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Efficacy of Oral Administration of Sodium Iodide to Prevent Bovine Respiratory Disease Complex

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Background: The prevention of bovine respiratory disease complex (BRD) in beef cattle is important to maintaining health and productivity of calves in feedlot operations. Objective: Determine whether BRD bacterial and viral pathogens are susceptible to the lactoperoxidase/hydrogen peroxide/iodide (LPO/H\(_2\)O\(_2\))/I\(_2\) system in vitro and to determine whether the oral administration of sodium iodide (NaI) could achieve sufficient concentrations of iodine (I) in the respiratory secretions of weaned beef calves to inactivate these pathogens in vivo.

Animals: Sixteen weaned, apparently healthy, commercial beef calves from the University of Missouri, College of Veterinary Medicine teaching herd.

Methods: In vitro viral and bacterial assays were performed to determine susceptibility to the LPO/H\(_2\)O\(_2\)/I\(_2\) system at varying concentrations of NaI. Sixteen randomly selected, healthy crossbred beef weanlings were administered 70 mg/kg NaI, or water, orally in a blinded, placebo-controlled trial. Blood and nasal secretions were collected for 72 hours and analyzed for I concentration.

Results: Bovine herpesvirus-1, parainfluenza-3, Mannheimia haemolytica and Bibersteinia trehalosi were all inactivated or inhibited in vitro by the LPO/H\(_2\)O\(_2\)/I\(_2\) reaction. Oral administration of NaI caused a marked increase in nasal fluid I concentration with a C\(_{\text{max}}\) = 181 (1.420 \(\mu\)M I), T\(_{12}\), a sufficient concentration to inactivate these pathogens in vitro.

Conclusions and Clinical Importance: In vitro, the LPO/H\(_2\)O\(_2\)/I\(_2\) system inactivates and inhibits common pathogens associated with BRD. The administration of oral NaI significantly increases the I concentration of nasal fluid indicating that this system might be useful in preventing bovine respiratory infections.

Key words: Hypoiodous acid; Lactoperoxidase; Bovine respiratory disease; cattle.

Bovine respiratory disease complex (BRD) is a multifactorial disease process that is currently thought to be initiated by a stressful event, such as transport and commingling of cattle, which results in immune...
compromise and reduced resistance to infection. Commingling of cattle also increases exposure to respiratory pathogens. Subsequent viral infections compromise respiratory defenses that normally limit the migration of pharyngeal flora into the lower respiratory tract. Bacterial bronchopneumonia of the lungs causes pulmonary damage compromising animal health and production. Methods to decrease BRD and BRD-associated losses include mitigating stress, preventing viral and bacterial infections, and antimicrobial treatment of microorganisms. While these methodologies can reduce BRD, they are often unsuccessful and require further antibiotic treatment of the bacterial bronchopneumonia.1 Concerns regarding antibiotic use continue to increase, and alternative means of maintaining animal health and welfare will be required as antibiotic use practices evolve.

A novel approach to preventing and treating BRD infections might be naturally present within the respiratory system. The upper respiratory tract utilizes innate defense mechanisms to protect the respiratory system. One component is an oxidative antimicrobial system in the airway surface liquid (ASL), composed of hydrogen peroxide (H2O2) produced by dual oxidase enzymes (DUOX), lactoperoxidase (LPO) from epithelial submucosal glands, and a halide/ pseudohalide ion.2 Lactoperoxidase (DUOX), lactoperoxidase (LPO) from epithelial submucous acid has potent antimicrobial activity.2 In in vitro conditions high enough to achieve effective in vivo antimicrobial treatment of the bacterial bronchopneumonia.2,4 A recent discovery in sheep required for this reaction are not present equally in all species. The upper respiratory tract utilizes innate defenses that normally limit the migration of pathogens. Subsequent viral infections compromise respiratory defenses that normally limit the migration of pharyngeal flora into the lower respiratory tract. Bacterial bronchopneumonia of the lungs causes pulmonary damage compromising animal health and production. Methods to decrease BRD and BRD-associated losses include mitigating stress, preventing viral and bacterial infections, and antimicrobial treatment of microorganisms. While these methodologies can reduce BRD, they are often unsuccessful and require further antibiotic treatment of the bacterial bronchopneumonia.1 Concerns regarding antibiotic use continue to increase, and alternative means of maintaining animal health and welfare will be required as antibiotic use practices evolve.

A novel approach to preventing and treating BRD infections might be naturally present within the respiratory system. The upper respiratory tract utilizes innate defense mechanisms to protect the respiratory system. One component is an oxidative antimicrobial system in the airway surface liquid (ASL), composed of hydrogen peroxide (H2O2) produced by dual oxidase enzymes (DUOX), lactoperoxidase (LPO) from epithelial submucosal glands, and a halide/pseudohalide ion.2 Lactoperoxidase catalyzes a reaction of H2O2 with thiocyanate (SCN–) in the ASL at concentrations because serum SCN– concentration is relatively greater than serum I concentration; therefore, OSCN– is preferentially formed. When I is supplemented as a single, high-dose bolus, iodide (I–) secretion in the upper respiratory tract increases. As I– concentration increases in the ASL, SCN– concentration decreases, indicating that I– and SCN– compete for secretion into the respiratory tract.2,3,5–7 Hypoiodous acid has potent antimicrobial activity.2 In in vitro models, increasing I– concentration in the LPO/H2O2/I– system to 500 μM results in a significant reduction in viral pathogens.6 The administration of oral potassium iodide (KI) resulted in ASL I– concentration of 500 μM in humans. Thus, the oral administration of KI was capable of providing I– in the ASL at concentrations high enough to achieve effective in vivo antimicrobial activity.6 The objectives of the presented study were to determine whether the selected BRD pathogens were susceptible to the oxidative antimicrobial system composed of LPO/H2O2/I– in vitro and determine whether the oral administration of NaI could achieve concentrations of I– in the respiratory secretions of weaned beef calves equivalent to those required to inactivate these pathogens in vivo.

Materials and Methods

Viral In Vitro Susceptibility

The in vitro susceptibilities of BPI3V, BoHV1, and BVDV to the LPO/H2O2/I– reaction were tested using the following procedure. All assays were performed in triplicate. The viral strains used were TVMD60, Colorado, and 125C, respectively. The titer of the stock virus for BPI3V and BoHV1 was 3.5 × 108 50% cell culture infective dose (CCID50), and the beginning titer for the BVDV stock concentration was 5.2 × 107 CCID50. In a 24-well plate, minimum essential media were added (720–860 μL) to each reaction well in the amount needed for a total reaction volume of 1,000 μL. Test assays were performed as previously described in the following manner.8 NaI was added to each well to achieve a final concentration of 0, 10, 100, or 250 μM NaI. Subsequently, 20 μg of LPO diluted to a concentration of 1 μg/μL in distilled water was added to each well followed by the addition of 100 μL of the appropriate stock virus solution. Finally, H2O2 was added to achieve a final concentration of 100 μM. Each viral assay was performed on a separate 24-well plate. Control reactions for LPO, H2O2, and NaI individually, as well as negative and positive control wells, were included on each plate. Plates were incubated at 37°C for 5, 15, or 60 minutes and then frozen at –80°C for a minimum of 48 hours. Following a thaw cycle, virus titration of each well was performed by direct observation of cytopathic effect in cultured cells as previously described.8

Bacterial In Vitro Susceptibility

Bacterial isolates of Mannheimia haemolytica and Bibersteinia trehalosi were obtained from clinical bovine pneumonia cases submitted to the California Animal Health and Food Safety Laboratory for necropsy. M. haemolytica and B. trehalosi were isolated from submitted necropsy samples and replicated in vitro. Attempts were made to assess P. multocida but it was not amenable to replication or consistent growth and thus was not utilized in this study. Isolates were identified via traditional biochemical tests and confirmed via matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. Identified isolates were stored at –80°C in glycerol broth. For each experiment, an aliquot of each isolate was subcultured to brain heart infusion agar (BHI) and incubated overnight at 37°C. Isolated colonies from the BHI agar plates were inoculated into 5 mL of BHI media and incubated at 37°C, and 250 rpm until log-phase growth was achieved. Colony forming units (CFU) per mL of culture media were estimated by optical density reading at 600 nm wave length, and media were diluted to the desired concentration of 105 CFU/mL for experimental treatments.

Experiments were carried out in deep well, 96-well plates. Reagents were filter sterilized with 0.22-μm filter. Experimental conditions were different than those of the viral assays and included: NaI at concentrations of 50, 100, 250, and 500 μM, LPO (200 μg/mL), bacteria (106 CFU/mL), and H2O2 (100 μM). Control conditions included: NaI at concentrations stated above (50, 100, 250, and 500 μM) with bacteria alone; LPO with bacteria alone; H2O2 with bacteria alone; phosphate-buffered saline (PBS) with bacteria alone; and a negative control with all reagents but without bacteria. A prereaction aliquot was taken for colony count determination before adding H2O2 (starting titer); additional aliquots were collected 5 minutes (T5), and 30 minutes (T30) after adding H2O2. Colony counts were determined using 10-fold serial dilutions of each aliquot in sterile PBS, which were plated on BHI media and incubated for 48 hours.
agar, incubated overnight at 37°C, and quantified to calculate CFU/mL. Experiments were carried out on each isolate in duplicate and on 2 separate days.

**Oral Sodium Iodide**

*Animals*

Sixteen weaned commercial beef calves (average weight 270.5 kg ± 18.2 kg) were selected from the University of Missouri, College of Veterinary Medicine teaching herd to be included in the randomized, blinded, placebo-controlled study. The group consisted of 4 steers and 12 heifers. The calves were housed at the College of Veterinary Medicine Middlebush Teaching Farm and sample collection occurred through standard cattle processing facilities on site. The calves were fed, watered, and cared for in accordance with standard farm protocols. Animal use and methods were approved by the University of Missouri Animal Care and Use Committee, protocol number 8207.

Calves were divided into 2 groups of 8 animals each, control and treatment, by random number generator. The 2 groups were maintained in the same herd and pasture. Both primary investigators were blinded to treatment and control groups throughout the study period. The treatment group was administered a single dose of 70 mg/kg, 200 mg/mL (20%) NaIb by ororumen intubation.

The control group was administered an equivalent volume of water by ororumen intubation. Samples were collected immediately before administration of treatments, T₀, then every 12 hours for 72 hours. Samples collected included blood and nasal secretions. Basic physical examination consisting of temperature, heart rate, respiratory rate, rumen auscultation, and general visual inspection of the cattle was performed at each sample collection. The calves were observed for indications of iodine intoxication which included but are not limited to: salivation, diarrhea, excessive coughing, nasal discharge, lacrimation, epiphora, alopecia, dermatitis, scaly hair coats, and hyperthermia. Nasal fluid was collected from all calves by use of a modified 1 mL serologic pipette attached to a sample collection tube and vacuum pump, as illustrated in Figure 1. Samples were stored at −20°C until final processing on all samples could be performed. Dithiothreitol (DTT) was used to lyse the mucous component of the ASL. This allowed for optimal iodine recovery and successful sample analysis, similar to the method described by Popov et al. Blood samples were collected by jugular venipuncture into 10 mL serum separator vacutainer blood tubes. Blood samples were centrifuged at 1,500 × g for 30 minutes at 4°C. The serum was removed and stored at −20°C until analysis. The nasal fluid and serum were submitted to the Michigan State University Diagnostic Center for Population and Animal Health and analyzed by inductively coupled plasma mass spectrometry.

**Data Analysis**

In vitro viral and bacterial growth data were analyzed by 2-way nonparametric ANOVA for time and treatment, with post hoc testing. The concentration of iodine measured in blood and nasal secretions was analyzed by repeated measures ANOVA in which the dependent variable was iodine concentration, and the independent variable was the treatment group. Tukey's post hoc analysis was applied between groups and treatment time points.

**Results**

**Viral In Vitro Susceptibility**

Viral titers for BPI3V were significantly decreased (P = 0.001) in all reactions containing even the lowest concentration of NaI, Figure 2. The reaction containing 10 μM NaI decreased the average viral titer by 2 logs compared to the positive control wells; the 100 μM reaction further reduced the mean titer by an additional 2 logs. The virus was completely inhibited in all wells containing a NaI concentration of 250 μM regardless of the presence of LPO or H₂O₂. However, the differences in inhibition between the various NaI concentrations were not statistically different. For BoHV1, significant reduction in viral titer was seen at reactions containing >10 μM NaI (P < 0.001). At the 100 μM NaI concentration, the mean viral titer decreased 4 logs compared to the positive control wells, Figure 3. As with BPI3V, a NaI concentration of 250 μM completely inactivated the virus, regardless of the presence of LPO or H₂O₂. Of the 3 viruses, BVDV showed the least susceptibility to the system with no observed differences (P = 0.33) in titer at even the highest concentrations of NaI, Figure 4. Titers of BPI3V (P = 0.44), BoHV1 (P = 0.14), and BVDV (P = 0.65) were not affected by incubation time.

**Bacterial In Vitro Susceptibility**

At all concentrations of NaI in combination with LPO and H₂O₂, growth of both *M. haemolytica* and *B. trehalosi* was inhibited by the complete reaction by 5 minutes after the reaction was started (T₅), Figures 5 and 6. There was no significant difference between bacterial numbers at T₀ for any of the treatment conditions, aside from the negative control. A 2-way ANOVA of results for *M. haemolytica* was significant for time (P < 0.0001), treatment (P < 0.0001), and interaction of time and treatment (P = 0.0008). Post hoc pairwise comparisons revealed significantly decreased bacterial counts at T₅ in all 3 NaI concentrations (50, 100, and 500 μM) that included H₂O₂ and LPO compared to the positive control. Results for
**B. trehalosi** were similar; a 2-way ANOVA showed significant effect of time ($P < 0.0001$) and treatment ($P < 0.0001$); however, effect of interaction of time and treatment was not significant ($P = 0.69$). NaI alone at higher concentrations (100 and 500 μM NaI), as depicted by the solid bars. The control conditions, represented by the checked and dashed bars, include virus only, LPO only, H$_2$O$_2$ only, NaI (250 μM) only, and media only. Standard error bars presented. BPI3V was significantly inhibited at all concentrations of NaI but no effect of time was detected.

**In Vitro Sodium Iodide Animal Model**

**Serum Iodine Concentration**

Both groups had no significant difference in serum I concentrations at $T_0$ with an average of 0.04 mg/L (0.3 μM) and 95% confidence interval (CI) of 0.03–0.05 mg/L. After NaI administration, the serum I concentrations were significantly increased ($P < 0.05$) at each collection time point beginning with $T_{12}$ through the final collection time point of $T_{72}$ post-NaI administration, as depicted in Figure 7. The highest measured average serum I concentration at $T_{12}$ was 106 mg/L (834 μM) and CI of 92–120 mg/L; subsequently, serum I concentration steadily declined during the remainder of the study period. During the 72-hour study period, at each collection time point, the control group did not have a significant change in serum I concentration.

**Nasal Secretion Iodine Concentration**

Both groups had similar average I concentrations at $T_0$ in nasal secretions: control 0.915 mg/L (7.21 μM), CI of...
0.067–1.76 mg/L, and treatment 0.529 mg/L (4.17 μM), CI of 0.185–0.873 mg/L. The concentration of I in the nasal secretions was significantly increased \((P < 0.05)\) after the administration of a NaI oral bolus as demonstrated in Figure 8. At the T 12 sample collection time point, the treatment group had a significantly higher average I concentration of 181 mg/L (1,420 μM) and CI of 155–206 mg/L in the nasal secretions that remained significantly different throughout the 72-hour study period of the treatment group. The I concentration of nasal secretions steadily declined during the study period. The control group nasal secretion I concentration did not change throughout the study period.

**Physical Examination**

Physical examination findings for temperature and respiratory rates between the 2 study groups were not statistically different. At the T 0 and T 48, the heart rates of the control group were significantly greater than the treatment group, 166 beats per minute and 145 beats per minute, respectively. Throughout the study period, evidence of I toxicity was not observed during routine physical examinations and sample collection. Herd caretakers...
Discussion

The results of the experiments presented in this report demonstrate that the LPO/H₂O₂/I⁻ system inactivates BoHV1, BPI3V, M. haemolytica, and B. trehalosi in vitro. This study also demonstrated that weanling beef calves are capable of secreting I in the upper respiratory tract after a single bolus of oral NaI. While more investigation into the application of these findings is required, the use of a single oral bolus of NaI might be a new methodology in the prevention or mitigation of BRD.

The one pathogen that was not found to be susceptible to inactivation by the LPO/H₂O₂/I⁻ system was BVDV, which is a member of the Flaviviridae family and of the genus Pestivirus. It is a single-stranded RNA, nonsegmented, enveloped virus with 2 biotypes that can cause immunosuppression. The reason for this difference in susceptibility to the LPO/H₂O₂/I⁻ system was unknown. One consideration might be that BVDV is fairly hardy in the environment and is resistant to most disinfectants and environmental conditions. If this is the case, a higher concentration of NaI might be required to inactivate BVDV. The importance of BVDV’s lack of inactivation by the LPO/H₂O₂/I⁻ system will require further investigation.

Bovine respiratory syncytial virus (BRSV) is another important viral pathogen of BRD. Because of its similarity to human respiratory syncytial virus (hRSV), BRSV was not examined in this study. BRSV was not examined in vitro in this study because of the previous reports of inactivation of hRSV in both in vitro studies and in vivo challenge model in sheep of the LPO/H₂O₂/I⁻ system. In the in vivo challenge model, lambs administered KI had decreased gross pulmonary lesions, viral antigen concentration, and clinical signs of respiratory disease compared to controls. Human respiratory syncytial virus and BRSV are remarkably similar, to the extent that BRSV has been used as an animal model for hRSV studies. Another limitation of this study was that the calves utilized were not stressed or commingled immediately before the onset of the study period. Commingling and stress are known factors of BRD’s pathogenesis. Extrapolating these results to stressed and/or commingled calves might be premature.

This study determined that beef weanlings are capable of secreting I in the upper respiratory tract after a single bolus of oral NaI. The highest measured nasal I concentration was 181 mg/L (1,420 μM). Based on the in vitro data, this concentration of iodine could inactivate BoHV1 and BPI3V and inhibit M. haemolytica and B. trehalosi in vivo. These results are similar to those previously reported in both humans and sheep.

In previous studies, HOI was measured directly. In the present study, the presence of I was measured as inorganic I⁻ as a proxy measurement of HOI in the nasal secretions of calves, as previously reported. Hypoiodous acid is formed by the reaction of H₂O₂ and I⁻ (catalyzed by LPO oxidation) identically to OSCN⁻ formation. Compared to OSCN⁻, HOI is considerably virucidal as well as antibacterial. In ovine models, both respiratory syncytial virus and adenovirus were rapidly inactivated by HOI. The proposed mechanism of action of HOI is that it modifies the surface proteins of the virus. These changes can inhibit binding and/or entry of the virus into the host cells. This is achieved through the oxidation of sulfhydryl groups, unsaturated double bonds, and iron-sulfur centers. A concern of any modified cellular process is whether the new output or reaction will result in cytotoxicity. By measuring lactate dehydrogenase, it has been determined that HOI is not cytotoxic. This was demonstrated by the application of LPO, H₂O₂, and NaI to well-differentiated epithelial cells in vitro. Based on these findings, HOI is a potent antimicrobial but does not damage the respiratory epithelium.
NaI has been used for the treatment of various infectious diseases in cattle since the beginning of the last century. Traditionally, NaI is administered as an intravenous bolus at a dose of 70 mg/kg. Signs of intoxication can be observed at this dose, especially when multiple doses are administered for treatment.

Fig 7. The serum iodine concentration of treatment (NaI, n = 8) and control (water, n = 8) groups. At T₀, grouping markers (darkened circle and triangle) overlapping. The highest measured concentration of iodine was 106 mg/L. Standard error of the mean bars graphed. * indicates statistically significant difference at P < 0.05.

Fig 8. The concentration iodine in nasal secretions treatment (NaI, n = 8) and control (water, n = 8) groups. At T₀, grouping markers (darkened circle and triangle) overlapping. The highest measured concentration of iodine was 181 mg/L. Standard error of the mean bars graphed. * indicates statistically significant difference at P < 0.05.
These signs include any degree or combination of the following: anorexia, salivation, diarrhea, excessive coughing, nasal discharge, pneumonia, laceration, epiphora, alopecia, dermatitis, scaly hair coats, and hyperthermia. Iodine toxicity usually resolves when the I⁻ source is removed, and clinical signs diminish over a period of 2–3 days without long-term affects.9–11

When considering an antimicrobial for administration in food animals, the effect on the animal and consumable products must be acknowledged. First and foremost is the effect the treatment has on the animal’s health. In this study, there were no clinical signs or measurable abnormalities as a result of the administration of oral NaI at the dose administered. The only significant change between the 2 treatment groups was the heart rates at T₀ and T₄₈ when the treatment group heart rates were statistically significantly lower than the control, 166 beats per minute and 145 beats per minute, respectively. Both of these values are greater than the standard heart rates of cattle. However, these heart rates were taken immediately upon entry to the processing chute and are likely attributable to physiological responses associated with handling. Secondly, there is concern as to the effect on the consumable tissues and potential harmful residues. Iodine toxicity of humans through food animal sources could present a potential risk to human health. Most of the soil throughout the world is considered I deficient.9 Currently, table salt is iodized to prevent the occurrence of I deficiency in people.20 Recent investigations into the I content of food products determined that beef is not a pertinent source of I to humans (<2% of requirements), even when the animal is fed chronic doses above natural homeostatic requirements.

In conclusion, LPO/H₂O₂/I⁻ is capable of inactivating BoHV1 and BPJ3V and inhibiting the growth of M. haemolytica and B. trehalosi based on the in vitro analyses performed. By reducing these viral and bacterial pathogens, the morbidity and mortality of BRD might effectively be reduced. Weanling beef calves are capable of secreting I in their upper respiratory tract after administration of a one-time oral bolus of NaI at 70 mg/kg. Further investigation of the application of oral NaI treatment as a prevention of BRD is warranted.

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: The use of NaI (20%) in the manner described above is considered extralabel drug use.

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