Effect of Dihydroheptaprenol on Nitroblue Tetrazolium Reduction by Swine Alveolar Macrophages

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Swine respiratory diseases such as mycoplasma pneumonitis and atrophic rhinitis are frequently encountered in swine practice, and these diseases are thought to result from the suppression of defense mechanisms against infectious agents in stressed animals [7]. In local pulmonary immunity as well as systemic nonspecific immunity, macrophages constitute a major defense mechanism through phagocytosis and superoxide generation, and these functions of macrophages were reported to be augmented by several adjuvants [2, 5]. A chemically synthesized polyprenol derivative, dihydroheptaprenol (DHP), has been recently developed and described to augment H₂O₂ generation by murine peritoneal macrophages, leading to the protection against experimental *Escherichia coli* infection [1]. In the present study, as one of the functions of macrophages, nitroblue tetrazolium (NBT) reduction by swine alveolar macrophages treated *in vivo* with various concentrations of DHP was examined and compared to that of untreated controls.

Seven specific pathogen-free pigs (Iwatani Camborough Inc.; Minimal disease pig) ranging from 100- to 120-day old and 32 to 47 kg in body weight, were used in the present experiment. After preliminary maintenance over a week on stock diet in individual cages, the pigs were divided into three groups. Two pigs were injected intramuscularly with 2.5 mg/kg body weight of DHP (Group A), and 3 pigs were with 5 mg/kg body weight of DHP (Group B). Two pigs were only with solvent as controls (Group C). To prepare swine alveolar macrophages, bronchoalveolar lavages were performed according to the method of Markham *et al.* [3] with a slight modification using fiber-optic bronchoscope

(OLYMPUS BF Type 6C). Briefly, after anesthesia with pentobarbital sodium, the bronchoscope was passed down into the trachea, and wedged into a bronchus of the right cranial lobe. The lobe was then lavaged with a total of 100 ml prewarmed sterile saline solution in five replicate lavages of 20 ml each. In all groups, 75% of total infused saline solution was recovered, and it contained approximately 7×10^7 cells. These lavage cells consisted mainly of macrophages (80%) and the remainder was lymphocytes and neutrophils, as judged from Giemsa staining and non specific esterase staining. The relative proportion of macrophages in the entire bronchoalveolar lavage cells did not significantly alter throughout the experiment.

NBT reduction by these cells was determined as previously described [4, 6]. The lavage cell suspensions (2.5×10^6 cells/0.5 ml) with and without 0.1 ml of 1% zymosan A (Sigma Chemical Co., St. Louis, Mo) were mixed with 0.4 ml of 0.1% NBT (Sigma) solution. After incubation for 30 min at 37°C, the mixtures were extracted by 3 ml of dimethylsulfoxide, and the supernatant fractions were then measured for its optical density at 565 nm. NBT reduction activity was calculated by subtracting the value of optical density of the sample prepared in the presence of zymosan A from that prepared in the absence of it.

Fig. 1 shows the NBT reduction of alveolar macrophages after *in vivo* treatment with DHP. Two days after the administration of DHP, the NBT reduction by alveolar macrophages from both Group A and B significantly increased as compared to that from untreated control (Group C), and then gradually decreased to the control level by 6 days after the administration. The enhancement of NBT reduction was more evident in Group A, which received 2.5 mg/kg of DHP, than in Group B which received 5 mg/kg,

Fig. 1. Effects of DHP on NBT reduction by swine alveolar macrophages. The pigs were administered intramuscularly with DHP (2.5 mg/kg (●) or 5 mg/kg (○)) or with solvent (■). NBT reductions on days 0 (before administration), 2, 4 and 6 days after treatment were indicated. The results are expressed as mean $\pm$ SD. **, $p < 0.01$.

suggesting that the optimal dosage of DHP to stimulate NBT reduction by swine alveolar macrophages was 2.5 mg/kg. In addition, a slight increase of NBT reduction by macrophages from untreated Group C was observed, which might be associated with the effect of repeated bronchoalveolar lavages on the respiratory tracts.

The data presented in this report indicates that *in vivo* administration of DHP induced the augmentation of NBT reduction by swine alveo-

lar macrophages without affecting the total cell number and relative proportion of alveolar macrophages, and this is the first evidence of enhancement of local pulmonary immunity by systemic administration of DHP. However, it remains unclear whether the activation of NBT reduction by alveolar macrophages is due to the direct effect of DHP on macrophages or the indirect effect via induction of soluble mediators such as colony stimulating factors and interferons produced by other types of cells. Araki *et al.* [1] have been reported that DHP administration augments peripheral blood neutrophil (PBN) function in mice, as well as increases the number of swine PBN (data not shown). Thus, further studies concerning the effect of DHP on swine alveolar macrophage functions as well as systemic immune responses are required to seek the possibility of clinical application of DHP to the prophylaxis of swine respiratory disorders.

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要 約

ジヒドロヘプタプレノール (DHP) の豚肺胞マクロファージ NBT 還元能に対する影響 (短報) : 亘 敏広・後飯塚僚¹⁾・小山秀一・左向敏紀・内野富弥・荒木誠一²⁾・長谷川篤彦¹⁾・本好茂一 (日本獣医畜産大学獣医内科学教室, ¹⁾東京大学農学部家畜内科学教室, ²⁾エーザイ株式会社)——健常豚に DHP を投与した後, 気管支肺胞洗浄を実施した。回収された肺胞マクロファージの細胞数及び構成比には DHP 投与による差は認められなかったが, NBT 還元能において DHP 投与群では対照群と比較して有意な高値を認めた。