidine = Sulfadimethoxine (Omnibon, Yamanouchi Co.; Sulxin, Chugai Co.).

(3) 3-Sulfanilamido-6-methoxypyridazine = Sulfamethoxypyridazine (Lederkyn, Japan Lederle Co.).

(4) 3-Sulfanilamido-2-phenylpyrazol = Sulfaphenazol (Merian, Dainihon, Co.).

(5) 3,4-Dimethyl-5-sulfanilamido-isoxazole = Sulfisoxazole (Sulfazin, Shionogi Co.). (Control).

The sulfonamides were dissolved in distilled water by adding sodium hydroxide solution to obtain the 1 mg per ml solution and added to media to obtain appropriate dilutions of the drugs.

The medium used was OGAWA's egg medium. The composition of the medium was as follows : Basal solution (1% KH_2PO_4 and 1% sodium glutamate), 100 ml; Eggs, 200 ml; Glycerin, 6 ml; 2% malachite green solution, 6 ml. The medium was dispensed in 8 ml amounts into tubes, 17 to 18×170 mm, and slanted by sterilization at 90°C for 60 minutes. The pH of the resultant medium was 6.8.

The strain used was *Mycobacterium tuberculosis* var. *hominis*, strain $H_{37}Rv$. Six-week-old cultures of the test strain grown on drug-free medium as discrete colonies were homogenized by shaking with glass beads and suspended in physiological saline. The cell suspension was tenfold diluted and the resultant dilutions were used for inoculation. Inoculation was conducted by the use of a whirl loop delivering 0.02 ml[10]. The tubes inoculated were incubated at 37°C for 4 weeks and counts were made.

The antituberculous action of the sulfonamides were expressed as the highest concentration on which small inocula can grow. The action was estimated on media with and without PABA.

Results

The results obtained are shown in table 1. As shown in the table, sulfisomezole, sulfadimethoxine and sulfaphenazol represented similar antituberculous activites and their activities were reversed by the addition of PABA, showing similar antagonistic ratios. It appeared noteworthy that these long-acting sulfonamides are never superior to sulfisoxazole when observed from both viewpoints of the antituberculous action and the antagonistic ratio

Table 1. Antituberculous action of sulfonamides on *M. tuberculosis* (H 37 Rv) and its reversal by p-aminobenzoic acid (PABA).

Sulfonamides Concent.	Sulfisomezole			Sulfadimethoxine (Omnibon)			Sulfadimethoxine (Sulxin)		
	PABA (μg/ml)			PABA (μg/ml)			PABA (μg/ml)		
	0	1	10	0	1	10	0	1	10
μg/ml									
100	—	—	*8	—	—	*27	—	—	36
50	—	11	20	—	2	20	—	48	40
40	—	21	25	—	38	46	—	67	55
30	—	30	28	—	42	46	—	61	48
20	—	39	29	—	49	41	—	71	57
10	—	46	29	—	56	47	—	70	51
5	—	31	55	—	62	59	—	71	49
2	24	42	40	43	54	45	19	49	43
1	88	40	43	32	53	47	52	63	59
0	76	63	52	60	54	66	54	72	66

Sulfonamides Concent.	Sulfamethoxypyridazine			Sulfaphenazol			Sulfisoxazole		
	PABA (μg/ml)			PABA (μg/ml)			PABA (μg/ml)		
	0	1	10	0	1	10	0	1	10
μg/ml									
100	—	16	39	—	—	67	—	—	*24
50	—	27	38	—	38	60	—	—	23
40	—	26	37	—	44	47	—	27	34
30	—	42	38	—	39	89	—	25	27
20	—	46	25	—	42	65	—	27	21
10	—	56	41	—	48	45	—	41	34
5	14	40	41	—	50	58	—	65	55
2	69	41	32	24	46	47	21	60	38
1	59	43	51	49	38	98	48	52	42
0	69	58	50	61	57	49	56	69	47

Numbers in the table represent numbers of surviving colonies.

* Small colonies.

against PABA. Among long-acting sulfonamides, sulfamethoxy-pyridazine was somewhat inferior to the other sulfonamides in the antituberculous activity.

Antimycobacterial activities of the sulfonamides against *Mycobacterium avium* (strain Jucho) were also tested by the serial dilution method utilizing SAUTON medium (pH 7.0). The results indicated that sulfisoxazole and sulfaphenazol were fourfold to eightfold more active to the organism than the other sulfonamides.

Discussion

The *in vitro* antituberculous action of new long-acting sulfonamides and sulfisoxazole were tested in OGAWA's egg medium, since the author believes that egg media can give experimental conditions better resembling human body than synthetic or semisynthetic media. The results indicated that sulfisomezole, sulfadimethoxine and sulfaphenazol represent antituberculous activities similar to the action of sulfisoxazole and antagonistic ratios similar to the ratio of sulfisoxazole. The results suggest that these long-acting sulfonamides may be useful for clinical use, if the long-acting sulfonamides have less side effects. NISHIMURA, *et al.*[11] reported that sulfisomezole was superior to sulfisoxazole when used singly in experimental tuberculosis in mice, but there was no significant difference between them when used in combination with isoniazid. Their results might be explained by the fact that blood levels of sulfisomezole were somewhat higher than those of sulfisoxazole[11], since the *in vitro* antituberculous activity and the antagonistic ratio of it against PABA were not superior to those of sulfisoxazole as shown in the present study.

Summary

In vitro antituberculous actions of new long-acting sulfonamides against *Mycobacterium tuberculosis* var. *hominis* ($H_{37}Rv$) were tested in OGAWA's egg medium and the following conclusions were obtained.

Sulfisomezole (5-methyl-3-sulfanilamido-isoxazole), sulfadimethoxine (6-sulfanilamido-2, 4-dimethoxy-pyrimidine), and sulfaphenazol (3-sulfanilamido-2-phenylpyrazol) represented antiuberculous activities similar to the activity of sulfisoxazole (3, 4-dimethyl-5-sulfanilamido-isoxazole) known previously as the most effective antituberculous sulfonamide. The antagonistic ratios of these long-acting sulfonamides against *p*-aminobenzoic acid (PABA) also were similar to the ratio of sulfisoxazole. It has been thus suggested that these long-acting sulfonamides may be useful for the chemotherapy of tuberculosis in combination with other antituberculous agents, if they have no significant side effect. Sulfamethoxypyridazine (3-sulfanilamido-6-methoxy-pyridazine) was somewhat less active than the other long-acting sulfonamides.

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