Comparison of the Inhibitory Effect of Sulfamonomethoxine and Other Sulfonamides on Capsule Formation of *Bordetella bronchiseptica*

Akira KUWANO, Takashi ITO, Hiroshi TACHI, and Takashi HIRAMUNE¹⁾

Developmental Research Laboratories of Daiichi Pharmaceutical Co., Ltd., 16-13 Kita-kasai 1-chome, Edogawa-ku, Tokyo 134 and Department of Epizootiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069, Japan

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ABSTRACT. The inhibitory effect of sulfamonomethoxine and other sulfonamides on the capsule formation of sulfonamide-resistant *Bordetella bronchiseptica* was investigated. All the sulfonamides having MeO(-OCH₃) groups inhibited the capsule formation of *B. bronchiseptica*. Strong inhibition was obtained with sulfamonomethoxine. Inhibition was not seen with sulfonamides having no MeO groups.—**KEY WORDS:** *Bordetella bronchiseptica*, capsule, sulfonamide.

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Bordetella bronchiseptica has been identified as a primary pathogen in swine atrophic rhinitis (AR) [2, 4, 10, 11, 13]. The organism shows four phase variations, phases I, II, III and a rough phase [6, 8], with phase variant organisms showing a characteristic loss of a capsular antigen [6, 9]. The encapsulated phase I organism is pathogenic and the non-encapsulated phase organism is non-pathogenic [9]. Furthermore, phase I organism is capable of attaching to pig nasal epithelial cells, whereas isogenic variants in phase III fail to attach [14]. The above reports suggest that the capsules of *B. bronchiseptica* have a close relation with the finding of AR lesion.

Recently, it has been shown that low concentration (1.56 µg/ml) of sulfamonomethoxine (6-MeO-SMMX) could inhibit the capsule formation of *B. bronchiseptica* [7]. These results indicate that a fixed concentration of each sulfonamide, i.e. 1.56 µg/ml of 6-MeO-SMMX, is necessary to inhibit the capsule formation of *B. bronchiseptica* and that the inhibitory effect of sulfonamides on the capsule formation of *B. bronchiseptica* is unrelated to their antibacterial activity.

It was reported about twenty years ago that many strains of *B. bronchiseptica* isolated from pigs in Japan were sensitive to sulfonamides [1, 12]. However, approximately 90% of the strains isolated from pigs in 1988 showed resistance to sulfadimethoxine and the minimum inhibitory concentration was higher than 400 µg/ml [5], which indicated a high incidence of sulfonamide resistance among *B. bronchiseptica* strains from pigs.

Nevertheless, sulfonamides are still useful to control AR in the field (unpublished data) and various sulfonamides are used worldwide for the prevention of AR. These contradictory phenomena may have some relation with the inhibitory effect of sulfonamides on the capsule formation of sulfonamide-resistant *B. bronchiseptica*. The purpose of the present experiment was to compare the inhibitory effect of 6-MeO-SMMX and other sulfonamides on the capsule formation of sulfonamide-resistant *B. bronchiseptica*.

Ten strains of sulfonamide-resistant *B. bronchiseptica* (BB113, SM2-4, K-5, MS-5, MY-931, YM-4, OKM-5, I-11, S-11 and SM24-3) isolated from the nasal cavity of pigs showing symptoms of AR were used. Phase I colonies were identified by colonial morphology on Bordet-

Gengou agar (BGA) [8] and agglutination with K anti-serum, which was prepared as reported previously [6]. The phase I colony of all the strains grown on BGA was suspended in 1 ml of phosphate-buffered solution (PBS; pH 7.0). The each cell suspension was diluted 1,000-fold with PBS, and the diluted cell suspensions (10³⁻⁴ CFU/ml) were examined.

The sulfonamides used were pure and in powder form; their abbreviations are indicated in Fig. 1. Ten mg of each test sulfonamide were dissolved in 10 ml of 0.1 N NaOH, which were then diluted in distilled water. According to the method standardized by the Japan Society of Chemotherapy [3], Mueller Hinton agar (MHA) plates were prepared with the drugs at final concentrations of 100–0.1 µg/ml in doubled dilutions, which were then inoculated into the cell suspension of each strain using a multipoint inoculator (Sakuma, Tokyo, Japan) and incubated at 37°C for 18 hr. Agglutination with K antiserum was tested both in PBS and in K antiserum and the minimum concentrations of sulfonamides which inhibited agglutination were determined.

The concentrations of sulfonamides which lose K-agglutinability are shown in Table 1. All the sulfonamides having MeO(-OCH₃) groups inhibited agglutination. Strong inhibition was obtained with 6-MeO-SMMX and 2-MeO-SMMX. Inhibition was not seen with sulfonamides having no MeO groups.

No difference in the minimum concentration of 6-MeO-SMMX was observed when BGA was used [7]. The results of the present study show that the inhibitory effect of sulfonamide on capsule formation of *B. bronchiseptica* is related to the MeO group of the pyrimidine ring. This observation supports the finding that sulfonamides which possess one MeO group such as 6-MeO-SMMX and 2-MeO-SMMX were more active than those having two MeO groups such as SDMX and SDX. These findings indicated that the number of MeO groups is especially important and that the position of MeO group on the pyrimidine ring is not.

Furthermore, non-encapsulated organisms changed by 6-MeO-SMMX regained the capsule present in phase I after the incubation on BGA without 6-MeO-SMMX [7]. It is therefore suggested that the capsule formation of *B. bronchiseptica* is inhibited temporarily by sulfonamides.

Fig. 1. Chemical structures and abbreviations of sulfonamides used.

Table 1. Minimum concentrations of sulfonamides which lose K-agglutinability for test strains of sulfonamide-resistant *Bordetella bronchiseptica*

strains	6-MeO-SMMX	2-MeO-SMMX	SDMX	SDX	SMXZ	STAZ	SMPD	SMTZ	SIXA
BB113	3.13 ^{a)}	3.13	25	25	>100	>100	50	>100	100
SM2-4	1.56	1.56	25	12.5	>100	>100	50	>100	>100
K-5	3.13	3.13	12.5	25	>100	>100	50	>100	>100
MS-5	1.56	3.13	12.5	12.5	100	100	12.5	>100	100
MY-931	1.56	1.56	12.5	12.5	100	>100	12.5	>100	100
YM-4	3.13	3.13	12.5	25	>100	>100	50	>100	>100
OKM-5	3.13	3.13	50	50	>100	>100	100	>100	>100
I-11	1.56	3.13	25	25	100	>100	25	>100	100
S-11	1.56	1.56	12.5	25	>100	>100	25	>100	>100
SM24-3	1.56	3.13	12.5	12.5	>100	>100	50	>100	>100

a) Unit: $\mu\text{g/ml}$.

Considering these facts, the relationship between the capsule formation of *B. bronchiseptica* and MeO group of sulfonamides should be elucidated in the future. For practical research, studies of the protective effect of 6-MeO-SMMX in pigs experimentally inoculated with sulfonamide-resistant *B. bronchiseptica* are now in progress and will be described elsewhere.

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