THE COMBINATION EFFECT OF SULFAMETHOXAZOLE AND TRIMETHOPRIM AGAINST ANIMAL INTESTINAL BACTERIA

Y. Nakai[1], H. Matsumoto[2] and K. Ogimoto[2]

Department of Animal Science
Ibaraki University, Ami, Ibaraki 300-03, Japan

Summary

Combination effects of sulfamethoxazole (SMX) and trimethoprim (TMP) against nine gram positive bacterial strains and 43 gram negative bacterial strains which included 40 strains of animal intestinal bacteria were studied *in vitro*. Minimum inhibitory concentrations (MICs) of SMX and TMP alone and 20:1 (SMX : TMP) mixture (ST) were investigated by the method recommended by Ad Hoc Committee of the Japan Society of Chemotherapy for the Evaluation of Sensitivity Testing Methods for Sulfamethoxazole and Trimethoprim. MICs of ST were more potentiated than those of SMX alone in 8 of 9 gram positive strains and 40 of 43 gram negative strains. Especially, 38 strains of 40 intestinal bacteria showed significant susceptibility to ST as compared to SMX. These results suggest a strong synergistic activity of ST mixture against animal intestinal bacteria. The activity was considered to be comparable to those of other current antibiotics.
(**Key Words**: Intestinal Bacteria, MIC, Sulfamethoxazole, Trimethoprim)

Introduction

Sulfonamides has been known to have a highly synergistic antibacterial activity in combination with a dihydrofolate reductase inhibitor (Bushby and Hitchings, 1968; Bushby, 1973). The mixture of sulfamethoxazole (SMX) and trimethoprim (TMP) shows synergistic effect by interfering with the folic acid metabolism in sequential synthetic pathway of nucleic acid of bacteria; namely, SMX interferes with dihydrofolic acid synthesis from p-aminobenzoic acid and dihydropteridine and TMP has antagonistic effects of dihydrofolic acid reductase which concerns tetrahydrofolic acid synthesis from dihydrofolic acid (Hitchings, 1969). The *in vitro* effects of the mixture was studied against many bacteria isolated from human patients (Awataguchi et al., 1973; Goto et al., 1973; Kamiya et al., 1973; Kawakami et al., 1973; Kosakai and Oguri, 1973; Nakazawa et al., 1973; Yokota, 1973).

In the present study, activity of the mixture against bacteria isolated from animal cases was investigated.

[1]Address reprint requests to Dr. Y. Nakai, Department of Animal Science, Ibaraki University, Ami, Ibaraki 300-03, Japan.

[2]Department of Animal Microbiology, Tohoku University, Sendai 981, Japan.

Received March 11, 1991
Accepted June 14, 1991

Materials and Methods

Bacterial strains

Bacterial strains were mainly originated from animal cases. *Staphylococcus aureus* FDA 209-P and *Escherichia coli* NIHJ JC-2 were used as the standard strains.

Drug susceptibility test

Susceptibility tests were performed by the agar plate dilution method recommended by the Japan Society of Chemotherapy (Ad Hoc Committee of the Japan Society of Chemotherapy, 1973). SMX was first dissolved in a small volume of 1/8N NaOH and brought into water solution. TMP was dissolved in a small volume of N, N-Dimethylformamide and also brought into water solution. Combination of these drugs (ST) was prepared by mixing 20 parts of SMX with 1 part of TMP. Gentamicin sulfate (GM: 546 μg gentamicin/mg) was dissolved in water and used as the reference drug.

Media

Medium used for preincubation of bacteria for drug susceptibility tests against GM was Trypto-Soya Broth (Nissui) and that against SMX, TMP and ST was Mueller-Hinton Broth

TABLE 1. COMPARISON OF THE ANTIBACTERIAL ACTIVITIES OF SULFAMETHOXAZOLE (SMX), TRIMETHOPRIM (TMP), SMX-TMP 20:1 MIXTURE (ST) AND GENTAMICIN (GM)

Organism	Minimum inhibitory concentration (μg/ml)			
	SMX	TMP	ST	GM
Bacillus subtilis 6044	3.13	0.2	0.39	0.2
Bacillus subtilis ATCC 6633	6.25	0.1	0.39	0.2
Bacillus anthracis Pasteur II	12.5	>100	12.5	0.39
Staphylococcus aureus FDA 209-P	25	0.78	1.56	0.78
Staphylococcus aureus Terajima	25	0.39	0.1	0.2
Staphylococcus aureus Smith SM	25	0.1	0.78	0.39
Staphylococcus aureus ATCC 6538P	6.25	0.39	0.78	1.56
Staphylococcus epidermidis ATCC 12228	>100	0.39	1.56	0.2
Micrococcus luteus ATCC 9341	0.39	0.78	0.2	1.56
Bordetella bronchiseptica ATCC 19395	>100	>100	12.5	1.56
Bordetella bronchiseptica ATCC 4617	>100	100	12.5	6.25
Pseudmonas aeruginosa	>100	>100	>100	1.56
Salmonella enteritidis 116-54	6.25	0.025	0.025	0.78
Salmonella abortus equi Shurei	1.56	0.025	0.05	3.13
Salmonella abortus equi	0.39	0.1	0.39	3.13
Salmonella typhymurium 3173	25	0.025	0.2	0.78
Salmonella typhymurium	6.25	0.2	0.39	1.56
Salmonella cholerae suis 1348	25	0.025	0.2	1.56
Salmonella pullorum L-60131	12.5	0.1	0.2	1.56
Salmonella pullorum K-18	12.5	0.05	0.2	0.1
Salmonella pullorum 971	12.5	0.05	0.39	6.25
Salmonella pullorum L-58572	>100	0.2	0.78	1.56
Salmonella pullorum 9-25	6.25	0.1	0.78	3.13
Salmonella typhi Type-O	25	6.25	1.56	0.78
Salmonella typhi T2	12.5	0.1	0.39	0.78
Salmonella paratyphi B var java	6.25	0.1	0.39	6.25
Salmonella paratyphi A 1015	0.05	0.025	0.1	0.2
Salmonella bovis alpha 1960	6.25	0.025	0.39	3.13
Salmonella thompson var berlin 2988	12.5	0.2	0.39	1.56
Salmonella gallinarum 416	25	0.1	0.39	1.56
Salmonella senftenberg 3007	6.25	0.05	0.2	1.56
Escherichia coli O-2	25	0.78	0.78	6.25
Escherichia coli O-2 wild	>100	0.2	3.13	3.13
Escherichia coli O-2 wild E-71	>100	0.1	1.56	3.13
Escherichia coli O-1	25	0.78	0.78	3.13
Escherichia coli O-1 wild	>100	0.1	3.13	3.13
Escherichia coli O-8	6.25	0.39	0.78	3.13
Escherichia coli O-8 wild	3.13	0.39	0.78	3.13
Escherichia coli O-11	6.25	0.39	0.78	6.25
Escherichia coli O-11 wild	>100	0.2	3.13	1.56
Escherichia coli O-78	6.25	0.1	0.39	6.25
Escherichia coli O-78 wild	>100	0.39	6.25	1.56
Escherichia coli O-6	12.5	0.025	0.39	1.56
Escherichia coli O-144	6.25	0.025	0.1	3.13
Escherichia coli NIHJ JC-2	6.25	0.025	0.1	0.39
Escherichia coli	1.56	0.2	0.39	3.13
Klebsiella pneumoniae 8167 NIEH	3.13	0.2	0.39	1.56
Klebsiella pneumoniae Kasuya MNU	6.25	0.78	1.56	1.56
Klebsiella pneumoniae ATCC 10031	25	0.2	0.78	1.56
Proteus vulgaris IAM 1203	6.25	3.13	0.78	1.56
Proteus morganii Kono	1.56	0.39	0.39	0.78
Serratia marcescense 19 ATU	25	6.25	1.56	1.56

(Difco). In the susceptibility test, Sensitivity Disk Agar-N (Nissui) was used; however, 7.5% hemolyzed horse blood was added into the medium especially for tests of SMX, TMP and ST.

Inoculation

Bacterial suspension was prepared to be 10^8 -10^9/ml after cultivation for 18 hr at 37℃ and used for the test against GM. In the tests against SMX, TMP and ST, the suspension was diluted 100 times for gram positive bacteria and 1,000 times for gram negatives. The suspensions were spotted onto the media by using the multi-inoculator. Minimum inhibitory concentrations (MICs) were determined after 18-20 hr cultivation at 37℃.

Results and Discussion

MICs of drugs were shown in table 1. MICs of SMX and TMP in the standard strains of *Staphylococcus aureus* FDA 209-P and *Escherichia coli* NIHJ JC-2 were in the ranges of MICs in these respective bacterial strains described in the recommendation of Ad Hoc Committee of the Japan Society of Chemotherapy for the Evaluation of Sensitivity Testing Methods for Sulfamethoxazole and Trimethoprim (Ad Hoc Committee of the Japan Society of Chemotherapy, 1973). All the strains tested were susceptible to GM.

MICs of ST were lower than those of SMX and slightly higher than those of TMP in most of gram positive bacteria and in *Bacillus anthracis* Pasteur II, *Staphylococcus aureus* Terajima, and *Micrococcus luteus* ATCC 9341, they were lower than those of SMX and TMP. The results show the synergistic effects in ST mixture against gram positives.

Of gram negative bacteria, although no effects of SMX, TMP and ST were seen in *Pseudmonas aeruginosa*, strong synergistic effects were observed in *Bordetella bronchiseptica*.

In almost of intestinal bacteria, MICs of ST were much lower than those of SMX and same or slightly higher than those of TMP. For example MICs of ST were lower than those of SMX and higher than those of TMP in 17 strains of 19 strains of *Salmonella* tested. In all strains of 15 strains of *E. coli* tested, MICs of ST were lower than those of SMX and those values in two of these strains were same values of those of TMP. MICs of ST were middle between those of SMX and TMP in *Klebsiella pneumoniae*; moreover, they were lower than those of SMX and TMP in *Proteus vulgaris* IAM 1203 and *Serratia marcescense* 19 ATU.

These results suggested strong synergistic effects of ST mixture in intestinal bacteria. ST mixture may be one of the suitable combination to control bacteria of the animal intestine.

Acknowledgements

We thank Mr. Harumoto Kawaguchi, Aburahi Loboratories, Shionogi Research Laboratories for his advice.

Literature Cited

Awataguchi, S., C. Shirane, M. Mayama, H. Nagata, K. Kanazawa, T. Yoshida and K. Katagiri 1973. Studies on sulfamethoxazole and trimethoprim. Fundamental research I. Antibacterial activities of sulfamethoxazole and trimethoprim alone and in combination *in vitro*. Chemotherapy 21: 137-149

Ad Hoc Committee of the Japan Society of Chemotherapy for the Evaluation of Sensitivity Testing Methods for Sulfamethoxazole and Trimethoprim, 1973. Methods of sensitivity testing for sulfamethoxazole-trimethoprim combination product. Chemotherapy 21:67-76.

Bushby, S. R. M. 1973. Trimethoprim-Sulfamethoxazole: *in vitro* microbiological aspects. J. Infect. Dis. 128:422-462.

Bushby, S. R. M. and G. H. Hitchings. 1968. Trimethoprim a sulfonamide potentiator. Br. J. Pharmacol. 33:72-90.

Goto, S., Y. Kaneko, K Harada and S. Kuwahara. 1973. *In vitro* antibacterial activity of the combination of sulfonamides and trimethoprim. Chemotherapy 21:77-87.

Hitchings, G. H. 1969. Species differences among dihydrofolate reductase as a basis for chemotherapy. Postgraduate Med. J. Suppl. 45:7-10.

Kamiya, H., K. Watanabe, K. Ninomiya, K. Ueno and S. Suzuki. 1973. The combination effect of trimethoprim and sulfamethoxazole against anaerobes. Chemotherapy. 21:128-136.

Kawakami, M., H. Yoshida, T. Fujii and S. Mitsuhashi 1973. Anti-microbial effect of combinations of trimethoprim and sulfamethoxazole *in vitro*. Chemotherapy 21:104-109.

Kosakai, N. and T. Oguri. 1973. *In vitro* bacteriological studies on pathogenic organisms isolated from clinical materials: with reference to their sensitivity to sulfamethoxazole-trimethoprim and their combination. Chemotherapy 21:110-116.

Nakazawa, S., H. Ono, M. Otsuki, Y. Kurokawa, K. Hashimoto, K. Tanoue and M. Enya. 1973. Bacteriological studies on the combination of sulfamethoxazole and trimethoprim. Chemotherapy 21:88-103.

Yokota, T. 1973. Critical evaluation of assay methods for antimicrobial activities of sulfamethoxazole and trimethoprim with a report on the potentiation of drug activities by the combination against clinically isolated bacterial strains. Chemotherapy 21:117-127