Reproduction of Turbinate Atrophy in Pigs by Intranasal Consecutive-Day Inoculation with Suspensions of a Toxigenic Serotype D Strain of *Pasteurella multocida*

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Porcine atrophic rhinitis (AR) is a disease characterized by hypoplasia or disappearance of the turbinate bones. *Bordetella bronchiseptica* has been identified as a primary causative agent of the disease. The dermonecrotic toxin (DNT) of *B. bronchiseptica* was considered as a major pathogenic factor responsible for the production of nasal turbinate atrophy [8]. de Jong *et al.* [5] reported that some strains of *Pasteurella multocida* produced DNT and caused severe atrophy of the nasal turbinates of pigs. The nasal lesion was induced by intranasal or intramuscular injection with the purified DNT from the organism [6, 9]. The importance of toxigenic *P. multocida* in AR has been accepted by researchers in many countries [4]. However, it was difficult to reproduce the turbinate atrophy by the experimental infections with toxigenic *P. multocida*, because the organism had a poor affinity to swine nasal mucosa and epithelial cells [7]. For colonization of the organism, combined infection with toxigenic *P. multocida* and *B. bronchiseptica* [1], damage with a nasal irritant such as diluted acetic acid [13] and consecutive-day inoculation [2, 3, 9, 12, 16] were required. It was thought that AR was transmitted by way of infective aerosols and that the severity of the clinical expression of AR was dependent on the frequency of the aerosol transmission of AR pathogens in the swine herd [3]. Therefore, the consecutive-day inoculation method was chosen in this study. By this method, older broth cultures of toxigenic *P. multocida* were generally used as inocula [2, 3, 9, 12]. Even with broth-culture filtrates, the turbinate atrophy was induced in piglets [2, 3, 12]. However, it was difficult to induce the nasal lesion by the inocula consisting of suspensions of the organism grown on agar medium [12]. These findings suggest that DNT released from the organism into broth culture medium may give some influences on the turbinate bones. Thus, the purpose of the present study is to determine the conditions of induction of turbinate atrophy in SPF pigs by intranasal consecutive-day inoculation with suspensions of a toxigenic strain of *P. multocida* capsular serotype D grown on agar medium.

Toxigenic *P. multocida* strain 70 of capsular serotype D, somatic serotype 11, which was isolated from a porcine pneumonic lesion at a slaughterhouse in Nagasaki Prefecture in 1983 was kindly supplied by Dr. S. Iwamatsu (Chuo Livestock Hygiene Service Center of Nagasaki Prefecture, Japan). This strain had no fimbriae or hemag-

glutinating property with human type O red blood cells. The DNT sonically extracted from strain 70 (concentration of 10^{10} cells per ml) had a titer of 32 in 0.1 ml by the guinea pig skin test [5]. The organism propagated on dextrose starch agar (Difco, Detroit, U.S.A.) plates at 37°C for 6 hr was harvested, suspended in heart infusion broth (Difco) and used for inoculum.

In experiment 1, eleven 1-day-old gnotobiotic piglets produced by hysterectomy were divided into 3 groups. The 1st and 2nd groups were inoculated intranasally with 10^7 viable organisms of strain 70 (0.5 ml/each nostril) during 3 and 6 consecutive days, respectively. The 3rd group was uninoculated control.

The piglets were reared in sterile stainless steel isolators until the age of 3 weeks and thereafter kept in individual rooms. The piglets were sacrificed at the age of 14, 21 or 35 days.

In experiment 2, ten 1-day-old SPF piglets were produced by immediate removal from sows at the birth and raised in individual rooms. Seven piglets were inoculated intranasally with 10^4 , 10^6 , 10^8 or 10^{10} viable organisms of strain 70 (0.5 ml/each nostril) during 6 consecutive days. The remaining three piglets were used as uninoculated controls. All the piglets were sacrificed at the age of 45 days.

In experiment 3, seven 80-day-old SPF pigs obtained from our breeding herd were raised in individual rooms. Six pigs were inoculated intranasally with 2×10^7 , 2×10^9 or 2×10^{10} viable organisms of strain 70 (1 ml/each nostril) during 6 consecutive days. The remaining one pig was used as an uninoculated control. All the pigs were sacrificed at the age of 122 days.

The snouts of the sacrificed pigs were sectioned transversely at the site of the first upper premolar teeth. Macroscopic findings of atrophy of nasal turbinates were graded according to the criteria described by Maeda *et al.* [10]. The recovery of strain 70 from the nasal cavities was performed with sterile cotton-tipped swabs at necropsy.

In experiment 1, the 2nd group inoculated with strain 70 during 6 consecutive days had a very severe turbinate atrophy, although the 1st group during 3 consecutive days had moderate gross lesions (Table 1). The organism was recovered from 2 of 4 piglets in small numbers (about 10^4 cfu/g of nasal exudate) in the 1st group and all of 5 piglets in large numbers (10^6 – 10^8 cfu/g) in the 2nd group. The 3rd group pigs as uninoculated controls had no gross nasal lesion and were bacteriologically negative for the organism. None of these groups piglets showed any clinical signs of AR during the experimental period.

Table 1. Results of intranasal inoculation with strain 70 of *P. multocida* serotype D in pigs

Experiment No.	Dose of inoculum	Age in days for inoculation	Age in days at necropsy	Recovery of strain 70 from nasal cavity	Grade of atrophy of nasal turbinates
1	10 ^{7a)}	1-3 ^{b)}	14	1/2 ^{c)}	++, ++ ^{d)}
	10 ⁷	1-3	35	1/2	++, ++
	10 ⁷	1-6	14	1/1	###
	10 ⁷	1-6	21	3/3	###, ###, ###
	10 ⁷	1-6	35	1/1	###
	—	—	35	0/2	—, —
2	10 ⁴	1-6	45	0/2	—, —
	10 ⁶	1-6	45	0/2	###, ###
	10 ⁸	1-6	45	0/2	###, ##
	10 ¹⁰	1-6	45	0/1	##
	—	—	45	0/3	—, —, —
3	2×10 ⁷	80-85	122	0/2	—, —
	2×10 ⁹	80-85	122	0/2	—, —
	2×10 ¹⁰	80-85	122	1/2	+, +
	—	—	122	0/1	—

a) Cells/dose. The bacterial concentrations were spectrophotometrically adjusted.

b) Inoculated daily during the ages.

c) No. of pigs which recovered the inoculated organism/No. tested.

d) Macroscopically graded according to the criteria described by Maeda *et al.* (—=normal, +=mild, ++=moderate, ###=severe, ####=very severe)[10].

In experiment 2, the piglets inoculated with 10⁴ viable organisms of strain 70 and uninoculated controls had no nasal lesions. However, the piglets inoculated with 10⁶ to 10¹⁰ viable cells had severe or very severe turbinate atrophy (Table 1). The degree of nasal lesions induced by inoculations with 10⁶ or more viable organisms was not dependent on the concentrations of viable organisms in the inocula. In spite of such severe nasal lesions, none of the piglets showed any clinical manifestations of AR. No inoculated organism was recovered from the nasal cavities of any piglets.

In experiment 3, the pigs inoculated with 2×10⁷ or 2×10⁹ viable organisms of strain 70 and an uninoculated control had no nasal lesions. Two pigs inoculated with 2×10¹⁰ viable organisms had a mild turbinate atrophy, and the inoculated organism was recovered from only one of the pigs (Table 1). No clinical signs were observed in these pigs.

Several reports showed that induction of nasal turbinate atrophy in pigs by broth cultures of toxigenic *P. multocida* was easier than that by the suspensions of viable organisms of toxigenic *P. multocida* grown on agar medium [11, 12]. In the present study, however, it was demonstrated that 1-day-old SPF piglets induced the nasal lesion by intranasal consecutive-day inoculation of the bacterial suspensions. The degree of the nasal lesions was dependent on the repeated inoculation times and the age of piglets used (Table 1). In relation to the age, de Jong and Akkermans [3] had already reported. The inoculated organism was recovered from only 3 of 16 pigs (18.8%) kept in individual rooms, though done from 5 of 6 pigs (83.3%) kept in isolators. It was indicated that some normal microbial flora eliminated *P. multocida* from the nasal mucosa and that the colonization by the consecutive-

day inoculation was as temporary as that by the inoculation following intranasal treatment with diluted acetic acids [15]. The temporality of colonization of *P. multocida* may be due to the poor affinity to swine nasal epithelial cells [7]. However, turbinate atrophy could be caused by the maintenance of sufficiently large numbers of toxigenic *P. multocida* in nasal cavity for a few days [1].

In Japan, there has been no report on the successful induction of turbinate atrophy in pigs using toxigenic *P. multocida* alone. Etiologically, no correlation between appearance of AR in pigs and DNT-producing ability of *P. multocida* was observed [16]. Thus, some Japanese researchers have questioned the role of the bacterium in AR [13, 14, 16]. Oyamada *et al.* [14] reported that no gross lesions of the nasal turbinate bones were observed in all the pigs inoculated with the bacterial suspensions of 5 × 10⁷ and 1.5 × 10⁸ viable organisms 1 and 3 times at weekly intervals, respectively, although the organism was constantly recovered in low numbers from the nasal cavities of the pigs during the experimental period. This may be due to the low grade of colonization. Nakagawa *et al.* [13] found no significant lesions in gnotobiotic piglets inoculated with serotype A strain Kobe 5 of *P. multocida*. The reason of their failure may be related to the inability of the strain used to produce DNT (data not shown).

The inoculation method demonstrated in the present study may be applicable to estimation of the efficacy of a toxigenic *P. multocida* vaccine maternally used.

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