Effects of Growth Hormone Releasing Hormone Delivered by Plasmid Injection and Electroporation on the Immune Function and Body Condition Scores in Holstein Heifers

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Introduction

Novel DNA-based technologies were recently introduced for various purposes, including to direct the *in vivo* production of hormones and other peptides for therapeutic or preventative applications. Growth hormone releasing hormone (GHRH) is a hypothalamic hormone with both direct and indirect effects on the maintenance of health and immune status under an array of conditions. Our previous studies have shown that delivery of optimized plasmids by intramuscular injection followed by electroporation is scalable and represents a promising approach for long-term stable production of GHRH in pigs and dogs. We have now tested this approach to optimize GHRH levels in Holstein heifers, and characterized the effects of the therapy on the immune and health status of the treated subjects and controls.

Materials and Methods

Thirty-two primiparous Holstein heifers, 18-20 months of age with an average weight of 1,203 ± 95 lb (547 ± 43 kg) received 2.5 mg pSP-HV-GHRH once during the third trimester of gestation. The heifers were injected in the trapezius muscle with a 21-gauge needle, and two minutes after injection the muscle was electroporated. Twenty pregnant heifers from the same source and of the same breed and age remained untreated and served as controls. Animals calved at age 23 months ± 24 days. Blood samples were taken for complete blood count (CBC) prior to treatment, and days 18 and 300 post-treatment. Samples for clinical chemistry, insulin measurements and IGF-I were collected at 0, 60 and 100 days in milk (DIM). Samples for immune markers were taken at days 0 and 18 post-treatment, and a subset of 20 treated and 10 controls were sampled at day 300 post-treatment. The immune marker analysis was performed using monoclonal antibodies developed in Dr. Davis's laboratory. Body condition scores (BCS) were assessed by two independent dairy scientists (blinded to treatment groups) prior to treatment, between 60 and 80 DIM, and between 100 and 120 DIM.

Results

No adverse effects were associated with either the plasmid delivery or GHRH expression. Compared to controls, GHRH-treated animals had increased numbers of CD2+ab T-cells by 14% ($P < 0.004$), increased CD25+CD4+ cells ($P < 0.001$), and CD4+CD45R+ cells by 53% ($P < 0.016$). These increases were maintained long-term after treatment and correlated with plasmid expression. At 300 days post-GHRH therapy, CD45R+/CD45R0- naïve lymphocytes were significantly increased in frequency. Natural killer lymphocytes were also increased. As a consequence of improved health status, body condition scores of treated animals improved significantly, hoof pathology was reduced with treatment, and morbidity and mortality of treated heifers was decreased. During the 360-day study, none of the treated heifers died, while 20% of control heifers had to be culled ($P < 0.003$).

Significance

Collectively, these results indicate that the myogenic GHRH plasmid can be successfully transferred into a 1100 lb (500 kg) mammal and expressed for prolonged periods of time, ensuring physiological levels of GHRH. The plasmid-mediated supplementation could prove an efficient method for the systemic production of therapeutic proteins and may provide a useful means for basic research in relevant animal models.