A New Diagnostic Indicator Using Concanavalin A Low-Affinity Lactoferrin Levels in Mammary Gland Secretion in Mastitic Drying Cows

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ABSTRACT. We examined the effective diagnostic indicator using the concanavalin A (Con A) low-affinity lactoferrin (Lf) to mastitic drying cows. The concentrations of both Lf and Con A low-affinity Lf in mammary gland secretions (MGSs) were lower than normal MGSs at the early and middle dry periods and colostrums. On the other hand, the levels of Con A low-affinity Lf in MGSs increased following the appearance of mastitis symptoms, and decreased when the mastitic symptoms were cured. Moreover, IgG1 concentrations of colostrums decrease on the quarters where a high level of Con A low-affinity Lf was determined after the onset of dry period. These results suggest that this method could be used as a useful indicator to mastitic drying cows.

KEY WORDS: dry period, lactoferrin, mastitis diagnosis.

NOTE Internal Medicine

The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the somatic cell counts (SCC) and microbiological status of the quarter [9, 12]. Mastitis changes the composition of milk, and the extent to which various compositional changes occurs depends on the inflammatory response [15, 17]. The degree of changes depends on the pathogenicity of the mastitis-causing bacteria and the amount of affected tissue in the gland, especially the affected epithelial area. The main changes in the udder include (1) leaking of ions, proteins and enzymes from the blood into the milk due to an increased permeability, (2) invasion of phagocytizing cells into the milk compartment, and (3) a decrease of the synthetic capacity of the gland, resulting in decreased concentrations of certain milk constituents [17]. The mastitic quarter may also produce substances related to the inflammatory reaction such as acute-phase proteins [6].

To determine bovine mastitis in lactating cows, many diagnostic indicators are employed, such as systematic and local symptoms, California Mastitis Test (CMT), SCC, clots, n-Acetyl-β-D-glucosaminidase (NAGase) activity, lactoferrin (Lf) concentration and bacterial tests [14, 22]. However, the diagnostic indicators of mammary gland secretions (MGSs) including CMT, SCC, clots, NAGase activity and Lf cannot be used in the diagnosis of mastitis in drying cows because the mammary gland of drying cows increases SCC, monocytes, macrophages, Lf, and NAGase activity during the physiological changes [2, 16, 18, 19]. Therefore, mastitis in drying cows has to be diagnosed with both systematic and local symptoms, and the diagnosis of mastitis in drying cows proves to be very difficult for the early stage of mastitis.

Lf is one of the diagnostic indicators of mastitis in lactating cows [14]. We have found concanavarin A (Con A) low-affinity Lf molecules in the mastitic MGS in both lactating and drying cows [16]. Lf has antibacterial [4, 13, 25], antiviral [11, 24] and anti-inflammatory effects [5, 8]. However, the Con A low-affinity Lf molecule has inflammation-inducing effects including proinflammatory cytokine induction, chemokine induction and leukocyte infiltration [16]. Moreover, the concentration of Con A low-affinity Lf in MGSs increases following the appearance of mastitic symptoms in the lactating and early dry period [16]. In this study, we examined whether Con A low-affinity Lf in MGSs can be used as a diagnostic indicator of mastitis in drying cows.

One hundred sixteen drying cows on 6 dairy farms aged 3 to 6 years were examined. We collected MGSs from these drying cows during the early dry period (1 to 20 days after the onset of the dry period), in the middle dry period (21 days after the onset of the dry period to 11 days before parturition), the late dry period (10 days before parturition), and during colostrums (1 to 3 days after parturition). These cows were normal (early dry period; n=9, middle dry period; n=7, late dry period; n=9, and colostrums; n=24.), acute mastitis (early dry period; n=6, middle dry period; n=5, late dry period; n=0, and colostrums; n=12.) and chronic mastitis (early dry period; n=6, middle dry period; n=11, late dry period; n=5, and colostrums; n=12). The acute mastitic cows showed both systemic clinical symptoms (pain and fever) and local clinical symptom (swelling). The chronic mastitis cows presented a local symptom (firmness) without systemic clinical symptoms. In the MGS we also detected a number of staphylococci (more than 200 colony formation units/ml), coliform bacteria, and streptococci and SCC...
(more than 500,000 cells/ml).

In the MGS samples, we examined SCC, bacterial tests, Lf concentration, the levels of Con A low-affinity Lf, and clinical symptoms. The SCC were made using a flow cytometer (FACSCalibur, Becton Dickinson, Co., Ltd., San Jose, CA), based on a cell count due to the specific binding of propidium iodide to DNA [10]. The causative bacteria in the mastitic cows was isolated from MGSs with staphylococcus No. 110 agar medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for staphylococci, desoxycholate hydrogen sulfate agar medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for coliform bacteria, and soybean casein digest agar medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% sheep blood cells for streptococci. The staphylococci counts were measured as described by Kai et al. [13]. Coagulase production was determined by tube test of rabbit plasma (Eiken Chemical Co., Ltd., Tokyo, Japan). The isolated causative bacteria were identified with commercial kits (Api staph system, Api 20 NE system and Api Strep 20 system, bioMe’rieux sa., Marcyl’Etoile, France). The concentration of Lf was measured by the single radial immunodiffusion (SRID) method [16].

In this experiment, the concentration of Con A low-affinity Lf in mastitic MGSs showed the same pattern of Lf concentration in mastitic MGSs on drying cows and colostrums (Fig. 2B). However, the levels of Con A low-affinity Lf in MGSs on mastitic drying cows increased following the appearance of mastitic symptoms at each early dry period (normal MGSs; 53.0 ± 4.8%, chronic mastitic MGSs; 63.1 ± 3.1%, acute mastitic MGSs; 69.2 ± 4.7%), middle dry period (normal MGSs; 54.2 ± 4.6%, chronic mastitic MGSs; 60.0 ± 3.5%, acute mastitic MGSs; 68.1 ± 3.6%), late dry period (normal MGSs; 49.2 ± 4.1%, chronic mastitic MGSs; 55.4 ± 2.7%) and colostrums (normal MGSs; 47.9 ± 1.2%, chronic mastitic MGSs; 57.9 ± 2.1%, acute mastitic MGSs; 60.2 ± 3.0%) (Fig. 2C).

On the other hand, healthy quarters with low levels of Con A low-affinity Lf (< 50%) after the onset of the dry period showed lower levels of Con A low-affinity Lf (41.3 ± 5.1%) and higher concentration of IgG1 (9.6 ± 1.8 mg/ml) in colostrums in comparison with the quarters with high levels of Con A low-affinity Lf (> 50%) after the onset of the dry period (levels of Con A low-affinity Lf; 55.0 ± 5.4%, IgG1; 4.4 ± 1.1 mg/ml). Moreover, mastitis on the low-content-rate quarters of Con A low-affinity Lf through the dry period to after parturition (9%) was approximately 7.8-fold lower than the high-content-rate quarters (70%) of Con A low-affinity Lf after the onset of the dry period (Table 1).

In the antibiotic therapy on staphylococcal mastitic quarters, the levels of Con A low-affinity Lf in MGSs at 7 days after antibiotic injection (37.3 ± 2.1%) decreased by approximately 2.2-fold compared to the levels of Con A low-affinity Lf in MGSs at 0 day (81.2 ± 3.1%) on the cured quarters. This level of Con A low-affinity Lf on the cured quarters was the same level of normal MGSs at early and middle dry period (Fig. 2C). On the other hand, the levels of Con A low-affinity Lf from MGSs from non-cured quarters had little decrease at 7 days after antibiotic injection in comparison with the MGSs at 0 day. The levels of Con A low-affinity Lf in MGSs on non-cured quarters was the same as clinical
mastitic MGSs at early and middle dry period in Fig. 2C. Moreover, both clinical symptoms and causative bacteria on cured quarters disappeared at 7 days after antibiotic injection. On the other hand, we detected clinical symptoms and causative bacteria in 66.7% of the cows at 7 days after antibiotic injection (Table 2).

In the dry period, diagnostic indicators of mastitis were indicated in some reports [10, 20]. However, these methods have not been used as indicators in dairy farms. In this study, the increase of Con A low-affinity Lf in MGSs in drying cows follows the appearance of mastitic symptoms. On the other hand, Lf concentration could not be used as a diagnostic indicator of mastitis in drying cows, including after parturition. Thus, the level of Con A low-affinity Lf is an effective indicator of mastitis through the dry period and after parturition. The producing mechanism of Con A low-affinity Lf is not clear. It was reported that Lf is cleavage by some enzymes including bacterial proteinase [1, 3]. Mastitic MGS show the proteolytic activity derived from inflammatory leukocytes and causative bacteria [3, 21]. Since concentration of Lf is physiologically changes in dry period [2]. Moreover, mastitic MGS in dry period show the lower level of population of neutrophil such as Lf production cells [7] than the mastitic MGS at lactating period [18, 23]. Therefore, we considered that the concentration of Con A low-affinity Lf, as possible cleavage products of Lf, did not correlate with mastitic symptoms. On the other hand, increases of proteolytic activity in MGS accelerate the cleavage of Lf, and content rates of Con A low-affinity Lf increase with deterioration of mastitis.

Moreover, the amount of Con A low-affinity Lf in MGSs decreased with recovery from mastitis after antibiotic therapy. These results suggest that the measurement of Con A low-affinity Lf in cows can be used as an effective diagnostic and prognostic indicator of mastitis. Moreover, we confirmed a decrease in colostral IgG1 concentration on the quarters where a high level of Con A low-affinity Lf was determined after the onset of dry period. Therefore, it is suggested that the measurement of Con A low-affinity Lf is an effective indicator of the health of the mammary gland of drying dairy cows.

REFERENCES

Fig. 2. The concentration and the levels of Con A low-affinity Lf in MGSs in mastitic dry cows. The MGSs were collected during the early dry period, middle dry period, late dry period and colostrums. (A) These MGSs were analyzed by Con A two-dimensional electrophoresis as described previously. (B) The MGSs were obtained from normal quarters (●), chronic mastitic quarters (■) and acute mastitic quarters (▲). The concentration of Con A low-affinity Lf was calculated as described previously. (C) The rate of Con A low-affinity Lf content was calculated by Lf concentration and Con A low-affinity Lf concentration. The data are expressed as means ± SD. A P value of less than 0.05 was considered statistically significant. * P<0.01, compared with the normal MGSs.
Table 1. The levels of Con A low-affinity Lf, IgG1 concentration in MGS and MGSs with mastitis with different levels of Con A low-affinity Lf after the onset of the dry period

<table>
<thead>
<tr>
<th>Con A low-affinity Lf content under 50% (n=11)</th>
<th>Con A low-affinity Lf content over 50% (n=10)</th>
</tr>
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<tbody>
<tr>
<td>Content of Con A low-affinity Lf (%)</td>
<td>Concentration of IgG1 (mg/ml)</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
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<tr>
<td>MGSs after the onset of dry period</td>
<td>41.8 ± 5.1*</td>
</tr>
<tr>
<td>Colostrum</td>
<td>41.3 ± 5.1</td>
</tr>
</tbody>
</table>

*a) mean ± SD.
* P<0.01, compared with the MGS after the onset of dry period.

Table 2. Changes in levels of Con A low-affinity Lf in mastitic MGSs after antibiotic therapy in drying cows

<table>
<thead>
<tr>
<th>Cured mastitic quarters (n=5)</th>
<th>Not cured mastitic quarters (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>3 days</td>
</tr>
<tr>
<td>Levels of Con A low-affinity Lf (%)</td>
<td>81.2 ± 3.1</td>
</tr>
<tr>
<td>Detection of clinical symptoms (%)</td>
<td>100.0</td>
</tr>
<tr>
<td>Detection of causative bacteria (%)</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*a) P<0.01 compared with the quarters before antibiotic therapy.
*b) P<0.01 compared with the quarters at 3 days after antibiotic therapy.
c) P<0.05 compared with the cured mastitic quarters at 3 days after antibiotic therapy.
d) P<0.01 compared with the cured mastitic quarters at 7 days after antibiotic therapy.