2009

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Recommended Citation
DOI: https://doi.org/10.31274/ans_air-180814-951
Available at: https://lib.dr.iastate.edu/ans_air/vol655/iss1/55
Activation of Vitamin D$_3$ in Bovine Mastitis Caused by *Streptococcus uberis*

A.S. Leaflet R2432

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Summary and Implications

Inflamed mammary tissue of three cows infected with *Streptococcus uberis* was found to have higher concentrations of 1α-hydroxylase than un-infamed control mammary glands. Increased levels of 1α-hydroxylase resulted in increased production of 1,25-dihydroxyvitamin D$_3$. Therefore, vitamin D$_3$ may have a role in the inflammation and resolution of bovine mastitis.

Introduction

*Streptococcus uberis*  
*S. uberis* is among the most common bacterial pathogens that cause clinical mastitis in dairy cows. Intramammary infections caused by *S. uberis* leads to an inflammatory response that includes increased cytokine expression and infiltration of immune cells. The inflammatory response functions to clear the mammary gland of infection.

*Vitamin D*  
Vitamin D$_3$ can be acquired in the skin by radiation from UVB light or in the diet and is readily converted to 25(OH)D$_3$ in the liver. The substrate for 1α-hydroxylase is 25(OH)D$_3$, which is converted to the active steroid hormone, 1,25(OH)$_2$D$_3$. Meanwhile, the vitamin D receptor (VDR) is activated upon binding of the active hormone. Activated VDR functions as a transcription factor by binding vitamin D response elements (VDRE) in promoters of vitamin D responsive genes.

Materials and Methods

**Intramammary Infection**  
Three mid-lactation Holstein cows were infused with 250 CFU of *S. uberis* in one quarter. The adjacent quarter was infused with phosphate buffered saline as a control. Bacteria were not detected in milk from any of the quarters prior to infection. Seventy-two hours after infection the cows were euthanized and tissue from three separate sites in the control and infected glands was collected.

**mRNA Quantification**  
Total RNA from mammary tissue was isolated and mRNA was reverse transcribed to cDNA. Quantitative real-time PCR using the $2^{-ΔΔCt}$ method was used to measure relative abundance of interleukin 8 (IL-8), 1α-hydroxylase and 24-hydroxylase cDNA. Ribosomal protein S9 (RPS9) was used as the reference gene.

Results and Discussion

Seventy-two hours after infection with *S. uberis*, all three cows developed clinical signs of mastitis. *S. uberis* was detected in milk from the infected mammary glands but not in milk from the control glands. Also, IL-8 mRNA was elevated in mammary tissue from the infected gland compared to mammary tissue from the control gland (p < 0.01) (figure 1). IL-8 is a chemokine that is expressed in milk and mammary tissue of inflamed mammary glands and is used here to verify activation of pro-inflammatory genes in the infected mammary glands.

![Figure 1](image-url). Relative expression of interleukin-8 (IL-8) mRNA in mammary tissue from three separate sites in control and infected mammary glands of three cows at 72 hours after infection with *S. uberis*. IL-8 was measured by quantitative RT-PCR and normalized to RPS9 gene expression.

The relative amount of 1α-hydroxylase mRNA in control and infected mammary tissue was measured and was found to be much higher in the infected mammary gland (p < 0.01) (figure 2). Production of 1,25(OH)$_2$D$_3$ in the infected mammary gland should be higher then since 1α-hydroxylase is the enzyme that converts 25(OH)D$_3$ to 1,25(OH)$_2$D$_3$. A marker of 1,25(OH)$_2$D$_3$ production is 24-hydroxylase expression. Expression of 24-hydroxylase is increased by 1,25(OH)$_2$D$_3$ via a VDRE in the 24-hydroxylase gene promoter. Therefore, it is shown that production of 1,25(OH)$_2$D$_3$ was higher in the infected mammary glands versus the control mammary glands by...
using 24-hydroxylase mRNA as a marker of 1,25(OH)2D3 production (p < 0.01) (figure 3).

Figure 2. Relative expression of 1α−hydroxylase (1α-OHase) mRNA in mammary tissue from three separate sites in control λ and infected σ mammary glands of three cows at 72 hours after infection with S. uberis. 1α-OHase was measured by quantitative RT-PCR and normalized to RPS9 gene expression.

In conclusion, we have found that vitamin D is activated in inflamed mammary tissue via the expression and activity of 1α-hydroxylase. The definite role of vitamin D in mastitis and the immune response of dairy cattle in general is not yet known. However, vitamin D is known as an anti-inflammatory hormone; so, it may be involved in regulating the inflammatory response in cattle. Vitamin D has also been shown to enhance bactericidal activity in human macrophages; so, it may also be involved in the resolution of bacterial infections in cattle. Therefore, studies regarding the role of vitamin D in mastitis are underway to better understand the fundamental mechanisms of its regulation of the bovine immune system.

Figure 3. Relative expression of 24-hydroxylase (24-OHase) mRNA in mammary tissue from three separate sites in control λ and infected σ mammary glands of three cows at 72 hours after infection with S. uberis. 24-OHase gene expression serves as a marker for 1,25(OH)2D3 production in inflamed mammary tissue. 24-OHase was measured by quantitative RT-PCR and normalized to RPS9 gene expression.