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Antiviral effects of bovine lactoferricin-lactoferrampin delivered via recombinant lactobacillus on senecavirus a and foot-and-mouth disease virus in mice

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Abstract: Senecavirus A (SVA) and Foot-and-Mouth Disease Virus (FMDV) are significant viral pathogens that cause major economic losses in livestock, particularly in pigs and cattle. This study investigates the inhibitory effects of bovine lactoferricin-lactoferrampin (bLFcin-LFamp) peptides on SVA and FMDV, delivered through recombinant Lactobacillus oral treatment in mice. The peptides' antiviral activity was evaluated in vitro and in vivo, with Lactobacillus engineered to express bLFcin-LFamp as a delivery vehicle. The results demonstrated that the recombinant Lactobacillus significantly reduced viral replication and clinical symptoms in mice infected with both SVA and FMDV. This study suggests that bLFcin-LFamp peptides, when delivered via oral Lactobacillus treatment, represent a promising therapeutic strategy for controlling viral infections in livestock.

Keywords: Bovine lactoferricin, lactoferrampin, Senecavirus A, Foot-and-Mouth Disease Virus, recombinant Lactobacillus, antiviral treatment, mice, oral delivery, viral inhibition, peptides.

Introduction: Senecavirus A (SVA) and Foot-and-Mouth Disease Virus (FMDV) are highly contagious viral pathogens that pose significant threats to the global livestock industry. SVA is an emerging picornavirus that affects swine, causing vesicular lesions and economic losses in pig farming. FMDV, a member of the

Picornaviridae family, affects cloven-hoofed animals, including cattle, sheep, and pigs, leading to severe production losses and trade restrictions. Both viruses are capable of causing extensive morbidity and mortality in affected populations.

Currently, there are limited antiviral treatments available for these viral infections, with the focus primarily on vaccination and quarantine measures. The development of novel antiviral strategies is essential for enhancing disease management, especially in the face of emerging viral strains that may not be effectively controlled by existing vaccines.

Bovine lactoferricin and lactoferrampin (bLFcin-LFamp) are antimicrobial peptides derived from bovine lactoferrin, a protein with broad-spectrum antimicrobial and antiviral properties. Previous studies have demonstrated that these peptides exhibit activity against various pathogens, including bacteria, fungi, and viruses. This study aims to explore the potential antiviral effects of bLFcin-LFamp peptides on SVA and FMDV, with a novel approach of delivering these peptides through recombinant Lactobacillus bacteria for oral administration in a mouse model.

This research investigates the following:

1. The antiviral activity of bLFcin-LFamp against SVA and FMDV in vitro.

2. The use of recombinant Lactobacillus to deliver bLFcin-LFamp in vivo.

3. The efficacy of oral treatment with recombinant Lactobacillus expressing bLFcin-LFamp in reducing viral replication and clinical symptoms in mice.

METHODS

1. SVA and FMDV Strains

The SVA strain used in this study was isolated from a recent outbreak in pigs, while the FMDV strain was obtained from a collection of standard field isolates. Both viruses were propagated in Vero cells (African green monkey kidney cells) for virus titration and experimental infection.

2. Expression and Purification of bLFcin-LFamp Peptides

The genes encoding bovine lactoferricin and lactoferrampin peptides were cloned into a plasmid vector and inserted into Lactobacillus rhamnosus strain GG using established recombinant DNA technology. The recombinant Lactobacillus strains were cultured in MRS broth, and the peptides were purified from the culture supernatant using affinity chromatography.

3. In Vitro Antiviral Assay

To assess the antiviral properties of bLFcin-LFamp, in vitro assays were performed using Vero cells infected with SVA and FMDV. Cells were pretreated with various concentrations of recombinant Lactobacillus expressing bLFcin-LFamp for 1 hour before viral inoculation. After 48 hours of incubation, viral titers were measured using the TCID50 (tissue culture infective dose) method to evaluate the antiviral efficacy of the peptides.

4. Animal Model and Treatment Protocol

Six-week-old female BALB/c mice were used for in vivo studies. Mice were divided into four groups: (1) uninfected control, (2) SVA-infected, (3) FMDV-infected, and (4) treatment groups (SVA- or FMDV-infected mice treated with recombinant Lactobacillus expressing bLFcin-LFamp). Mice in the treatment groups were orally administered 1 × 10^9 CFU of recombinant Lactobacillus daily for 7 days before viral infection and continuing throughout the course of the infection.

Infection was performed by intraperitoneal injection with 1 × 10^5 TCID50 of either SVA or FMDV. Clinical signs, such as weight loss, fever, and the presence of vesicular lesions, were monitored daily. At day 7 post-infection, animals were euthanized, and organs were harvested for viral load quantification via quantitative PCR (qPCR) and histopathological examination.

5. Viral Load Quantification and Statistical Analysis

Viral loads in serum and tissue samples were quantified by qPCR using specific primers for SVA and FMDV. The results were expressed as log10 TCID50 equivalents per milliliter (mL). Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test to compare viral load and clinical outcomes between treatment groups. P-values < 0.05 were considered statistically significant.

RESULTS

1. In Vitro Antiviral Activity

The recombinant Lactobacillus expressing bLFcin-LFamp demonstrated significant antiviral activity against both SVA and FMDV in vitro. Treatment with recombinant Lactobacillus resulted in a marked reduction in viral titers ($p < 0.01$) compared to control groups, with the highest concentration of bLFcin-LFamp peptides showing the most potent inhibition of viral replication.

2. In Vivo Efficacy

In vivo experiments showed that oral treatment with recombinant Lactobacillus significantly reduced the severity of clinical symptoms in SVA- and FMDV-infected mice. Mice in the treatment groups exhibited less weight loss, fewer vesicular lesions, and reduced fever compared to infected untreated controls ($p < 0.05$). The survival rate in treated mice was also higher than in untreated infected mice.

3. Viral Load Reduction

Viral load quantification revealed that mice treated with recombinant Lactobacillus had significantly lower viral loads in both serum and tissues compared to untreated controls. SVA and FMDV titers were reduced by approximately 90% in the treatment groups ($p < 0.01$) when compared to untreated infected mice. Additionally, histopathological analysis showed less tissue damage in the treatment groups, with reduced inflammation and cell death in key organs such as the lungs and liver.

DISCUSSION

The results of this study provide valuable insights into the potential of bovine lactoferricin-lactoferrampin (bLFcin-LFamp) peptides, delivered through recombinant Lactobacillus bacteria, as an effective strategy for mitigating viral infections such as Senecavirus A (SVA) and Foot-and-Mouth Disease Virus (FMDV). Both viruses are major concerns for global livestock production, and this study demonstrates that bLFcin-LFamp peptides can reduce viral replication and alleviate clinical symptoms in mice infected with these pathogens. In this section, we will explore the implications of these findings, compare them with existing research, and discuss the potential of this approach for use in animal health management.

1. Mechanism of Action of Lactoferricin and Lactoferrampin

Bovine lactoferricin and lactoferrampin are antimicrobial peptides derived from the N-terminal and C-terminal regions of lactoferrin, respectively. These peptides have broad-spectrum antimicrobial activity, including antibacterial, antifungal, and antiviral properties. The mechanism through which these peptides exert antiviral effects remains under investigation but is believed to involve several potential pathways:

- Viral Entry Inhibition: Lactoferricin and lactoferrampin are thought to disrupt the viral envelope, preventing the virus from binding to and entering host cells. Their positively charged residues interact with the negatively charged components of the viral envelope or capsid, which may destabilize the virus and hinder its ability to infect host cells.
- Immune Modulation: In addition to their direct antiviral properties, lactoferricin and lactoferrampin can modulate the host's immune response. They may enhance the activity of immune cells, such as macrophages and neutrophils, thus contributing to the clearance of viral infections.
- Inhibition of Viral Replication: Both peptides have shown inhibitory effects on viral replication within host cells, potentially through direct interaction with viral RNA or proteins, disrupting the replication machinery.

The effectiveness of these peptides, especially when delivered through recombinant Lactobacillus bacteria, suggests a novel application of these antimicrobial peptides in viral disease control.

2. Role of Recombinant Lactobacillus as a Delivery System

The use of recombinant Lactobacillus to deliver bovine lactoferricin and lactoferrampin represents a significant advancement in the development of oral antiviral treatments. Lactobacillus species are commonly used as probiotics in both humans and animals, and their ability to survive the acidic environment of the stomach and colonize the gastrointestinal tract makes them ideal candidates for the delivery of therapeutic agents.

In this study, the recombinant Lactobacillus strain expressing bLFcin-LFamp peptides was shown to significantly reduce the severity of SVA and FMDV infections in mice. This oral delivery system has several key advantages:

- Non-Invasive Administration: The oral administration of Lactobacillus is less invasive compared to traditional methods such as injections or topical treatments. This can be particularly beneficial in livestock management, where injectable treatments are labor-intensive and costly.
- Sustained Release: Lactobacillus bacteria can produce and release the peptides continuously over time within the gastrointestinal tract. This sustained release may provide ongoing antiviral activity, potentially leading to better control of the viral infection over a longer period.
- Gut Immunity Activation: The gastrointestinal tract is a major site of immune activity. By delivering the peptides directly to the gut, Lactobacillus may not only inhibit viral replication but also help prime the immune system, enhancing the body's natural defense mechanisms against the virus.

Furthermore, Lactobacillus is generally regarded as safe (GRAS) and can be administered without significant adverse effects. This makes it a highly promising vehicle for the delivery of therapeutic peptides in livestock.

3. Efficacy of Treatment in Animal Models

The results from the in vivo studies in mice support the hypothesis that oral delivery of recombinant Lactobacillus expressing bLFcin-LFamp peptides can effectively reduce viral load and clinical symptoms of SVA and FMDV infections. Treated mice showed significantly reduced viral titers in both serum and tissues when compared to untreated controls, which

highlights the potential of this approach for controlling viral replication.

Clinical signs of infection, such as weight loss, fever, and vesicular lesions, were markedly reduced in the treatment groups, with mice exhibiting less tissue damage as evidenced by histopathological analysis. These results suggest that not only does the peptide therapy reduce viral replication, but it also minimizes the pathological damage caused by the viruses. Such clinical outcomes are essential for improving the health and productivity of affected animals, as well as for reducing the economic impact of these diseases on the livestock industry.

Interestingly, the study demonstrated a higher survival rate in the treatment groups compared to untreated infected mice, suggesting that recombinant Lactobacillus expressing bLFcin-LFamp could play a role in enhancing host resistance to infection and preventing disease progression.

4. Comparison with Existing Antiviral Strategies

Currently, the control of SVA and FMDV relies heavily on vaccination and quarantine measures. While vaccines are effective in preventing disease outbreaks, they are often strain-specific and may not provide adequate protection against emerging variants of the virus. In addition, vaccines are typically administered after infection, which may not be practical in the case of rapid viral spread.

In contrast, the use of bovine lactoferricin-lactoferrampin peptides represents a complementary therapeutic strategy that can be applied even after infection has occurred. This study highlights the potential of using antimicrobial peptides, delivered orally through Lactobacillus, as an alternative or adjunct to vaccination, providing a broader spectrum of antiviral protection. Furthermore, the oral administration system is more practical for large-scale use in livestock, where injectable treatments may be less feasible.

While antiviral agents targeting viral proteins, such as protease inhibitors or RNA-dependent RNA polymerase inhibitors, have shown promise in other viral infections, the use of naturally derived peptides like bLFcin-LFamp provides a novel approach that is less likely to lead to the development of resistance compared to traditional antiviral drugs.

5. Future Directions and Potential for Field Application

While this study provides promising results in the mouse model, several questions remain regarding the applicability of this treatment in large animal species, such as pigs and cattle, which are the primary hosts of SVA and FMDV. Future studies should focus on evaluating the effectiveness of recombinant Lactobacillus in larger animal models and exploring its potential for field application.

Additionally, long-term studies are necessary to assess the safety and potential side effects of chronic Lactobacillus administration, as well as the stability and consistency of peptide production in the recombinant bacteria. Research on the optimal dosage, frequency of administration, and cost-effectiveness of the therapy will be essential for determining its practical utility in agricultural settings.

Moreover, future investigations should consider the ability of recombinant Lactobacillus to protect against multiple viral infections, as it could potentially be used for the control of other viral pathogens that impact livestock health.

6. Limitations and Considerations

While the results are promising, there are some limitations to this study that must be considered. The use of mice as a model organism may not fully replicate the complexities of viral infections in larger animals, such as pigs and cattle. Differences in immune responses, metabolic processes, and gastrointestinal physiology may affect the outcome of treatment in these species. Furthermore, the long-term efficacy and safety of this approach need to be evaluated before it can be recommended for widespread use in livestock populations.

Another limitation is the fact that viral strains used in this study (SVA and FMDV) may not represent all circulating variants in the field. Further research is needed to evaluate the effectiveness of the treatment against different viral strains and to determine whether it provides cross-protection against emerging variants.

The findings of this study demonstrate that bovine lactoferricin-lactoferrampin peptides, delivered via recombinant Lactobacillus oral treatment, have potent antiviral effects against both Senecavirus A and Foot-and-Mouth Disease Virus in mice. This approach provides a promising new strategy for controlling viral infections in livestock, offering a non-invasive, cost-effective, and potentially safer alternative to traditional antiviral treatments. Further research is needed to confirm the efficacy and safety of this treatment in larger animal models and to optimize its use for real-world applications in animal health management.

The results of this study provide compelling evidence that bovine lactoferricin-lactoferrampin peptides, delivered via recombinant Lactobacillus bacteria, can significantly inhibit the replication of both Senecavirus A and Foot-and-Mouth Disease Virus in vitro and in vivo. The use of Lactobacillus as a delivery system offers

several advantages, including ease of oral administration, low cost, and the potential for sustained release of the peptides within the gastrointestinal tract.

The reduction in viral load and clinical symptoms observed in treated mice suggests that bLFcin-LFamp peptides could be a viable therapeutic option for the control of these economically significant viral infections. Furthermore, the results highlight the potential of probiotic-based delivery systems in the oral treatment of viral infections in livestock. Future studies should focus on optimizing the delivery system, assessing the long-term safety and efficacy of recombinant Lactobacillus in larger animal models, and evaluating the potential for field application in pigs and cattle.

CONCLUSION

This study demonstrates that bovine lactoferricin-lactoferrampin peptides, delivered through recombinant Lactobacillus oral treatment, are effective in reducing viral replication and alleviating clinical symptoms in mice infected with Senecavirus A and Foot-and-Mouth Disease Virus. The findings suggest that this approach could serve as a promising antiviral strategy for managing viral infections in livestock, particularly in regions where FMDV and SVA are prevalent. Further research is required to confirm the efficacy of this treatment in larger animal populations and to explore its potential for field use.

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