Sequential changes in serum iron concentration and hepatic hepcidin mRNA expression in LPS-challenged calves

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Introduction

Inflammatory diseases caused by lipopolysaccharide (LPS) are the most frequently observed diseases in dairy and feedlot cows. The acute-phase response to LPS may regulate transcription factors and the release of pro-inflammatory cytokines, including IL-6 and hepcidin. Iron is known to play roles in many enzymatic activities, and is an essential trace element for the host and pathogen. Previous reports demonstrated that serum Fe levels decrease in inflammatory diseases due to intestinal absorption and reduction of the release of Fe by reticuloendothelial cells.

In this study, we measured sequential changes in the serum Fe concentration in calves after LPS challenge in order to clarify whether the serum Fe concentration is useful as a marker of inflammation. We also examined sequential changes in mRNA levels of IL-6 and hepcidin to evaluate the inflammatory response.

Materials and Methods

Five female and 5 male Holstein calves, aged 176.8±23.7 days old and weighing 394 ± 73.4 lb (178.7±33.3 kg), were enrolled in this study. These calves were divided into the LPS group and control group (n=5 each group). The calves in the LPS group each received 2.5 µg/kg of BW ultrapure O111:B4 LPS in 10 ml of autologous serum via a catheter in the jugular vein, whereas the control calves received a similar volume of saline. Blood samples were collected from the contralateral jugular vein before the endotoxin challenge, and at 0.5, 1, 2, 4, 8, 12, 24, and 48 hours after administration, and were stored in tubes containing EDTA or heparinized tubes. Liver samples were simultaneously collected by ultrasound-guided liver biopsy using a True-Cut needle. Plasma endotoxin activity was measured by the limulus amebocyte lysate kinetic turbidimetric assay (LAL-KTA). Serum iron concentrations were measured by the 2-nitroso-5-(N-propyl-N-sulphopropylamino) phenol (nitroso-PSAP) method using an auto-analyzer (LABOSPEC 003, Hitachi Medical Co., Japan) at 753 nm with a commercial kit. The mRNA expression levels of hepcidin and IL-6 in the liver samples from endotoxin-challenged calves were measured using real-time PCR. Reverse transcription and real-time PCR were carried out in 1 step using commercial kits. GAPDH was used as an endogenous control.

Normally distributed data were reported as the mean ± standard deviation (SD), and non-normally distributed data were expressed as the median and range. For non-normally distributed data, such as endotoxin activity and iron concentration, the Mann-Whitney U test was employed for comparison between groups. The mean values for each dependent variable were compared with the pre values using Dunnett's t-test after 2- way ANOVA as the F-test.

Results

The endotoxin activity in the plasma was significantly increased, peaking (0.425±0.251 EU/ml) at 15 min after the LPS challenge ($p<0.001$), and then returning to the baseline level at 4 hr after administration. The infusion of 2.5-µg/kg O111B4 LPS induced significant and progressive increases in the plasma Fe concentration, reaching 210.2±62.7 µg/dl at 4 hr after endotoxin challenge. The Fe concentration in plasma then significantly decreased, reaching 47.7±29.4 µg/dl at 24 hrs after endotoxin challenge ($p<0.001$). Significantly low plasma Fe concentrations in calves that received endotoxin were maintained from 12 to 48 hrs after the challenge, as compared with the pre-values ($p<0.001$). In the hepatic tissues, significantly high expression of IL-6 and hepcidin mRNA in calves that received endotoxin were maintained from 2 to 8 hr after the challenge as compared with the pre-values ($p<0.001$).

Significance

We found that IL-6 and hepcidin mRNA levels increased in the liver. Furthermore, the Fe level decreased in calves with LPS. Therefore, our study demonstrated that the serum Fe concentration is useful as a marker of inflammation in calves with inflammatory diseases associated with LPS.