

## Effect of intramammary injection of RbG-CSF on milk CL activity of subclinical mastitis cows\*

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### ABSTRACT

The effect of intramammary injection of rbG-CSF ( $4 \times 10^5$  units/10 ml saline) on quarter milk levels of CL activity and SCC and shedding patterns of total bacteria and *S. aureus* in an udder suffering from *S. aureus* subclinical mastitis was investigated. The cytokine injection induced significant increases in milk CL activity and SCC and significant decrease in milk *S. aureus* count.

KEY WORDS: dairy cows, *S. aureus*, subclinical mastitis, rbG-CSF, milk CL activity

### INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) mastitis is known to be highly resistant to therapy with antibiotics, since the organism forms microabscesses or granulomas in the mammary gland tissues, survives in the host cells and easily becomes chronic. Granulocytes such as neutrophils play an important role in the host defences of cattle against pathogens (Swain et al., 2000). When granulocytes release active oxygens in oxygen-dependent mechanisms, a trace amount of intense light (photons) is emitted in proportion to the amount of active oxygens (Horan et al., 1982). Milk chemiluminescence (milk CL) assay measures the ability of a phagocyte in milk to produce the light (Takahashi et al., 2001). Granulocyte-colony stimulating factor (G-CSF), which is a glycoprotein with a

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molecular weight of 18 to 25 kDa, is reported to stimulate the production and function of granulocytes (Kehrli et al., 1991). In previous study, administration of the recombinant human G-CSF (rhG-CSF) to dairy cows induced resistance against bacterial infections in mammary glands experimentally infected with *S. aureus* (Kehrli et al., 1991). Nagata et al. (2003) established a high level expression and purification system for recombinant bovine G-CSF (rbG-CSF) using a baculovirus system. They demonstrated that the subcutaneous injection of the recombinant cytokine to dairy cows improved the phagocytic activity of neutrophils in blood (Nagata et al., 2003).

In this study, we investigated the effect of intramammary injection of the rbG-CSF on quarter milk levels of CL activity and SCC and counts of total bacteria and *S. aureus* in an udder with subclinical mastitis as a result of spontaneous infection with *S. aureus*.

## MATERIAL AND METHODS

Six Holstein dairy cows in the second to fourth lactation were used in the experiment. The cows were all in mid lactation, weighed  $616 \pm 30$  kg, and were milked twice daily throughout the study. The cows had no clinical mastitis, but they had been at least 1 quarter subclinically and spontaneously infected with *S. aureus* and elevated milk SCC for 2 to 6 months. The rbG-CSF was produced in baculovirus expression system (Nagata et al., 2003). A sterile polyethylene catheter (outer diameter; 1 1/3 mm) was inserted into the cistern of a subclinical *S. aureus* infected quarter through the teat canal. As a control experiment, 10 ml of sterilized saline was injected within 30 sec into the cistern through the catheter just after the morning milking. Seven days after the single injection of the saline,  $4 \times 10^5$  units of rbG-CSF in 10 ml of saline was injected within 30 sec into the same cistern. Milk samples of the test quarters were collected prior to the treatment and 6 h, 1, 2, 3 and 7 days after injection of saline or rbG-CSF solution. Rectal temperature and daily milk yield were measured to evaluate the effects of each treatment on the physiological homeostasis or side effects of the animals. Measured constituents of milk were CL activity, SCC and counts of total bacteria and *S. aureus*.

## RESULTS

Saline and rbG-CSF injections did not induce any abnormal clinical symptoms or any visible local reactions in the injected areas. Feed intake was unchanged throughout the experiment. Rectal temperature and daily milk yield did not show any adverse effects after the injections. Milk CL activity had not any changes after the saline but had a marked increase with a maximum at 6 h after the cytokine

injection (Figure 1). Milk SCC did not change after the saline but increased significantly after the cytokine injection. Total milk bacterial count did not change after the saline but tended to decrease after the cytokine injection. Milk *S. aureus* count did not change after the saline but was significantly decreased for 48 h after the cytokine injection.

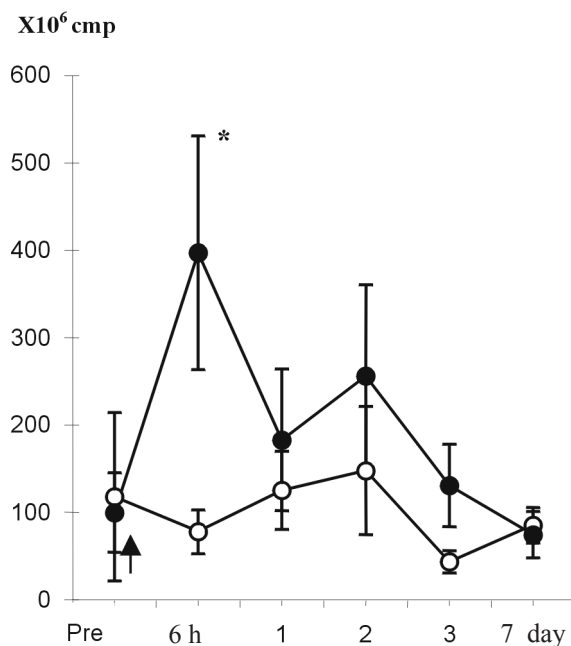


Figure 1. Change in milk CL activity following intramammary injections of saline (○; n=6) and rbG-CSF (●; n=6). Each point and vertical bar represents the mean±S.E. of 6 animals. Significance of difference from the value at pre-injection time is indicated as follows: \* P<0.05

## DISCUSSION

Although current antibiotic therapy programs can be effective in the control of some mastitis, therapy of *S. aureus* is only moderately efficacious and requires 3-5 day milk withdrawal due to antibiotic residues (Daley et al., 1993). Furthermore, antibiotic therapy can also inhibit the normal defense mechanisms of the host by decreasing phagocytic function and thereby further exacerbate the inefficiency of the phagocytes and subsequent relapse of the infection (Ziv et al., 1983). On the other hand, cytokine therapy is fundamentally different from conventional chemotherapies in that it cures disease by activating or controlling the basic part of the infection protective mechanism of the body, although its effects are somewhat pleiotropic and may include side effects (Daley et al., 1993). These potential beneficial effects to the host must be balanced with the potential local and

systemic side effects that cytokines have been known to elicit (Daley et al., 1993), and the injecting dose of rbG-CSF which holds the side effects to a minimum must be selected in order not to disturb homeostasis and productivity of the animals. In this study, rectal temperature and daily milk yield had not any adverse effects by the injections of saline and cytokine. Other side effects such as diarrhoea, external glandular swelling and loss of appetite were not produced by the dose of cytokine used in this experiment. In the present experiment, the injection of rbG-CSF to an udder with subclinical mastitis caused profound increases in milk CL activity and SCC and significant decrease in milk *S. aureus* count. The increase in milk CL activity reflects the promotion of bactericidal activity of milk granulocytes since CL activity moves parallel with the amount of active oxygens released from granulocytes (Horan et al., 1982). In this experiment, intramammary injection of the rbG-CSF induced a significant increase in neutrophil CL activity of peripheral blood. Nagata et al. (2003) demonstrated that subcutaneous injection of the rbG-CSF to dairy cows had a strong phagocytic activity on the peripheral neutrophils. The results suggest that the cytokine stimulated the phagocytic activity of neutrophils in the peripheral blood not only *via* subcutaneousness but also *via* intraudder.

From these results, it is suggested that the rbG-CSF causes bactericidal effects in mammary glands spontaneously infected with *S. aureus*.

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