Enhancement of Neutrophil Function by Dihydroheptaprenol in Adult Cows

Osamu YONEYAMA, Satoshi OSAME, Makoto KIMURA¹⁾, Seiichi ARAKI¹⁾, and Sigeru ICHIJO

Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080 and

¹⁾Eisai Co., Ltd. Bunkyo, Tokyo, 112, Japan

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It was reported that dihydroheptaprenol (DHP), a synthetic polyprenol derivative, increases the number of peripheral blood neutrophils and enhances their phagocytic killing activity in normal healthy calves [8]. However it remains unknown whether DHP enhances the antibacterial activity of neutrophils in adult cows which have a more mature immune system than calves at a lower dose. The present paper describes the effect of intramuscular administration of DHP on neutrophil counts, nitroblue tetrazolium (NBT) reduction and comparison of phagocytic killing of *Staphylococcus aureus* by neutrophils between adult cows and calves.

Fifteen clinically healthy Holstein cows aged 3 to 9 years were used. They were housed on the farm affiliated to the university and were randomly assigned to 3 groups of 5 cows each; the two groups were given a single injection of DHP [1] at doses of 1 and 2 mg/kg body weight, respectively, and one group received placebo (vehicle) only.

Fig. 1 shows the changes in total leukocyte counts obtained by a hemocytometer and the neutrophil counts estimated from the differential counts determined on Giemsa-stained blood smears. The neutrophil counts in the placebo group were $890 \pm 390/\mu\text{l}$ and $1,720 \pm 750/\mu\text{l}$ just before and a half day after administration, respectively, showing a slight increase within the range of diurnal rhythm [5].

In the group receiving 1 mg/kg of DHP, the neutrophil count was $550 \pm 100/\mu\text{l}$ before treatment, and this increased remarkably to $2,550 \pm 1,290/\mu\text{l}$ a half day after treatment. After that it returned almost to the initial level one day after treatment.

In the cows receiving 2 mg/kg of DHP, the neutrophil count was $530 \pm 410/\mu\text{l}$ before treatment and this increased to $3,310 \pm 1,720/\mu\text{l}$ and $3,430 \pm 1,860/\mu\text{l}$, a half day and one day after administration, respectively. The values re-

Fig. 1. Effect of DHP and placebo on the total number of blood leukocytes and neutrophils. Significant difference from before injection; * $P < 0.05$.

mained at high levels of $1,650 \pm 1,860/\mu\text{l}$ and $1,100 \pm 890/\mu\text{l}$, 2 and 3 days after treatment, respectively.

Neutrophils were isolated by the method by Roth and Kaeblerle [7] with slight modification. Briefly, neutrophil fraction containing eosinophils, which is obtained by the method of Roth and Kaeblerle [7], was suspended in 10 ml cold RPMI 1640 medium (Nissui, Japan). The suspension was layered on top of 5 ml of a Ficoll-Hypaque cushion (specific gravity; 1.135 g/cm^3) and centrifuged at $500 \times g$ for 45 min at 25°C . After centrifugation, RPMI 1640 medium, eosinophil and Ficoll-Hypaque layers were discarded and the remaining neutrophil layer was washed twice with cold RPMI 1640 medium. Viability of neutrophils was determined by trypan blue (0.5%) exclusion [3]. The cells were resuspended in RPMI 1640 medium to the final concentration of 5×10^6 viable neutrophils/ml and this was used for the NBT reduction test and phagocytic killing

assay against *S. aureus*.

The NBT reduction assay was performed according to the method described previously [8]. The results are shown in Fig. 2. No changes in NBT reduction activity were observed in the placebo group. In the group injected 1 mg/kg of DHP, the difference between the phagocytosing value (P) and the resting value (R), that is, the Δ value representing the phagocytic activity, significantly increased ($p < 0.05$) by 0.06 ± 0.04 and 0.07 ± 0.06 , 1 and 2 days after the administration of DHP, respectively in comparison with the value obtained before injection. These returned to normal on the fourth day. In the cows given 2 mg/kg of DHP, the Δ value significantly increased ($p < 0.05$) by 0.10 ± 0.05 and 0.06 ± 0.01 , 1 and 2 days after the administration of DHP, respectively and returned to normal after the third day.

The NBT reduction assay in the method was

used to evaluate the metabolic activity of phagocytes [2, 6, 9]. A remarkable increase in the reduction activity 1 and 2 days after the administration of DHP was considered to show that DHP enhanced the generation of reactive oxygen by neutrophils resulting in higher killing ability.

The phagocytic killing assay against *S. aureus* was carried out according to the method described by Hori *et al.* [4] with a slight modification as mentioned previously [8]. The ratio of neutrophils to bacteria was 1:4.

In the placebo group, the survival rates of the bacteria were from 29.0 to 40.1% and from 21.5 to 27.0% for 60 and 120 min incubation periods, respectively, showing no change attributable to administration of DHP.

In the group given 1 mg/kg DHP, those before administration were $43.1 \pm 18.1\%$ and $30.2 \pm 9.7\%$ for 60 and 120 min incubation periods, respectively. The phagocytic killing

Fig. 2. Effect of DHP and placebo on NBT reduction activity. Significant difference from before injection; * $P < 0.05$, ** $P < 0.01$.

activity of neutrophils was markedly enhanced 1 and 2 days after drug administration. On the second (showing peak activity), the survival rates of the bacteria were $22.6 \pm 10.1\%$ and $15.4 \pm 7.5\%$ for 60 and 120 min incubation periods, respectively.

In the group injected 2 mg/kg of DHP, the survival rates of bacteria before administration were $40.5 \pm 6.4\%$ and $30.0 \pm 3.5\%$ for 60 and 120 min incubation periods, respectively. The phagocytic killing activity of neutrophils was markedly enhanced 1, 2 and 3 days after drug administration. On the second day (showing peak activity), the survival rates of the bacteria were $22.3 \pm 9.2\%$ and $13.1 \pm 4.2\%$ for 60 and 120 min incubation periods, respectively (Fig. 3).

The authors reported in the previous paper [8] that an effective dose of DHP for calves was 6 mg/kg or more. The present study has revealed that in case of adult cows, a dose of 1 mg/kg was less effective for increasing the number of neutrophils than that of 2 mg/kg. However, the NBT reduction and phagocytic killing of *S. aureus* were clearly enhanced with no difference between the doses. The above findings suggest that the effective dose of DHP for adult cows may be 2 mg/kg or more and the administration of DHP to adult cows may enhance the mobilization and the phagocytic killing activity of neutrophils, by which the self-defense mechanisms against infectious diseases would be reinforced.

In the previous study on phagocytic killing of *S. aureus* in calves [8], the ratio of neutrophils to bacteria was 1:2. In the present study, the ratio was changed to 1:4, favoring the number of the bacteria, while the phagocytic killing activity of neutrophils from adult cows appeared higher than that from calves. Further studies should be pursued to clarify the difference in phagocytic killing activity of neutrophils between ages because the effective dose of DHP for adult cows was lower than that for calves.

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Fig. 3. Bactericidal activity by neutrophils after DHP 2mg/kg injection with *Staph. aureus* 209P.

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要 約

ジヒドロヘプタプレノールの成牛好中球数および機能に及ぼす影響（短報）：米山 修・納 敏・木村 誠¹⁾・荒木誠一¹⁾・一条 茂（帯広畜産大学家畜内科学教室，¹⁾エーザイ株式会社）——成牛にジヒドロヘプタプレノール（DHP）を投与し，末梢血の好中球数および好中球機能への影響を検討した．末梢血好中球数は，DHP 投与（1 mg または 2 mg/kg 筋注）0.5 および 1 日後に著明に増加し，NBT 還元能は投与 1 および 2 日後に，また *S. aureus* に対する貪食・殺菌能は投与 1，2 および 3 日後に著明に上昇した．以上の成績から，DHP には成牛に対し好中球の増数，貪食・殺菌能の増強などの非特異的免疫能を増強する効果があるものと考えられた．