Epigenetic Modulation of Avian Host Defense Peptide Synthesis

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Abstract

Attributed to 35,000 annual U.S. deaths,[1] antibiotic resistance has catalyzed an increasing demand for novel antibiotic alternatives.

Host defense peptides (HDPs) provide the potential to upregulate the innate immune response to microbial infections.[2]

This study analyzes the capacity for S-adenosylhomocysteine (SAH) and all-trans retinoic acid (ATRA) to epigenetically induce HDP expression, hypothesizing that they will independently and synergistically upregulate HDPs in chicken macrophage cell lines.

SAH and ATRA were introduced independently and in combination with butyrate to analyze HDP gene expression. Current results suggest minimal HDP response to SAH and ATRA; however, further studies must be conducted.

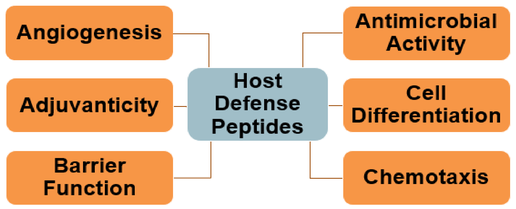

Figure 1. Pleiotropic Effects of HDPs. Recent research indicates HDPs nonspecific mechanisms allow for immune activity beyond antimicrobial response.

Introduction

- HDPs are cationic, amphipathic, short-chain peptides that interfere with bacterial macromolecular synthesis.[3]
- Inhibition of DNA and histone methylation increases gene expression.
- Cellular accumulation of SAH inhibits further methylation by S-Adenosylmethionine.[4]
- ATRA acts as a transcription factor influencing histone methylation.[5]
- Butyrate is a short chain fatty acid that has previously interacted synergistically with other epigenetic modulators to induce *AvBD9*. [2]

Methods

Culture and Stimulation of Chicken Macrophage Cells

- Chicken HTC macrophage cells were cultured in complete RPMI 1640 containing 10% fetal bovine serum, and 1% penicillin/streptomycin.
- HTC cells were seeded into 12-well tissue culture plates at 5×10^5cells/mL overnight.
- Cells were stimulated in duplicate with final concentrations of 0, 10, 20, and 40uM SAH for 24 hrs and 0, 0.1, 1, and 1uM ATRA for 24 hrs.
- After the optimal dosage was determined, HTC cells were treated with 2mM butyrate and SAH or ATRA for 24 hrs.

Isolation of Total RNA

- Total RNA was isolated using RNAzol RT and quantified using the Nanodrop Spectrophotometer.

Gene Expression Analysis by RT-qPCR

- Reverse transcription of RNA was conducted with an iScript cDNA Synthesis Kit (Bio-Rad).
- Gene expression levels of *AvBD9* were analyzed by quantitative real-time polymerase chain reaction (qPCR).

Data Analysis

- The fold change of gene expression relative to treatment was calculated using the ΔΔCt method normalized with *GAPDH*.

Results

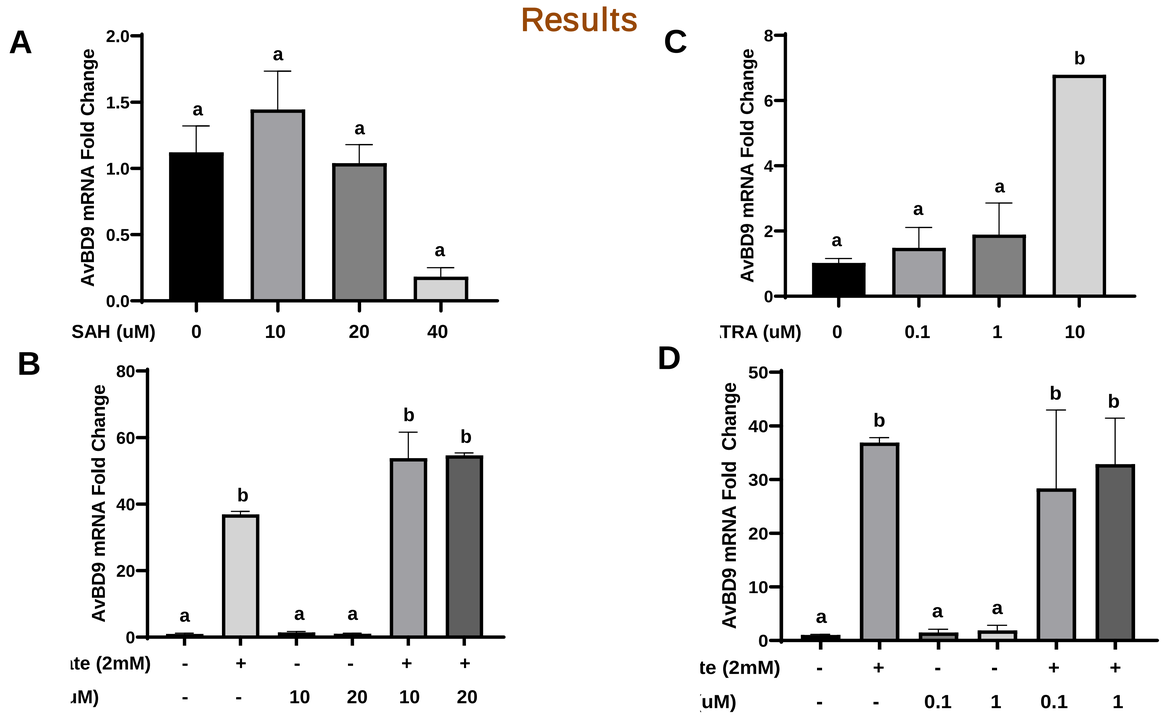

Figure 2. Effect of S-adenosylhomocysteine (SAH) and all-trans retinoic acid (ATRA) on innate animal immunity. Chicken HTC cells were independently treated with SAH **(A)** and ATRA **(C),** and in synergy with butyrate **(B, D)**. Immune response was quantified by the expression of a representative avian HDP gene *(AvBD9)*. The treatments without common superscripts are considered statistically different ($P \leq 0.05$) based on one-way ANOVA and post hoc Turkey's test.

Discussion

- Results indicated optimal dosages of 10uM SAH and 1uM ATRA at 24 hrs.
- Independent SAH and ATRA trials induced minimal *AvBD9* expression.
- SAH and butyrate interacted with minimal synergy.
- ATRA and butyrate interacted antagonistically with observed toxicity at high concentrations.

Conclusion

- Current results indicate that SAH and ATRA do not significantly induce *AvBD9* when used independently or synergistically with butyrate.
- The observed expression is not statistically significant for translation to *in vivo* studies.
- Further dosage and time trials must be performed for optimal HTC treatment.

References

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