Topically Applied Nicotinamide Inhibits Human Hair Follicle Growth Ex Vivo

TO THE EDITOR

The effective inhibition of unwanted human hair growth by safe and well-tolerated topical agents remains a major drawback of clinical and cosmetic dermatological treatments (Somani and Turvy, 2014). One key challenge is to promote hair follicle (HF) regression (catagen) with negligible skin toxicity or irritation (see Supplementary Figure S1 online). Pilot evidence suggests that nicotinamide, an amide form of vitamin B3 (Forbat et al., 2017), may be capable of doing this. The fact that nicotinamide is used as a cosmetic and a dermatotherapeutic agent (Chen et al., 2016; Forbat et al., 2017; Niren, 2006; Walocko et al., 2017) (see Supplementary Materials and Supplementary Figure S2 online) makes it an attractive candidate for hair growth inhibition. Therefore, we have investigated the hypothesis that nicotinamide may be an effective hair growth inhibitor.

Microdissected human scalp HFs were obtained with institutional approval and written informed patient consent (Langan et al., 2015). These were cultured in the presence of 200 μmol/L or 10 mmol/L (see Supplementary Materials online) nicotinamide for 6 days, and key human hair biology read-out parameters were assessed (Kloepper et al., 2010; Ramot et al., 2014).

HFs treated with nicotinamide (10 mmol/L) showed significantly decreased hair shaft production (Figure 1a) and entered catagen more rapidly than vehicle control HFs (Figure 1b, and for example staging see Supplementary Figure S1). This was confirmed by double-immunohistomorphometry (Ki-67/TUNEL), which showed significantly decreased hair matrix keratinocyte proliferation (Ki-67) below Auber’s line and increased apoptosis (TUNEL) within the hair matrix in anagen phase HFs. This independently corroborated a catagen-promoting effect of nicotinamide (Figure 1c and d) with no effect on the malnin content of anagen VI HFs (see Supplementary Materials and Supplementary Figure S2).

Because nicotinamide added to culture medium imitates systemic application, we next asked if topical

Figure 1. Nicotinamide inhibits hair growth and induces catagen. Human hair follicles were cultured for 6 days with nicotinamide (200 μmol/L:10 mmol/L vehicle); we found that (a) hair shaft elongation was decreased by 10 mmol/L nicotinamide with a (b) corresponding increase in catagen hair follicles, (c, d) a significant decrease in Ki-67+ cells below Auber’s line, and an increase in TUNEL+ cells in the matrix compared with vehicle. When nicotinamide (10 mmol/L) was topically applied to human scalp skin, (e) a higher proportion of catagen hair follicles were detected (day 3), with (f, g) more mid-/late catagen at day 6 and a matching decrease in Ki-67+ cells below Auber’s line compared with vehicle in anagen HFs. Data are mean ± standard error of the mean, one-way analysis of variance with Bonferroni post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars = 50 μm. M, mol/L; Nic, nicotinamide.

Abbreviations: HF, hair follicle; MC, mast cell; PEA, palmitoylethanolamide

Accepted manuscript published online 26 December 2017; corrected proof published online 13 March 2018

© 2017 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology.
application to organ cultured human scalp skin (see Supplementary Materials) (Lu et al., 2007) also inhibited human hair growth. Results showed that HF treated with topical nicotinamide (10 mmol/L) entered and progressed through catagen faster than vehicle-treated skin (days 3 and 6) (Figure 1e, and see Supplementary Figure S1) with a corresponding significant decrease in proliferation (Ki-67) (Figure 1f and g), mirroring HF organ culture experiments.

To exclude potential leaching of topical formulations into the medium, we refined this assay by suspending the skin within an air-permeable membrane (see Supplementary Materials). In addition, we tested a modified topical nicotinamide formulation (i.e., 1%, matching 10 mmol/L nicotinamide [Figure 1a–g]) or 4%, the common cosmetic concentration [Navarrete-Solís et al., 2011] [see Supplementary Figure S2]), using a hydrosome-based vehicle, which is a clinical grade vehicle that promotes skin penetration (see Supplementary Figure S3 online).

In this assay, the nicotinamide-hydrosome preparations (1% and 4%) significantly increased the number of catagen-like HFs in organ-cultured human scalp skin (Figure 2a, and see Supplementary Figure S1) (Oh et al., 2016). In line with this, the percentage of apoptotic (TUNEL⁺) HF matrix keratinocytes was increased (Figure 2b and d), whereas the percentage of proliferating (Ki-67⁺) HF keratinocytes was significantly decreased (Figure 2c and d), with no effect on melanocyte proliferation (see Supplementary Figure S2).

Moreover, topical nicotinamide (4%) also decreased the intrafollicular protein immunoreactivity for keratin 85, a surrogate marker for hair shaft production (Figure 2e and n, and see Supplementary Figure S4 online), showing that hair growth inhibition was maintained using this vehicle.

Because epilation devices and depilatory creams typically induce skin irritation and/or pruritus (see Supplementary Figure S4 online), we examined potential adverse effects of nicotinamide by investigating mast cell (MC) degranulation as an indicator of skin irritation, in the interfollicular dermis and HF connective tissue sheath using immunohistomorphometry of MC-tryptase. Contrary to previous reports that nicotinamide reduced MC degranulation (Navarrete-Solís et al., 2011; Niren, 2006), 1% nicotinamide added to the HF medium significantly increased MC degranulation (see Supplementary Figure S3a and b) in the HF connective tissue sheath.

Comparing this to skin organ culture, no significant change in MC degranulation resulted from 1% nicotinamide-hydrosome applied topically, although a significant increase by 4% nicotinamide-hydrosome was detected (Figure 2g and h). Comparatively, nicotinamide-PEG6000 induced degranulation at a
concentration of 1% (Figure 2i), suggesting that a hydrosome vehicle may have some ability to reduce degranulation when used in combination with lower concentrations of nicotinamide (see Supplementary Materials).

To further probe skin inflammation by topical nicotinamide, the number of CD68⁺ dermal macrophages was also investigated, and we found a significant decrease by 4% nicotinamide-hydrosome with a trend toward decreased numbers by 1% (see Supplementary Figure S4c and g). This suggests that nicotinamide does not induce macrophage-associated inflammation, but may induce MC-dependent itch inflammation but may induce itch and/or neurogenic inflammation. For this reason, a method for reducing MC degranulation while maintaining hair growth inhibition was examined. For this purpose, palmitoylethanolamide (PEA) (30 μmol/L), an antipruritic endocannabinoid used in clinical dermatology, was applied to our nicotinamide/PEG6000 formulation (see Supplementary Materials) and applied topically to ex vivo scalp skin ex vivo to reduce MC degranulation (Parrella et al., 2016) (see Supplementary Figure S4 online).

The addition of PEA significantly reduced nicotinamide-induced dermal MC degranulation ex vivo (Figure 2i) without altering the hair growth inhibitory effect (Figure 2i). This supports the addition of PEA to future nicotinamide products to reduce MC degranulation-associated itch/inflammation while maintaining hair growth inhibition (see Supplementary Materials).

To examine the potential for topical nicotinamide-hydrosome (1% and 4%) formulations to cause additional epidermal adverse effects that would render them unsuitable as hair growth-inhibitory products (Cerchia and Lavechcia, 2017), epidermal proliferation, apoptosis, and pigmentation were assessed by quantitative immunohistomorphometry (Ki-67/TUNEL/Masson-Fontana). Neither the 1% nor 4% topical nicotinamide-hydrosome formulation significantly altered epidermal proliferation, apoptosis, or pigmentation (see Supplementary Materials and Supplementary Figure S4a, b, and d–f).

Taken together, these data present clear evidence that nicotinamide can effectively inhibit human hair growth and promote catagen in our clinically relevant models. The fact that this occurred at commercially/clinically relevant concentrations without negatively affecting epidermal vitality or pigmentation makes nicotinamide an excellent cosmeceutical hair growth inhibitor for subsequent clinical testing. Data also suggest that hydrosomes provide a suitable vehicle and that PEA addition may reduce any potential skin irritation/itch that might be seen after repetitive application in vivo.

ORCIDs
Iain S Haslam: http://orcid.org/0000-0002-1008-2447
Jonathan A Hardman: http://orcid.org/0000-0002-3653-7908
Ralf Paus: http://orcid.org/0000-0002-3492-9358

CONFLICT OF INTEREST
Data presented in Figure 2a–h and Supplementary Figure S3 were supported by a grant to RP from Reckitt Benckiser, UK in the form of a research associate position for JAH.

ACKNOWLEDGMENTS
This study was supported by University of Manchester Intellectual Property and continued with support from RB, UK. Study completion was facilitated by the National Institute for Health Research Manchester Biomedical Research Centre (Inflammatory Hair Diseases Programme). The authors thank Stella Pearson for technical assistance.

iain S Haslam, Jonathan A Hardman and Ralf Paus

1 Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, UK; 2 Centre for Dermatology Research, The University of Manchester, Manchester, UK; and 3 National Institute for Health Research Manchester Biomedical Research Centre, Manchester, UK

Corresponding author e-mail: ralf.paus@manchester.ac.uk

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.12.019.

REFERENCES
Walocko FM, Eber AE, Keri JE, Al-Harbi MA, Noori K. The role of nicotinamide in acne treatment. Dermatol Ther 2017;30(5);