Resveratrol Increases Cathelicidin Expression and Offers a Novel Approach to Combat Staph aureus Invasion.

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INTRODUCTION

- Cathelicidin (CAMP), an antimicrobial peptide, is commonly described in literature as a byproduct of the Vit D receptor pathway and is a key component of the epidermal barrier. The epidermal barrier is a refined product of billions of years of evolution that serves to protect vital tissues from microbial pathogens and mechanical damage.
- We previously described a novel, VitD receptor independent pathway that showcased the role of sphingosine-1-phosphate (S1P) in de novo production of cathelicidin. Moreover, we have also shown that keratinocytes prevent apoptosis by de novo production of sphingomyelin from ceramide.
- Here, we investigate if ceramide expression can be induced by resveratrol (RESV), a polyphenol known to increase ceramide expression, and if induced ceramide can modulate downstream cathelicidin production in order to enhance the innate skin barrier and combat staph aureus invasion.

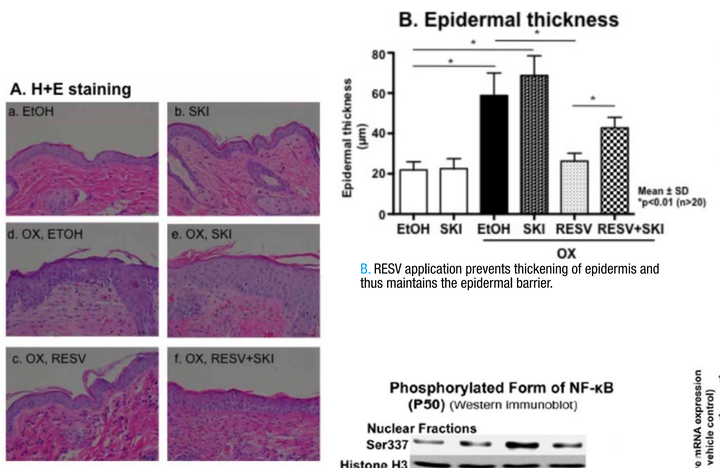
METHODS

- We used the well established oxazolone (Ox) model to perturb the epidermal skin barrier and study the effects of cathelicidin production in restoring the skin barrier and combating staph aureus penetration/invasion.
- Six mice model groups were created using the hairless SKH1 mice, including two control groups:
- RESV was applied to the flanks of the mice 2x/day for 21 days. Skin samples were obtained at the end of the 21 day period. qT-PCR and Western blot techniques were used on homogenized skin samples in order to quantify cathelicidin expression.
- Further, NF-κB receptor assay was utilized to assess whether RESV increased activation of the NF-κB nuclear transcription factor. Finally, skin tissue biopsies were gram stained to investigate the effects of cathelicidin in preventing invasion of Staph aureus into and through the innate skin barrier. Statistical comparisons were performed using an unpaired Student t-Test.

Study Groups	
Control	**Experimental**
EtOH (ethanol)	Ox + EtOH
Sphingosine-kinase-inhibitor (SKI)	Ox + SKI Ox + RESV Ox + RESV + SKI

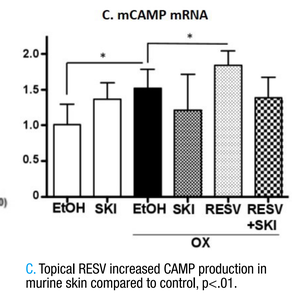
RESULTS

- RESV group maintained epidermal integrity.
- Topical RESV increased CAMP production in murine skin compared to control, p<.01.
- This increased production is secondary to activation of NF-KB pathway as revealed by:
  - Increased phosphorylation of NF-KB (marker of activation) in the RESV group.
  - Attenuation of phospho-NF-KB by introduction of SKI.
  - Attenuation of CAMP production with introduction of BAY11-7082, a NF-KB inhibitor, in cultured keratinocytes.
- RESV prevents invasion of Staph aureus into the epidermal barrier.

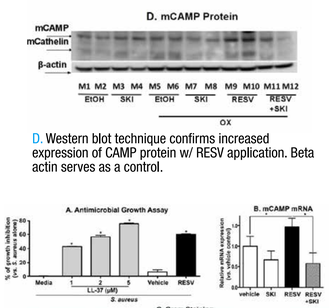


A. Haemotoxylin and Eosin stain of murine skin with application of RESV and EtOH (vehicle).

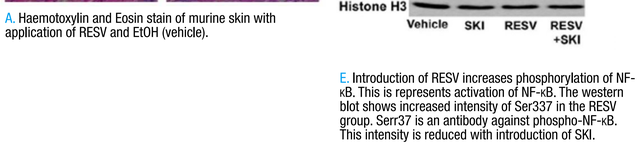
B. RESV application prevents thickening of epidermis and thus maintains the epidermal barrier.



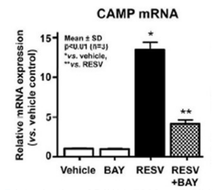
C. Topical RESV increased CAMP production in murine skin compared to control, p<.01.



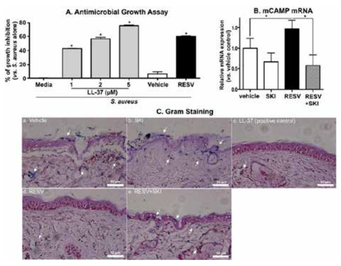
D. Western blot technique confirms increased expression of CAMP protein w/ RESV application. Beta actin serves as a control.



E. Introduction of RESV increases phosphorylation of NF-κB. This is represents activation of NF-κB. The western blot shows increased intensity of Ser337 in the RESV group. Serr37 is an antibody against phospho-NF-κB. This intensity is reduced with introduction of SKI.



F. Introduction of BAY11-7082, an inhibitor of NF-κB, markedly reduces CAMP mRNA production.



A. Introduction of LL-37, which is the active form of CAMP in-vivo, to cultured keratinocytes inhibited growth of Staph aureus. In a similar fashion, RESV also inhibited growth of Staph aureus.

B. RESV increased CAMP production.

C. H&E stain of murine biopsies colonized with Staph aureus. RESV group prevented invasion of Staph aureus. This effect was neutralized with co-application of SKI.

CONCLUSIONS

- Our results display strong evidence that:
  - RESV increases CAMP production.
  - CAMP upregulation follows the activation cascade ceramide→sphingosine→ S1P metabolism and is mediated via NF-κB activation.
  - CAMP modulation offers a novel method to combat Staph aureus invasion in an era of ever-increasing antimicrobial resistance.

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