EFFECTS OF MELENGESTEROL ACETATE ON INFLAMMATORY RESPONSE DURING *MANNHEIMIA HAEMOLYTICA* CHALLENGE

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Summary

Previous trials conducted at Kansas State University demonstrated that melengesterol acetate (MGA) increased growth rates and tended to reduce chronic sickness in heifers naturally challenged with undifferentiated bovine respiratory disease. Our study was conducted to gain further insight into the mode of action of MGA. Crossbred heifers (n=47; 511 lb) were used to evaluate effects of MGA on lung pathology and markers of inflammation in cattle after an intrabronchial *Mannheimia haemolytica* challenge. On day 0, cattle were assigned to diets (54% concentrate) that provided 0 or 0.5 mg MGA per heifer daily. On day 14 each heifer was intrabronchially inoculated with *M. haemolytica*. Blood samples were collected from each heifer immediately before inoculation and 12, 24, 48, 72, 96, 120, and 138 hours after inoculation. Heifers were then euthanized for postmortem examination. After the challenge, heifers fed MGA had greater numbers of neutrophils and white blood cells, as well as greater serum haptoglobin and fibrinogen concentrations. The incidence of post-challenge lung lesions was greater in heifers fed MGA, and lung lesion scores tended to be more severe in heifers fed MGA, compared with those of controls. These data indicate that MGA does not reduce inflammation in heifers 138 hours after *M. haemolytica* challenge, suggesting that there are other modes of action for the beneficial effects on growth and reduction of chronicity in feedlot heifers.

Introduction

Undifferentiated bovine respiratory disease is the most costly disease plaguing the beef industry today. Roughly 75% of sickness and 50% of death losses in feedlot cattle are attributable to bovine respiratory disease, with an estimated annual cost of $1 billion. Bovine respiratory disease is a multifactorial complex that is influenced by viral infection, weaning, transportation, commingling, and temperature extremes. Stress, coupled with a primary viral infection, can allow bacteria often found in the upper respiratory tract of healthy animals to proliferate in the lower respiratory tract. *Mannheimia haemolytica* is generally considered the most virulent and important pathogen associated with severe respiratory disease in feedlot cattle, inducing severe pneumonia characterized by local production of many inflammatory mediators and the influx and activation of inflammatory cells. The first inflammatory cells to arrive are neutrophils, which accumulate at the site of infection in response to proteins called cytokines. Pro-inflammatory cytokines, which include tumor necrosis factor-alpha (TNF-α), activate the endothelium, causing it to express receptors (called cell-adhesion molecules) for inflammatory cells. These receptors allow inflammatory cells to attach to the endothelium and emigrate out of the blood and into tissue spaces. Overly activated inflammatory cells cause damage to the lung, and prolonged inflammation can lead to extensive and irreparable damage, leaving these areas of the lungs non-functional.

Previous research from our laboratory indicated that MGA, a synthetic progestin commonly used to suppress estrous in feedlot cattle, improved growth rates and reduced chronic sickness in heifers naturally challenged with respiratory disease. Similar compounds have also been shown to alter inflammation in other animal species. Our study was conducted to evaluate the effects of MGA on biological markers of inflammation and lung-tissue damage in heifers experimentally infected with *M. haemolytica*.

Procedures

Forty-eight crossbred heifers were purchased from a local sale barn and transported to the K-State Beef Cattle Research Center in Manhattan, Kansas. No antibiotics or vaccines were administered to the calves any time after arrival to avoid interfering with progression of the experimental infection, and heifers were acclimated to the facility for a period of 1 to 5 weeks before being placed on the study. On day 0 of the experiment, calves were stratified by weight and randomly assigned, within strata, to one of two diets formulated to provide 54% concentrate:46% roughage and either 0 or 0.5 mg MGA per heifer daily. On day 14, all heifers were weighed and *M. haemolytica* was intrabronchially inoculated into the lungs. Blood samples were collected immediately before inoculation and 12, 24, 48, 72, 96, 120, and 138 hours after inoculation. After the final blood collection, calves were euthanized and transported to the KSU College of Veterinary Medicine Diagnostic Lab for necropsy. Each animal was given a lung-lesion score based on the percentage of each lung lobe affected by lesions according to the formula: total calculated lung-lesion score = [(left cranial % x 0.05) + (left posterior cranial % x 0.06) + (left caudal % x 0.32) + (right cranial % x 0.06) + (right posterior cranial % x 0.05) + (right middle % x 0.07) + (right caudal % x 0.35) + (intermediate % x 0.04)]. Complete blood cell counts were performed on smear slides.

One heifer in the MGA treatment group died between 48 and 72 hours after inoculation, and data from this heifer were excluded from the statistical analysis. This heifer had severe pneumonia, which was considered to be the cause of death.

Results and Discussion

All heifers in this study exhibited mild signs of respiratory disease after challenge. Heifers that did not receive MGA had elevated circulating white blood cells at 12 and 24 hours after the inoculation, but the increase was smaller than that observed for heifers fed MGA ($P<0.01$, Figure 1). This rise in white blood cells was due in large part to an increase in circulating neutrophils at 12 and 24 hours after the challenge, with MGA leading to a greater increase in circulating neutrophils ($P<0.01$, Figure 2). These findings are consistent with an earlier trial in which MGA caused increased numbers of circulating neutrophils in heifers 4 hours after injection with *E. coli* lipopolysaccharide. Progesterone and synthetic progestins, in some animal species, reduce endothelial expression of cell-adhesion molecules. We speculate that the larger number of circulating neutrophils in cattle fed MGA could be caused by a decrease in endothelial cell-adhesion molecules, thus decreasing subsequent neutrophil emigration into the lung tissue. This reduced influx of neutrophils early after challenge in heifers fed MGA may have allowed bacteria to proliferate more readily in the lung, potentially explaining why MGA increased incidence of lung lesions (60.9% vs. 25.0%; $P<0.02$) and tended to increase severity of lung lesions (lung-lesion score: 3.08% vs. 1.04%; $P=0.06$: Table 1) 138 hours post-inoculation. The serum TNF-α concentrations were marginally higher ($P=0.13$) for heifers fed no MGA (Figure 3). Increased serum TNF-α would be expected after *M. haemolytica* challenge, but no post-challenge increase in TNF-α was detected. Possible explanations of this include a low virulence of the bacteria used, as evidenced by

the limited severity of lung lesions, or the challenge may have been insufficient to stimulate higher systemic concentrations of TNF-α. The lack of an effect of MGA on serum TNF-α does not rule out the possibility that MGA decreased secretion of TNF-α or other pro-inflammatory cytokines by blood cells in the lung, but this cannot be determined from our study because concentrations of TNF-α in the lungs were not measured.

Concentrations of the acute-phase proteins (fibrinogen and haptoglobin) were elevated in both treatment groups after inoculation. However, concentrations of fibrinogen (Figure 4) and haptoglobin (Figure 5) were not greater in cattle fed MGA, compared with controls, until 72 and 96 hours after inoculation, respectively. These data further support the contention that the bacterial pathogens proliferated more readily in the lungs of heifers fed MGA, thus causing a more severe acute-phase response later in the challenge period.

Chronic inflammation is the result of an exaggerated inflammatory response that is often characterized as a reaction to products produced during the inflammatory response rather than to the initial pathogen. Anti-inflammatory agents have been shown to be useful in attenuating chronic inflammation, but are not generally accepted as beneficial in bacterial infections or low-severity inflammation because they hinder the body's ability to clear the pathogen. Heifers in our study exhibited mild clinical signs of respiratory disease after challenge. The bacteria used had a limited ability to initiate an inflammatory response, and it is possible that this played a role in the greater incidence of lesions in heifers fed MGA. The beneficial effects of MGA observed in a previous trial may have been due to greater incidence and severity of the natural disease challenge. Heifers in the previous trial experienced a morbidity rate of 75.6% and a mortality rate of 9.9%.

MGA increased incidence and severity of lung lesions in heifers subjected to a mild experimental challenge with *M. haemolytica*. Results of our study suggest that the previously observed improvements in growth and reduction of chronicity in heifers with more severe, naturally occurring, undifferentiated respiratory disease was not due to an attenuation of the inflammatory response to *M. haemolytica*. Further studies need to be conducted to examine the effects of MGA on respiratory disease in cattle.

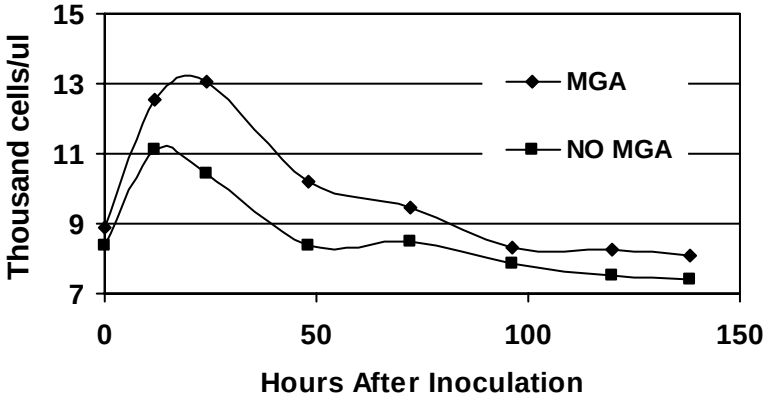

Figure 1. Circulating White Blood Cell Concentration of Heifers Fed Either 0 or 0.5 mg of MGA Daily for 20 Days and Inoculated on Day 14 with *M. haemolytica*. Effect of treatment, P<0.01; effect of sampling time, P<0.01; effect of interaction between treatment and sampling time, P=0.80.

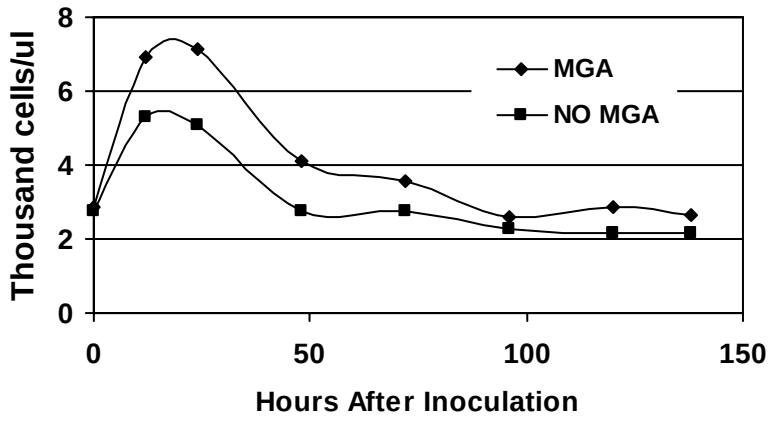

Figure 2. Circulating Mature Neutrophils of Heifers Fed Either 0 or 0.5 mg of MGA Daily for 20 Days and Inoculated on Day 14 with *M. haemolytica*. Effect of treatment, P<0.01; effect of sampling time, P<0.01; effect of interaction between treatment and sampling time, P=0.61.

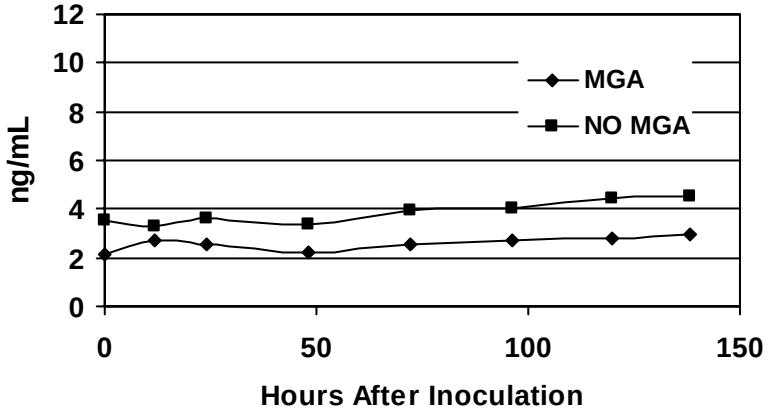

Figure 3. Serum TNF-α of Heifers Fed Either 0 or 0.5 mg of MGA Daily for 20 Days and Inoculated on Day 14 with *M. haemolytica*. Effect of treatment, P=0.13; effect of sampling time, P=1.00; effect of interaction between treatment and sampling time, P=1.00.

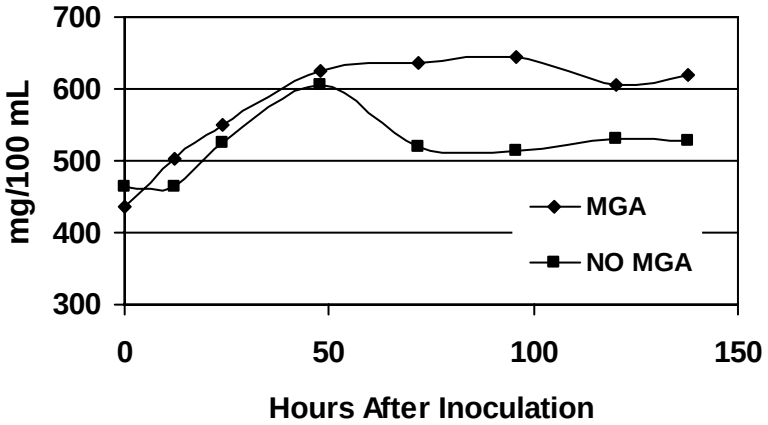

Figure 4. Circulating Fibrinogen of Heifers Fed Either 0 or 0.5 mg of MGA Daily for 20 Days and Inoculated on Day 14 with *M. haemolytica*. Effect of treatment, P<0.01; effect of sampling time, P<0.01; effect of interaction between treatment and sampling time, P=0.20.

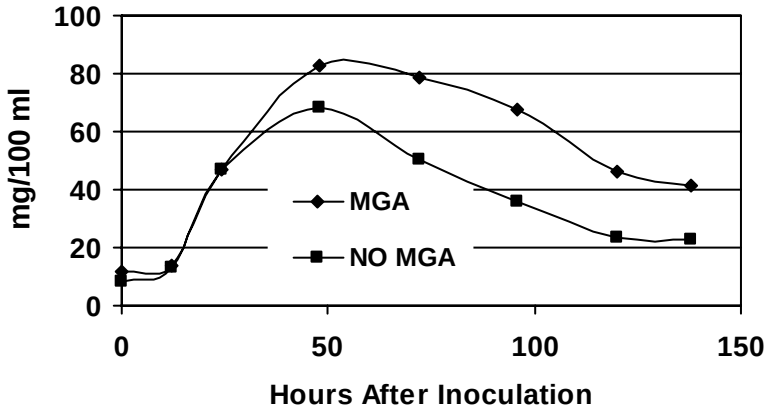

Figure 5. Serum Haptoglobin of Heifers Fed Either 0 or 0.5 mg of MGA Daily for 20 Days and Inoculated on Day 14 with *M. haemolytica*. Effect of treatment, P<0.01; effect of sampling time, P<0.01; effect of interaction between treatment and sampling time, P=0.56.

Table 1. Average Lung Scores 138 Hours After Inoculation with *M. haemolytica* for Heifers Fed 0 or 0.5 mg of MGA

	MGA, mg/day		
Item	0.5	0	P-value
Number of heifers	23	24	-
Average score[a]	3.08	1.04	<0.06

[a]Total calculated percentage lung-lesion score = [(left cranial % x 0.05) + (left posterior cranial % x 0.06) + (left caudal % x 0.32) + (right cranial % x 0.06) + (right posterior cranial % x 0.05) + (right middle % x 0.07) + (right caudal % x 0.35) + (intermediate % x 0.04)].