Livestock Science 159 (2014) 117–122

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Changes in parathyroid hormone-related protein concentrations in bovine milk from the early stage of lactation

R. Sato [a], K. Onda [a,*], K. Kazama [a], M. Ohnishi [b], H. Ochiai [a], K. Kawai [a], K. Kaneko [a], T. Ohashi [c], T. Miyamoto [c], Y. Wada [a]

[a] Azabu University, School of Veterinary Medicine, 17-71 Fuchinobe 1-chome, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan
[b] Veterinary Clinical Laboratory, Nemuro Agricultural Mutal Aid Association, Hokkaido 086-1105, Japan
[c] Pathology and Pathophysiology Research Area, National Institute of Animal Health, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan

ARTICLE INFO

Article history:
Received 3 June 2013
Received in revised form
21 October 2013
Accepted 24 October 2013

Keywords:
Age
Calcium
Cattle
Colostrums
Parathyroid hormone-related protein

ABSTRACT

The concentration of parathyroid hormone-related protein (PTHrP) in the blood of healthy animals is extremely low. However, milk contains a relatively large amount of PTHrP, and the changes in its levels in the early stages of lactation in cows remain unclear. To understand the characteristics of parturient changes in milk PTHrP content and the biological implications thereof, changes in milk PTHrP concentrations were measured at 7 time intervals between parturition and 21 days postpartum in 8 primiparous and 8 multiparous Holstein cows. Based on these results, milk samples were collected from 47 primiparous and 66 multiparous Holstein cows at 3 days postpartum to investigate the relationship between milk PTHrP concentration and the variables of cow age, milk yield, and milk calcium concentration. Milk PTHrP concentration in both parity groups was found to be lowest on the day of parturition (primiparous, 3.1 ± 0.5 nM and multiparous, 1.6 ± 0.3 nM) but to significantly increase on day 14 of lactation in primiparous cows (6.2 ± 0.8 nM) and day 7 of lactation in multiparous cows (4.1 ± 0.2 nM). Comparison of the 2 groups revealed that milk PTHrP concentrations in primiparous cows were higher than those of multiparous cows in the first 3 days of lactation. Although a significant negative relationship was found between milk PTHrP concentration and both age ($r = -0.65$) and milk calcium concentration ($r = -0.19$) at 3 days postpartum, no significant correlation was found between milk PTHrP concentration and milk yield. The study thus identified 3 unique characteristics of milk PTHrP concentration in the early stages of lactation: milk PTHrP concentration is higher in primiparous than multiparous cows, milk PTHrP concentration is lower in colostrum than later milk, and the difference in milk PTHrP concentration between primiparous and multiparous cows at 3 days postpartum is more strongly influenced by age than milk yield.

1. Introduction

Parathyroid hormone-related protein (PTHrP), which is produced by human and animal tumours, was initially identified as an important factor in the pathogenesis of malignant hypercalcaemia. PTHrP was later detected in normal tissues, including the mammary glands (Philbrick et al., 1996). Studies of PTHrP or its main receptor, now referred to as type 1 parathyroid hormone (PTH) receptor or PTH1R, in gene knockout mice, which die at birth or in utero, emphasise the critical role of PTHrP in maintaining overall homoeostasis (Clemens et al., 2001). PTHrP gene expression in the lactating mammary gland is high, and recent experiments have demonstrated that PTHrP plays an important role in maternal calcium (Ca) homoeostasis.

* Correspondence to: Laboratory of Internal Medicine 3, Azabu University, School of Veterinary Medicine, 17-71 Fuchinobe 1-chome, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan. Tel.: +81 42 754 7111x658; fax: +81 42 850 2464.
E-mail addresses: onda@azabu-u.ac.jp, kenondaken@gmail.com (K. Onda).

1871-1413/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.livsci.2013.10.022

Namely, Ca-sensing receptors in the lactating mammary glands detect decreases in the circulating Ca concentration of the mother and stimulate the production of mammary PTHrP. This action results in the secretion of PTHrP into the blood, which then acts in a similar manner to PTH to increase Ca concentrations by affecting the bone and kidneys (VanHouten, 2005).

PTHrP concentrations in the blood of healthy animals are extremely low or below the detection limit. A number of research groups have reported that circulating PTHrP concentrations are elevated during lactation (Dobnig et al., 1995; Lippuner, et al., 1996; Sowers et al., 1996), and it has been suggested that PTHrP contributes to Ca homoeostasis during lactation. Filipović et al. (2008) and Kocabagli et al. (1995a) detected very low blood PTHrP levels in adult cattle, and found the concentrations in some samples to be below the detection limits of the test used. In our previous study, we also reported that the plasma PTHrP concentrations of adult cows were below the detection limit (Onda et al., 2006). It is not clear whether circulating PTHrP concentrations in cows increase during lactation and influence the Ca metabolism of the mother. Although it is known that milk contains a relatively large amount of PTHrP, the biological role of milk PTHrP remains unclear (Thiede, 1994). The mean percentage of PTHrP entering the maternal circulation from the lactating mammary gland is 9% (range 1–25%) of that secreted into the milk in goats (Ratcliffe et al., 1992). Our estimation using a Jersey cow demonstrated that if all the PTHrP in the milk and blood circulation is derived from the lactating mammary gland, the percentage of PTHrP entering the maternal circulation is 2%, and the milk contains 98% of the PTHrP produced by the mammary gland (data not shown). VanHouten et al. (2003) found that litter size and growth rate were not affected by lack of PTHrP in milk from the dams of conditional knockout mice in which PTHrP expression in lactating mammary glands had been abrogated. Moreover, Kukreja et al. (1991) observed that oral administration of the N-terminus of PTHrP [1−34] had no significant effect on serum Ca levels in 3-day-old mouse pups. Although the biological role of milk PTHrP is unclear, it is not believed to be an essential factor for neonates.

One important aspect of the PTHrP studies in bovine milk is that regardless of the species difference, bovine milk contains 100–1000-fold higher levels of PTHrP than those found in the circulation of human patients with humoral hypercalcaemia of malignancy (Thiede, 1994). Another important aspect is the relationship between PTHrP and hypocalcaemia in periparturient cattle, as PTHrP produced in the mammary gland is speculated to influence maternal Ca metabolism (VanHouten, 2005). Because hypocalcaemia is known to induce a variety of parturient disorders in dairy cows, preventing periparturient hypocalcaemia is extremely important (Horst et al., 1997). Moreover, concentrations of several components of colostrum observed during the early stage of lactation differ from those observed during established lactation. To understand the characteristics of changes in milk PTHrP levels during early lactation, we measured the concentration of milk PTHrP in primiparous and multiparous cows during 7 time periods. Moreover, to confirm the findings above and identify the factors that affect the difference in milk PTHrP concentrations between the 2 parity groups, we investigated the relationship between milk PTHrP concentration and the variables of age, milk yield, and milk Ca concentration on day 3 of parturition.

2. Materials and methods

2.1. Farm management

This study was performed at a 740-head dairy farm in Hokkaido, Japan, from March 2007 to April 2008. All cows used for the experiments were Holstein dairy cattle determined to be clinically healthy by daily physical check-ups by farm crew. Cows were housed in a 4-row free-stall barn and separately kept in the following 5 groups according to age and/or milk production: fresh cows; cows with low, medium, and high milk production; and dry cows. In accordance with the standards of the National Research Council (2001), cows were fed a total mixed ration (TMR) consisting of corn silage, grass silage, and concentrated mixtures and had ad libitum access to water. Dry cows were also fed TMR consisting of the same silages and Italian ryegrass hay, soybean meal, and beet pulp. All animals were treated in compliance with the Guidelines for the Care and Use of Laboratory Animals of Azabu University, School of Veterinary Medicine, Sagamihara, Japan (approval number: 110310-3).

2.2. Milk sampling

To observe postpartum changes in milk PTHrP concentrations, milk samples were collected from 8 primiparous (age, 1.7–2.6 years) and 8 multiparous (age, 3.8–8.1 years) Holstein cows at 7 time intervals after calving (on the calving day and 1, 2, 3, 7, 14, and 21 days after calving). Parities of multiparous cows were as follows: 3rd, $n=3$; 4th, $n=3$; 5th, $n=1$, and 6th, $n=1$. Lactating cows were milked 3 times daily and milk samples were collected between 0900 and 1300 h during the cows' regular milking routine except for on the calving day, when samples were collected immediately after calving, and stored at −80 °C until analysis. To identify the features affecting milk PTHrP concentration, milk samples were collected from 47 primiparous and 66 multiparous Holstein cows at 3 days postpartum for determination of the relationship between milk PTHrP concentration and the variables of age, milk yield, and milk Ca concentration.

2.3. Milk PTHrP and Ca analysis

Milk PTHrP concentrations were measured using a commercial assay kit (PTHrP IRMA, Mitsubishi Chemical Medience, Tokyo, Japan) that had been previously modified for the measurement of bovine milk PTHrP concentrations (Onda et al., 2010). This assay employs 2 antibodies—rabbit-anti human PTHrP [50–83] polyclonal antibody and mouse-anti human PTHrP [1−34] monoclonal antibody—and thus recognises N-terminal PTHrP consisting of at least the first 83 amino acid residues. The milk samples were subjected to pretreatment consisting of