J. Dairy Sci. 100:1–12
https://doi.org/10.3168/jds.2017-12783

Inhibiting prolactin by cabergoline accelerates mammary gland remodeling during the early dry period in dairy cows

M. Boutinaud,*†[1] N. Isaka,‡ E. Gandemer,*† P. Lamberton,*† S. Wiart,*† A. I. De Prado Taranilla,‡ L. M. Sordillo,§ and V. Lollivier*†#
*INRA, UMR1348 PEGASE, 35590 Saint-Gilles, France
†Agrocampus Ouest, UMR1348 Pegase, F-35000 Rennes, France
‡Ceva Santé Animale, F33500 Libourne, France
§Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing 48824
#Université Européenne de Bretagne, F-35000 Rennes, France

ABSTRACT

The inhibition of prolactin release using cabergoline, a dopamine agonist, is an effective strategy to accelerate the changes in mammary secretion composition after drying-off. The objective of this study was to determine how cabergoline may affect mammary tissue remodeling during early involution. Holstein dairy cows were treated with either a single i.m. administration of 5.6 mg of cabergoline (Velactis, Ceva Santé Animale, Libourne, France, n = 7) or placebo (n = 7) at the time of drying-off. Mammary biopsy samples were collected 1 wk before drying-off (d −6), after 30 h of milk accumulation (d 1), and again 8 d following drying-off (d 8) to determine changes in gene expression, lactoferrin content, and cell turnover. Blood and mammary secretion samples were collected at d −6 and again at d 1, 2, 3, 4, 8, and 14 following the abrupt cessation of lactation to evaluate indicators of blood-milk barrier integrity and other markers of mammary tissue remodeling. Cabergoline induced less *SLC2A1*, *BAX*, *CAPN2*, and *IGFBP5* mRNA expression. In contrast, cabergoline did not modify changes in cell proliferation and apoptosis. Following the cessation of lactation, changes in mammary secretion composition (Na^+ and K^+) and blood lactose concentrations were indicative of a loss in the blood-milk barrier function in both treatment groups. Cabergoline treatment affected only Na^+ and K^+ concentrations at d 1, suggesting a moderate increase in tight junction permeability. The increase in the activity of MMP9 and in mammary epithelial cell concentration in mammary secretions was greater in cabergoline-treated cows than in control cows, suggesting more mammary tissue remodeling. The increase in lactoferrin immunostaining in the mammary tissue occurred earlier for cabergoline-treated cows than for control cows, and was essentially localized in the stroma. Changes in some key markers of mammary involution suggest that cabergoline accelerates mammary gland remodeling. Thus, a single injection of cabergoline after the last milking would facilitate drying-off by enhancing mammary gland involution.
Key words: cow, drying-off, prolactin, mammary involution

INTRODUCTION

The cessation of lactation causes physiological and morphological changes within the bovine mammary gland. The initial phase of the dry period corresponds to not only the cessation of milk synthesis and secretion, but also the onset of tissue remodeling. Intense remodeling of mammary parenchymal tissue is essential for the regeneration of secretory tissue between consecutive lactations that is necessary for optimal milk production (Capuco and Akers, 1999). The speed at which the mammary gland reaches steady-state involution is also essential to optimize the defense mechanisms against mastitis-causing pathogens (Oliver and Sordillo, 1989). Thus, acceleration of mammary gland involution following the abrupt cessation of lactation may not only expedite the tissue remodeling process, but also reduce the susceptibility of the mammary gland to new IMI.

Previous studies identified several biomarkers that can accurately assess the progression of mammary gland involution. The early dry period is characterized by a rapid and intense decrease in the expression of milk protein genes and cell survival genes (Wilde et al., 1997; Singh et al., 2008). The downregulation of genes involved in milk synthesis during early stage of the dry period is a clear indication of the cessation of milk secretion by mammary epithelial cells (**MEC**). The early nonlactating period is a time of mammary tissue re-

Received February 22, 2017.
Accepted August 15, 2017.
[1]Corresponding author: marion.boutinaud@inra.fr

modeling characterized by significant cellular turnover needed to achieve steady-state involution. Cessation of lactation also is associated with partial loss of the MEC (Wilde et al., 1997). Some MEC die by apoptosis as indicated by the presence of MEC with DNA fragmented nucleus and upregulation of the expression of genes involved in cell death during mammary gland involution (Singh et al., 2008).

The early dry period also is characterized by an increased tight junction permeability that is indicated by the transfer of components between blood and alveolar lumen. Hallmarks of a loss in blood-milk barrier function include in changes of Na^+ and K^+ concentrations in mammary secretions (Oliver and Sordillo, 1989) and the presence of lactose in plasma (Stelwagen et al., 1997). Moreover, the increased permeability of tight junctions also facilitates the migration of leukocytes into mammary tissues that participates in the clearance of apoptotic cells and the removal of residual milk components (Atabai et al., 2007). Remodeling of the mammary tissue is also linked with a destruction of the extracellular matrix. During mammary involution, the metalloproteinase (**MMP**) system is activated and induces the proteolysis of extracellular matrix components (Rabot et al., 2007; Tremblay et al., 2009). An example of extracellular matrix degradation biomarker is the increase in the MMP9 activity in milk analyzed by zymography (Tremblay et al., 2009).

Involution of the mammary gland after the abrupt cessation of milking can be controlled by both local and systemic factors. Accumulated milk within the alveolus is able to induce some remodeling processes due to local factors within the secretory tissues (Tremblay et al., 2009; Weaver and Hernandez, 2016) or due to the increase in the intramammary pressure (Wilde et al., 1999). The cessation of milking, however, also is induced by changes in systemic factors such as the galactopoietic hormones that are released at milking. Milking-induced prolactin (**PRL**) release, for example, was demonstrated to have a role in regulating MEC activity and turnover (Boutinaud et al., 2012; Lollivier et al., 2015). The inhibition of PRL by quinagolide, a dopamine agonist, was shown to hasten bovine mammary involution (Ollier et al., 2013). Finally, we recently showed that the inhibition of PRL release using cabergoline, another potent dopamine agonist, also is an effective strategy to accelerate mammary involution as suggested by concomitant changes in mammary secretion components (Boutinaud et al., 2016). Thus, inhibiting PRL release at drying-off may accelerate the mammary remodeling process and hasten mammary involution. The present study was carried out to better understand how cabergoline affects mammary tissue involution processes by analyzing key biomarkers that indicate the main mechanisms responsible for secretion cessation and mammary tissue remodeling.

MATERIALS AND METHODS

Animals and Experimental Design

All the procedures applied to animals were approved by the Animal Care Committee of the French Ministry of Agriculture and in accordance with French regulations (decree no. 2001–464, May 26, 2001). The study was designed and performed in compliance with the VICH GL9 European Guidelines relating to Good Clinical Practice (EMA, 2000).

Fourteen multiparous Holstein cows (644 ± 16.8 kg of BW) with 323 ± 21.6 DIM producing 16.8 ± 0.91 kg of milk at dry-off were randomly assigned to 1 of 2 treatments balanced for milk production, age, BW, lactation rank, pregnancy status, DIM and the 3 last SCC measured at d −13, −10, and −7. The cows were housed in individual tie-stalls at the INRA Méjusseaume experimental dairy farm, UMR1348 PEGASE (Le Rheu, France). Before drying-off, the cows were milked twice daily at 0730 and 1630 h. All cows were treated with an intramammary antibiotic (Cobactan, Intervet, Beaucouzé, France) after the last milking (at 0730 h on d 0). The cows were surgically equipped with a silastic permanent catheter (Silclear medical-grade silicone tubing, i.d. 1.02 mm, o.d. 2.16 mm; Degania Silicone, Degania Bet, Israel) inserted in the jugular vein the day before the first blood sampling and kept there for the duration of the study.

The treatment consisted of a single aseptic 5 mL i.m. administration in the neck region of a solution containing 5.6 mg of cabergoline (Velactis, Ceva Santé Animale, Libourne, France; CAB) or an i.m. injection of 5 mL of the same excipient used in CAB as a placebo (control) within 4 h after the last milking before drying-off (d 0). The cows were fed according to INRA guidelines as previously described (Boutinaud et al., 2016).

Blood Sample Collection and Analyses

Blood samples were collected in the morning at 0720 h, before feeding. Blood samples were collected at d −6, 1, 2, 3, 4, 8, and 14 to determine lactose concentrations. Plasma was collected from heparin-coated tubes (Sarstedt, Nümbrecht, Germany) for lactose determination. All plasma samples were separated by centrifugation at 4°C and 2,264 × *g* for 15 min and then stored at −20°C for subsequent analysis. Plasma concentrations of lactose were measured on a multiparameter analyzer