Serum Concentrations of Copper, Iron, and Zinc During Escherichia coli-Induced Mastitis

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ABSTRACT

Six Holstein cows were intracysterically challenged with 50 cfu of Escherichia coli to induce acute mastitis. Clinical status, milk concentrations of bacteria, and serum albumin concentration were determined to monitor the progress and severity of infection for 72 h after bacterial challenge. Blood samples were also collected throughout infection to determine serum concentrations of Zn, Fe, and Cu. Experimental E. coli mastitis resulted in mean serum concentrations of Zn, Fe, and Cu of 28, 35, and 52% of prechallenge concentrations. These decreases first occurred 4 to 12 h after peak bacterial concentration in milk. Changes in serum trace elements may occur too late in the pathogenesis of infection to decrease peak bacterial numbers in milk. However, mediation of infection and inflammation may occur in later stages of the infection process.

(Key words: iron, zinc, copper, mastitis)

INTRODUCTION

Acute inflammation is the response of tissue to injury or infection. Increased capillary permeability and phagocytic infiltration result in clinical signs most often associated with inflammation, including heat, swelling, pain, redness, and loss of function (18). Humoral factors and cytokines released in this process mediate systemic events, collectively termed the acute phase response (4). Acute phase phenomena that occur in cattle as a result of mastitis include mobilization of granulocytes into blood, fever, increased serum cortisol, and increased hepatic synthesis of certain proteins, termed acute phase reactants, that subsequently are released into blood (3, 13, 14, 17). Additionally, a transient depression of plasma Zn and Fe occurs (10, 11, 12). Serum Cu increases during acute phase reactions in other species, in part because of increased serum concentrations of the Cu-binding protein, ceruloplasmin (6).

Binding proteins, such as lactoferrin, or chelators, such as deferoxamine, decrease available Fe and Zn, thus decreasing the availability of these divalent cations needed for Gram-negative bacterial growth (10, 19). Lactoferrin concentrations in milk increase following mammary inflammation (8, 17), suggesting that Fe, and possibly Zn, sequestration during acute infections may be a mechanism to eliminate or to reduce pathogenic agents (2, 9, 20). Sequestration of Fe may also be a mechanism to reduce generation of oxygen radicals, which are potent mediators of tissue damage during inflammation (7).

Infusion of 2 × 10⁶ cfu of Escherichia coli into two quarters of cows markedly decreased plasma Fe and Zn 9 h later (12). Further studies (10) determined that numbers of E. coli in milk following challenge were positively correlated with severity of systemic clinical signs and decreased plasma Zn and Fe. However, because of the large inoculation dose and
rapid course of clinical disease, when changes in plasma Fe and Zn occurred in relation to other events was difficult to discern. Additionally, the dynamics of serum Cu during an experimental E. coli mastitis have not been reported.

In the present study, a minimum infective dose (smallest dose of bacteria that would consistently result in infection) was used, which yielded a slower progression of infection and, consequently, permitted an improved understanding of the relationship between pathogenesis and changes in serum trace minerals. Additionally, this study describes the effect of experimental E. coli mastitis on serum concentration of Cu, Zn, and Fe.

MATERIALS AND METHODS

Cows

Six healthy Holstein first lactation cows, which were at least 10 wk postpartum and producing 20 to 35 kg/d of milk, were used for the trial. Cows were milked twice daily at 0630 and 1530 h. All cows had access to pasture and were fed a ration consisting of coastal bermudagrass hay and a corn and oat concentrate supplemented with protein and minerals. During the trial, all cows were housed in individual box stalls, which permitted clinical condition and feed and water intake to be monitored.

Experimental Challenge

Two hours after the afternoon milking, 50 cfu of E. coli (MacDonald 487), prepared as described (5), were infused intracisternally into 1 quarter of each cow. Quarters selected for inoculation were considered to be negative for bacterial pathogens when cultures of weekly milk samples did not yield growth. Additionally, SCC, as determined by direct microscopic methods (15), were <150 × 10³ cells/ml.

Sampling Procedures

Coccygeal blood samples were collected prior to challenge and at 6, 12, 16, 20, 24, 36, 48, and 72 h after challenge. Samples were collected in vacuum blood tubes designed for analysis of trace minerals (Beckton-Dickenson, Rutherford, NJ). Samples were centrifuged for 20 min at 4°C to remove the clot, and the serum was frozen at −20°C until analyzed for trace minerals. Milk samples were collected for the determination of bacterial and serum albumin concentrations immediately before and 6, 12, 14, 16, 20, 24, 36, 48, and 72 h after challenge. Clinical status, including rectal temperature, presence of anorexia, dehydration, depression, and swelling of the affected quarter, was monitored when milk was collected. The SCC of the challenged quarters were not monitored because of unsuitable quality of the secretion for accurate analysis.

Assays

Numbers of E. coli (colony-forming units per milliliter) in milk were determined as described (5). Whey samples for determination of serum albumin concentration were prepared by removal of cells and fat; subsequent acidification to remove caseins was as described previously (5). Total serum concentrations of Fe, Zn, and Cu were determined at the State of Alabama Animal Diagnostic Laboratory by atomic absorption spectrophotometry according to routine methods described by the manufacturer (Varian 375; Varian, Inc., Sunnyvale, CA).

Statistical Analysis

The experiment was a complete block design with cow as block. The study was intended to determine whether Fe, Zn, and Cu concentrations decreased after bacterial challenge compared with the time zero control. Dunnet’s contrasts were performed, comparing concentrations of each element at time zero to those taken at 6, 12, 16, 20, 24, 36, and 48 h after bacterial challenge (16). Similar analysis was completed to determine whether milk serum albumin concentrations and rectal temperature increased after bacterial challenge compared with the time zero control.

RESULTS

Infection, indicated by isolation of bacteria in milk, was established in all challenged cows. All cows had fever, transient anorexia, and depression. Mean bacterial numbers in milk (log₁₀ ± SEM) was 4.97 ± .72 cfu/ml.
The mean peak bacterial number for each cow during the course of infection was $5.41 \log_{10} \text{cfu/ml}$. No bacteria were isolated from cows more than 72 h after the inoculation. The mean rectal temperatures during the first 48 h after challenge are presented in Figure 2. Mean temperatures at 14 and 16 h after the challenge (39.9 and 40.6°C, respectively) were significantly higher ($P < .01$) than at bacterial challenge.

Serum albumin concentrations in milk at 16, 20, 24, 36, and 48 h after challenge (Figure 3) were significantly higher ($P < .01$) than at bacterial challenge. The mean peak concentration attained by each cow during the course of infection was $518 \pm 65 \text{ mg/dl}$.

Mean Zn concentration in serum ranged from $1.26 \pm .09 \text{ µg/ml}$ at 6 h to $.40 \pm .09 \text{ µg/ml}$ at 20 h postinoculation (Figure 4). Mean Zn concentration in serum following an intramammary challenge of 50 cfu of *Escherichia coli* was $5.41 \log_{10} \text{cfu/ml}$.

No bacteria were isolated from cows more than 72 h after inoculation. The mean rectal temperatures during the first 48 h after challenge are presented in Figure 2. Mean temperatures at 14 and 16 h after the challenge (39.9 and 40.6°C, respectively) were significantly higher ($P < .01$) than at bacterial challenge.

Serum albumin concentrations in milk following an intramammary challenge of 50 cfu of *Escherichia coli*. Bars indicate standard error of the treatment mean; $n = 6$. **Means differ significantly ($P < .01$) from prechallenge sample.

Mean Zn concentration in serum following an intramammary challenge of 50 cfu of *Escherichia coli*. Bars indicate standard error of the treatment mean; $n = 6$. **Means differ significantly ($P < .01$) from prechallenge sample.

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During the trial, serum Zn, Fe, and Cu concentrations decreased to 27.6, 34.5, and 51.6% of prechallenge concentrations, respectively. Mean Cu concentration was significantly lower than mean concentration at challenge. Mean Fe concentration in serum ranged from .55 ± .06 μg/ml at 6 h to .31 ± .06 μg/ml at 24 h after challenge. Mean Cu concentration did not significantly decrease during the trial compared with concentration at challenge. During the trial, serum Zn, Fe, and Cu concentrations decreased to 27.6, 34.5, and 51.6% of prechallenge concentrations, respectively.

**DISCUSSION**

The acute phase response encompasses a wide variety of host reactions to physiological stress, such as infection, surgery, neoplasia, and burns (4). Mobilization of leukocytes, fever, increases in serum cortisol, increases in serum concentrations of proteins (such as fibrinogen, complement, haptoglobin, and ceruloplasmin), and transient decreases of serum Fe and Zn are acute phase events that have occurred in cows as a result of mastitis (5, 10, 11, 12, 13, 14, 17). Lohuis et al. (12) demonstrated that an intramammary challenge of 2 × 10⁶ E. coli in 2 quarters decreased serum Fe and Zn concentrations to 24 and 21% of preinoculation values, respectively. Decreases below 50% of preinoculation values were observed 9 h after inoculation, although the temporal relationship with peak bacterial numbers was unclear (12). Furthermore, the numbers of E. coli in milk secreted over time were correlated with severity of clinical signs of infection and with decreased plasma Zn and Fe concentrations (10). The present study found similar results; serum Fe and Zn concentrations decreased as a result of experimental E. coli mastitis. Additionally, a time lag was apparent between peak bacterial numbers in milk and the decrease in serum trace elements. This effect may have been more pronounced in this study because of the smaller inoculum and slower progression of infection than in earlier studies (10, 12).

Gram-negative bacteria require Fe for growth, and decreased Fe may be a host defense mechanism to limit bacterial growth (2, 20). In vitro growth of E. coli in milk is suppressed by lactoferrin, or deferoxamine, a potent Fe chelator (11, 19). The bovine polymorphonuclear leukocyte is a source of lactoferrin in milk, and both polymorphonuclear leukocyte and lactoferrin concentrations in milk and the decrease in serum trace elements.
milk increase during inflammation (8). Sequestration of available serum Fe during acute mastitis may augment local Fe binding and play an important role in antimicrobial mechanisms (2, 20). However, our study suggests that the potential antibacterial role of decreased serum Fe during acute coliform mastitis may be limited to the later stages of infection.

Oxygen radicals are released as a result of phagocytic function and from endotoxin-induced inflammatory mediators (5, 7). Free radicals are potentially harmful to host cytosol membranes; thus, defenses to neutralize them exist (5, 7). Iron is a catalyst for lipid peroxidation and radical formation; therefore, Fe sequestration during acute Gram-negative mastitis also plays an antioxidative role (7).

The potential role of Zn sequestration during acute mastitis is more obscure. Although specific binding proteins in milk are unknown, addition of Zn to milk enhances growth of *E. coli* in vitro (11), which suggests an antibacterial role of Zn sequestration.

In this study, the percentage of decreases in serum concentration of Cu were similar to that of Fe and Zn. Earlier reports in rats and hamsters (1, 6) suggested that acute phase stimuli, including endotoxin, induce interleukin-1 release, which, in turn, stimulates a 30 to 50% increase in serum Cu concentration. Much of this increase in serum Cu results from increased synthesis of the Cu-binding protein ceruloplasmin, which is thought to act as a scavenger for superoxide anion radicals (1, 6). Conner et al. (3) reported ceruloplasmin increases during the acute phase in cattle. The results of our study disagree with previous studies (1, 6); serum Cu decreased to 75% of prechallenge levels 24 h after inoculation. The unexpected decrease in serum Cu during the infection could be explained by differences in species. A previous report (1) indicated that interleukin-1 injections could not stimulate increased serum ceruloplasmin activity in Cu-deficient rats but would increase ceruloplasmin activity in Cu-sufficient rats (1). Thus, Cu deficiency may alter serum Cu dynamics during the acute phase. Mean serum Cu at challenge was 50 μg/ml. This concentration is at the low end of the reference range, for serum analyzed at the laboratory in this study, from cattle fed dietary Cu at 10 ppm. However, this study was not designed to determine the effect of dietary Cu on changes in serum trace elements induced by acute phase stimuli. Furthermore, nutritional and tissue status of Cu in cattle is difficult to assess from one baseline (prechallenge) value. Consequently the relationship between the unexpected decrease in serum Cu during this study and dietary Cu remains unclear.

CONCLUSIONS

A model of experimental *E. coli* mastitis, designed to study the sequence of changes in serum Fe, Zn, and Cu in relation to bacterial growth and local inflammation, resulted in decreases in serum Fe and Zn concentrations about 4 to 8 h after peak bacterial concentrations in milk. Contrary to earlier reports in other species, serum Cu concentrations also decreased following infection.

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REFERENCES

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