Bovine myeloid antimicrobial peptide-28 (BMAP-28) mRNA expression by bovine cells and effects of synthetic MAP-28 on bovine respiratory disease pathogens

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Introduction

Mannheimia haemolytica (MH) is the principal bacterial pathogen associated with bovine respiratory disease (BRD) in beef cattle. Bovine Herpes virus type 1 (BHV-1) can cause BRD by itself or it can predispose cattle to BRD due to MH or other opportunistic bacteria. Existing antimicrobials do not consistently prevent BRD due to MH, and do not have an effect against viruses; bovine antimicrobial peptides (AMP) have immune stimulating and nonspecific antimicrobial effects that could improve BRD control. Messenger RNA (mRNA) treatment could be used to induce AMP expression in cattle, but efficacy must first be confirmed in vitro. Synthetic AMP can be generated to use as standards when characterizing mRNA-expressed AMP. We hypothesized that bovine cells can express synthetic mRNA coding for the AMP BMAP-28 and that synthetic BMAP-28 can inhibit the growth of MH and elicit antiviral effects against BHV-1 virus.

Materials and methods

Madin-Darby bovine kidney cells were cultured and transfected with mRNA coding for BMAP-28 linked to the reporter nanoluciferase. After 4, 12, 24, 48 and 72 hrs, relative light units (RLU) and protein concentration were measured. Results were expressed as RLU/µg of protein. MH at 500 cfu/ml and synthetic BMAP-28 at 10 or 100 µg/ml were incubated for 0, 12 or 24 hrs in a shaker incubator and quantitative culture was performed. BHV-1 at 103 and 104 IU/ml were treated with synthetic BMAP-28 at 10 or 100 µg/ml and incubated at 37°C for 2 hr. A TCID50 assay on Madin-Darby bovine kidney cells was performed for each treatment. TCID50 units were calculated after 5 days post infection (dpi). The Effect of BMAP-28 on MH was tested in 2 trials using a mixed procedure with trial as a random effect and the effect of BMAP-28 on BHV-1 was tested using a Kruskal-Wallis test.

Results

Bovine cells expressed synthetic mRNA coding for BMAP-28 with peak expression occurring at 24 hr in cell lysates and supernatants. Synthetic BMAP-28 at 10µg/ml and 100 µg/ml inhibited MH growth at 12 and 24hr post-treatment ($P < 0.0001$). Synthetic BMAP-28 at 100 µg/ml killed BHV-1 at both viral concentrations ($P = 0.0085$).

Significance

Treatment of bovine cells with synthetic mRNA induces BMAP-28 expression in vitro. BMAP-28 can significantly inhibit MH growth and BHV-1 replication. These results provide support for further research to test the mRNA expressed AMP against BRD pathogens in vitro and in vivo. mRNA treatment to induce AMP expression could lead to new BRD control strategies.

