POSSIBLE EFFECTS OF DIHYDROHEPTAPRENOL ON NEUTROPHIL FUNCTION OF POSTPARTUM DAIRY COWS

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INTRODUCTION

The parturient cow is highly susceptible to environmental mastitis. Severe clinical mastitis may occur shortly after parturition and is more severe than most cases of mastitis occurring in mid and late lactation periods (3). The enhanced susceptibility to mastitis may be attributable to the suppression of host defense mechanisms (3,4). The function of neutrophils is important for protection in the early phase of infection. However, little data are available on the function of neutrophils in dairy cows during the perinatal period (6, 7,10). Immunomodulators that might abrogate the development of periparturient immunosuppression have not been evaluated. Dihydroheptaprenol (DHP) is a polyprenol derivative and is known as a stimulator of neutrophils under experimental systems of mice (1,11) and swine (2). The present study was performed to determine whether DHP administration alters neutrophil function in dairy cows during the postpartum period.

MATERIALS AND METHODS

Animals- Ten Holstein cows, 3.5-8.0 years old, were studied during the postpartum period (Parturition to 7 days after parturition). They were divided into the following two groups: Five cows which at parturition were given one dose of DHP (DHP-treated group) and 5 untreated cows (Untreated group).
Dihydroheptaprenol (DHP)- DHP(C_{35} H_{60} O, M.W.= 496.86, Research Lab., Eisai Co., Tokyo) was administered subcutaneously at parturition at a dosage of 0.5 mg/kg of body weight (11).
Blood- Heparinized blood samples were collected at parturition before administration of DHP, and at 1,2,3,5 and 7 days after parturition from the tail vein. Total and differential leukocyte counts were performed.
Isolation of neutrophils from blood- Polymorphonuclear cells were separated from the heparinized blood by Ficoll-Conray density gradient centrifugation (8). The isolated cells were suspended in Earle's solution to a concentration of 5 x 10^6neutrophils/ml (85-95% purity).
Chemotactic assay- The chemotaxis under agarose was perfomed according to the method of Nelson et al (9). with slight modifications. Zymosan activated serum was used as chemotactic factor. Random migration and chemotaxis were measured. Chemotactic index (C.I) was calculated and chemotactic differential was the chemotactic distance minus random migration distance.
Luminol-dependent chemiluminescence (CL) assay- Whole blood CL assay was performed according to the method described previously (8). A blood sample (100µl) was mixed with 400µl of Hanks balanced salt solution in a plastic vial (14x55mm). This mixture was incubated at 37°C for 10 minutes in a chemiluminometer and 20µl of luminol (final

$1x10^{-4}$M) were added. This was equilibrated for 10 min, then 20µl of zymosan solution (final 925.9µg/ml) were added. The absolute peak response (Peak CL, cpm) and the time showing peak CL (peak time, min) were read from the recorder. The CL index was calculated as follows: (peak CL/number of neutrophils in 100µl) x 1000.

Analysis- Data were analyzed by use of two- way analysis of variance and where appropriate Student t- test were performed.

RESULTS

Significant ($P<0.05$) differences in number of total leukocytes were observed between the value at parturition and the values at 1-7 days after parturition in both groups. No significant differences were detected in total leukocytes between untreated and DHP-treated groups. The neutrophil concentration in both groups at parturition was increased significantly ($P<0.05$) compared to those of cows at 1,3 and 5 days after parturition in untreated group and 2-7 days after parturition in DHP-treated group (Fig. 1).

Lower values for random migration of neutrophils were observed during the first 5 days after parturition with untreated group and first 3 days after parturition in the DHP-treated group, however, the changes were not significant. Chemotaxis was decreased at 1 and 2 days after parturition in untreated and DHP-treated groups compared with those at 7 days after parturition in both groups. The chemotactic differential in DHP-treated group at 1 day after parturition increased compared to that at parturition; in contrast, the chemotactic differential in the untreated group was shortened, but the difference was not significant (Fig. 2).

Higher peak CL was observed in whole blood of cows at parturition compared with those at 2-7 days after parturition in both groups. The mean CL index increased significantly ($P<0.05$) from 58.1 at parturition to 114.9 at day 1 after parturition in cows treated DHP (Fig. 3). The mean peak time of chemiluminescent response was significantly ($P<0.05$) prolonged at parturition in both groups compared with those at 3 and 7 days after parturition (Fig. 4).

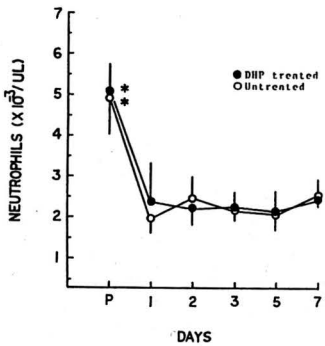

Fig.1. Changes in neutrophils in the blood from untreated and DHP-treated cows from parturition (P) to 7 days after parturition.

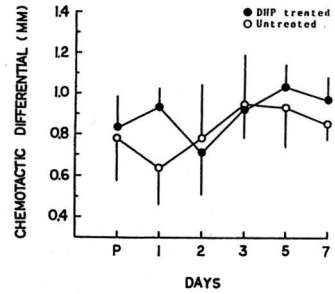

Fig.2. Changes in chemotactic differential of neutrophils P= parturition

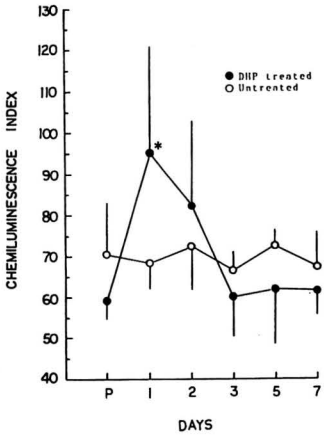

Fig.3. Luminol-dependent CL response in whole blood of untreated and DHP-treated cows. P= parturition.

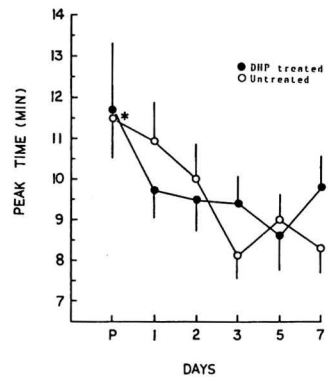

Fig.4. Peak time of CL response. P= parturition.

DISCUSSION

Dihydroheptaprenol is a low molecular weight synthetic polyprenol derivative which enhances nonspecific resistance to infection (1,2). Araki et al (1) reported that DHP increased neutrophils in peripheral blood in cortisone-treated mice. The neutrophils significantly enhanced clearance of Escherichia coli from the blood stream and the neutrophils and peritoneal macrophages were activated for H_2O_2 generation. Possible mechanisms of action of the DHP for enhancing resistance may be, at least, related to its abilities to stimulate the neutrophils and also to activate macrophages for the uptake function, H_2O_2 generation and tumor cytotoxicity.

A few attempts have been made to evaluate clinical application of DHP to domestic animals (2,12,13). Yoneyama et al (13) reported markedly increased neutrophil counts, enhanced nitroblue tetrazolium reducing activity, and increased phagocytic-killing activity in neutrophils from normal lactating cows(not postpartum period) at 1-3 days after DHP administration (1-2 mg/kg).

In mice (1), the number of neutrophils increased significantly after DHP administration, however, in this bovine study no drug related changes in total leukocyte and neutrophil counts were observed in the immediate postpartum period. The effects of physiologic changes of parturition on neutrophils may have obscured any leukocyte changes induced by administration of DHP. A rapid decrease in total leukocytes and neutrophil counts occurred after parturition that was similar to the reported pattern (5).

Migration is an important functional characteristic of neutrophils in response to an inflammatory stimulus such as bacterial invasion. In the present study, the random and chemotactic responses of neutrophils were considerably reduced in cows during the immediate postpartum period. Random and chemotactic responses decreased during the first 2 to 3 days, however, chemotactic index and chemotactic differ-

ential in the DHP-treated group at day 1 after DHP administration increased. This finding suggests that possible effects may arise from activating neutrophils stimulated by DHP.
Significantly ($P<0.05$) increased chemiluminescence index was observed in the blood of the DHP-treated group at day 1 after DHP administration , suggesting that DHP stimulated the neutrophils and enhanced the respiratory burst of bovine neutrophils. This finding is in agreement with the previous report that DHP increased O_2^- generation in murine neutrophils (1).
As the enhancing effects of DHP appeared to be short, longer treatment may be required for clinical application of DHP as the immunomodulator to abrogate the immunosuppresion.

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SUMMARY

Neutrophil responses were evaluated in dairy cows at parturition and on 1,2,3,5 and 7 days after they were given dihydroheptaprenol (DHP) (0.5 mg/kg body weight) at parturition. The chemiluminescence index increased significantly ($P<0.05$) from parturition to 1 day after parturition in cows injected with DHP. The total leukocytes and neutrophils in blood in untreated and DHP-treated groups at parturition were increased significantly ($P<0.05$) compared with postparturient period in both groups and decreased to normal levels by day 1 after parturition. Decreased random migration of neutrophils was detected during the first 5 days after parturition in the untreated group and the first 3 days after parturition in the DHP-treated group. Chemotaxis was decreased at 1 and 2 days after parturition in both groups as compared with those at 7 days after parturition. Results from this study suggest that luminol-dependent chemiluminescence response increased markedly during first 1,2 days when administered DHP to cows at parturition. One of the possible effects of DHP is to activate the respiratory burst of bovine neutrophils.