Increasing follicular resistance to bacterial contamination: A novel antimicrobial therapy?

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BACKGROUND

Olfactory receptors (OR) are part of an evolutionarily ancient chemosensory signaling system regulating many cell functions beyond olfaction. We have shown that human hair follicle (HF) epithelium engages in **OR-dependent chemosensation** via the **OR2AT4** receptor and that continuous signaling through this receptor is required for anagen maintenance *ex vivo*. Intriguingly, microarray analysis revealed that Sandalore® strongly up-regulates transcription of **dermcidin** (DCD), an antimicrobial peptide, in microdissected HFs *ex vivo*¹.

AIM

We asked whether Sandalore® I) actually up-regulates intrafollicular DCD peptide production, and II) renders organ-cultured human HFs less susceptible to bacterial contamination/infection.

EXPERIMENTAL PROCEDURE

Amputated or full-length HFs were microdissected from occipital scalp skin or follicular unit explants and cultured in William's E complete medium only, or stimulated with Sandalore® (500µM), for 6 days². For the contamination experiment both groups were kept with or without antibiotics, respectively. In human scalp skin *ex vivo*, Sandalore® was applied topically (using PEG6000) or systemically into the medium every other day³.

RESULTS

1) DCD RNA can be found in the HF epithelium, while protein is nearly undetectable, in human skin *in vivo*

RNA *in situ* hybridization

Immuno-fluorescence

Qualitative observation from one biopsy/donor immediately frozen after collection from **n=3 healthy donors**, using a probe specifically designed for human dermcidin

Qualitative observation from one biopsy/donor immediately frozen after collection from **n=3 healthy donors**. HM= hair matrix, ORS= outer root sheath, HS= hair shaft, yellow star = excretory duct of sweat gland

DCD expression has been reported in eccrine and sebaceous glands of human skin, therefore we applied this as internal positive control⁴.

4) Topical Sandalore® treatment tendentially increases dermcidin secretion in the HF outer root sheath in skin *ex vivo*

n=2 punches from one healthy donor, 2-9 HF/group; mean±SEM GraphPad Prism 6; DCD spots were counted in the HF epithelium.

These data confirmed the results of our pilot study only using 500µM topically applied Sandalore® (data not shown).

5) Culture medium of Sandalore® treated scalp skin affects microbial balancing and influences microbial communication

Preliminary data from n=2 punches of one healthy donor (see above) on day 3 and 5 of organ-culture.

Different bacterial and fungal strains were incubated with the culture medium and survival was quantified using an MTT assay. The data show the strain survival compared with the DCD concentration in the medium.

2) Sandalore® treatment up-regulates intrafollicular DCD peptide production in the HF epithelium *ex vivo*

DCD+ cells in HF epithelium

n=4 healthy donor, mean±SEM GraphPad Prism 6; D'Agostino & Pearson omnibus normality test, no Gaussian distribution, Mann-Whitney test, *p < 0.001**
DCD positive spots were counted in the HF epithelium

3) Sandalore® reduces microbial contamination in microdissected HF *ex vivo* in the absence of antibiotics

PAS staining in combination with Lightgreen can detect different sorts of fungi and mucous yeast films. In another experiment with amputated HF, treatment with Sandalore® prevented bacterial contaminations (data not shown).

n=7-8 HF/group from one healthy donor, mean±SEM GraphPad Prism 6; D'Agostino & Pearson omnibus normality test, no Gaussian distribution, Mann-Whitney test, *p<0.001 compared to vehicle respectively; number and mean area of the colonies was quantified per hair follicle**

CONCLUSION & OUTLOOK

DCD exerts broad antimicrobial activity against pathogenic microorganisms by interacting with bacterial membrane phospholipids and forming pores in the cell membrane. Our data show that stimulation of OR2AT4 by Sandalore® increases the antimicrobial activity of human HFs by stimulating DCD production. Thus, the data suggest that “olfactotherapy” of hair follicle diseases characterized by major microbial imbalances such as acne vulgaris or bacterial folliculitis, with selected odorants is a realistic possibility.

¹ Chéret J, Bertolini M, Ponce L, et al., Olfactory receptor OR2AT4 regulates human hair growth. *Nat Commun* 2018; **18**;9(1):3624
² Langan EA, Philpott MP, Kloepper JE, Paus R. Human hair follicle organ culture: theory, application and perspectives. *Exp Dermatol* 2015; **24**(12): 903–911.
³ Gherardini J, Wegner J1, Chéret J, et al., UV radiation on scalp skin surface induces scalp hair follicle damage *ex vivo* and is alleviated by the topical treatment with caffeine. *Int J Cosmet Sci* 2019; doi: 10.1111/ics.12521.
⁴ Dahlhoff M, Zouboulis CC, Schneider MR. Expression of dermcidin in sebocytes supports a role for sebum in the constitutive innate defense of human skin. *J Dermatol Sci* 2016; **81**(2):124-6.