



Original Article

N,N-dimethylglycine sodium salt prevents human dermal papilla senescence and promotes hair growth in patients with pattern hair loss



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ABSTRACT

Background: In our previous studies, N,N-dimethylglycine sodium salt (DMG-Na) was found to exert anti-inflammatory and anti-oxidant effects in human epidermal keratinocytes and promote the synthesis of regenerating growth factors (e.g., vascular endothelial growth factor).

Objective: In the current investigation, we assessed the effects of DMG-Na to modulate hair follicle-derived dermal papilla (DP) senescence induced by oxidative (H₂O₂) or non-oxidative (Bromodeoxyuridine) stimuli using *in vitro* cultured human DP cells. Further, in two randomized, double-blind, placebo-controlled, parallel-group, sex-stratified *in vivo* studies, we determined the efficacy of a topical DMG-Na containing formulation on more than three hundred patients with hair loss.

Methods: In the *in vitro* arm, intracellular Ca²⁺ measurements, senescence-associated β-galactosidase determinations, qRT-PCR assays, and various immunolabeling methods were employed whereas multiple hair loss parameters were assessed in the *in vivo* human studies.

Results: DMG-Na (0.05%) significantly prevented the effects of H₂O₂ and Bromodeoxyuridine to upregulate the number of SA-β-Gal positive cells. Likewise, DMG-Na significantly normalized the expressions of several upregulated (e.g., matrix metalloproteinases 1 and 3, p21, interleukin-1β, tumor necrosis factor-α) or downregulated (e.g., Ki67, lamin B1, collagens 1A1 and 3A1) senescence-associated markers by the inducers. In line with these anti-senescence effects, in the human trials, topical formulations containing 0.5% DMG-Na significantly improved hair loss parameters.

Conclusion: Our findings – together with our prior evidence that topically applied DMG-Na enhanced skin blood flow in humans – collectively suggest that DMG-Na is an effective and promising active for the management of hair conditions characterized by impaired cellular growth, repair, and dysregulated oxidative metabolic status.

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1. Introduction

Senescence is defined as a cellular process characterized by a predetermined growth arrest that bears both physiological and pathological impacts. Under homeostatic conditions, mostly via the release of numerous molecules collectively referred to as senescence-associated secretory phenotype (SASP), it contributes to cell

and organ development as well as tissue regeneration, repair, and remodeling by the SASP-mediated elimination of senescent cells. However, if and when senescence cells are not completely eradicated, SASP negatively affects the neighboring healthy cells of the given tissue leading to overall biological dysfunctions which may lead to premature and/or pathological degenerative conditions seen e.g., in aging [1–3].

Scalp hair density generally declines with age in both sexes by the mid- to late twenties [4,5]. In addition, female (FPHL) and male (MPHL) pattern hair loss, affecting 25–50% of individuals by the age of 50 and exhibiting increasing frequency with age [6,7], have a considerable impact on the quality of life and self-esteem of the patients. Both MPHL and FPHL are polygenic and multifactorial conditions, which involve progressive miniaturization and shortening of the anagen (growth) phase of affected hair follicles (HFs) [8,9]. Several pathogenetic factors have been identified to impair the normal hair cycle, including (but not limited to) inflammation, certain hormonal changes, stress, and nutritional deficiency due to reduced blood supply [10].

Notably, premature and/or pathological senescence of the dermal papilla (DP), the key HF compartment boosting and orchestrating the hair cycle [10,11], has emerged as another main pathogenetic factor promoting hair loss. Indeed, balding DP cells from the affected region of MPHL (androgenetic alopecia, AGA) patients exhibited robust senescence phenotype (e.g., decreased proliferation, increased senescence-associated β -galactosidase (SA- β -Gal) activity, decreased oxidative stress resistance, and the upregulation of multiple senescence-associated markers) both *in vitro* and *in vivo* when compared with their non-balding counterparts [12]. Moreover, when premature DP cell senescence was induced by the administration of H_2O_2 *in vitro* and *in vivo*, it compromised HF epithelial-mesenchymal interaction, induced the loss of HF inductive potential, and blocked telogen to anagen transition [13]. Of further importance, spontaneous DP cell senescence and the concomitant loss of HF inductive potential was found in human DP cells upon *in vitro* culturing (as early as after 5 passages); this phenomenon could be effectively prevented by the application of known senolytics (agents that increase senescence cell clearance) which also restored the HF inductive potential [14].

In our previous investigations, we have been assessing the cutaneous effects of the sodium salt of N,N-dimethylglycine (DMG-Na). DMG is a naturally occurring glycine derivative and a component of the endogenous homocysteine pathway. DMG and its sodium salt are both used as food supplements in animal husbandry and are considered safe, non-fuel food supplements for human use [15–17]. In cultured human epidermal keratinocytes, we have previously demonstrated that DMG-Na exerts positive effects on cellular proliferation, migration and wound closure as well as induction of the synthesis and release of growth factors, including vascular endothelial growth factor (VEGF). In addition, we found that DMG-Na decreased inflammation and oxidative stress response in various *in vitro* skin disease models, most probably via the increase of the intracellular calcium concentration in keratinocytes *in vitro* [18]. Furthermore, we have recently shown that topical application of a gel containing 1% DMG-Na to male and female subjects enhanced skin blood flow. The suggested mechanism is mediated by the increase of (i) calcium influx, (ii) the expression and activation of endothelial nitric oxide synthase (eNOS) as well as (iii) the production of nitric oxide (NO), the master regulator of endothelial-derived vasodilation, as shown in primary human dermal microvascular endothelial cells (HDMECs) [19].

In the current study, we hypothesized that the above beneficial biological effects of DMG-Na may be transferred to the HFs, especially under conditions resulting in hair loss. To probe this hypothesis, we employed a combined *in vitro* and *in vivo* approach. First, we assessed the effect of DMG-Na on *in vitro* cultures of human scalp

HF-derived primary (HF-DPCs) and immortalized DP cells (i-DPCs) challenged by oxidative (H_2O_2) and non-oxidative (Bromodeoxyuridine, BrdU) pro-senescence stimuli. Next, in two randomized, double-blind, placebo-controlled, parallel-group, sex-stratified *in vivo* studies – which extended our recently published human trial limited to a smaller cohort of only male patients by using a different application mode [20] – we determined the efficacy of a topical DMG-Na containing formulation on more than three hundred patients with FPHL or MPHL. Our results clearly show that DMG-Na prevents DP senescence and that it offers significant benefits in the future management of pattern hair loss.

2. Materials and methods

Detailed description of the materials and methods used in the current study can be found in the [Supplementary Material](#).

3. Results

3.1. N,N-dimethylglycine sodium salt (DMG-Na) prevents DP cell senescence *in vitro*

First, we investigated whether DMG-Na induces calcium influx to DPCs, similar to our previous findings on human epidermal keratinocytes and HDMECs [18,19]. As detected by microfluorimetry, in normal (1.8 mM) Ca^{2+} -containing medium, DMG-Na elevated the intracellular calcium concentration ($[Ca^{2+}]_i$) in both HF-DPCs and i-DPCs in a dose-dependent manner; namely, 0.02% and 0.05% DMG-Na exerted statistically significant responses when compared with control. In contrast, DMG-Na was unable to increase $[Ca^{2+}]_i$ in a Ca^{2+} -free medium supplemented with 5 mM EDTA ([Supplementary Figure 1](#)). The fact that DMG-Na was able to increase $[Ca^{2+}]_i$ only in the presence of extracellular Ca^{2+} suggests that DMG-Na induced Ca^{2+} -influx to DPCs from the extracellular space.

Among the multiple cellular senescence markers recognized and previously reported [1,3], we first assessed possibly the most often studied one, i.e. the number of SA- β -Gal positive cells within the cultures [21,22]. Based on previous publications [23,24], both the oxidative (H_2O_2) and non-oxidative (BrdU) senescence inducers profoundly and statistically significantly upregulated the percentage of SA- β -Gal positive cells after 48 and 72 h of treatment in both HF-DPCs and i-DPCs ([Fig. 1](#)). Notably, 0.05% DMG-Na treatment markedly and, except for the 72-hour time point on i-DPCs, statistically significantly prevented the effects of the two inducers ([Fig. 1](#)) suggesting that DMG-Na may exhibit anti-senescence actions (representative images can be seen in [Supplementary Figure 2](#)).

To further investigate the anti-senescence effects of DMG-Na, we performed multiple qRT-PCR experiments in which we assessed the expression of several markers which have been previously described to be up- or downregulated during cellular senescence. (These were selected based on data from the literature and on results from the optimization experiments with both senescence inducers. Their senescence-associated functions and expression patterns as well as the related references are summarized in [Supplementary Table 1](#)). In both HF-DPCs and i-DPCs treated with H_2O_2 , oxidative stress upregulated the expression of *MMP1*, *MMP3*, *P21* (*CDKN1A*), *IL1B*, and *IL6* ([Table 1](#)). These elevated marker levels, which are known to rise during cellular senescence, indicate senescence induction. Likewise, H_2O_2 treatment of both HF-DPCs and i-DPCs downregulated the expression levels of *MKI67*, *MTCO1*, *COL1A1*, *COL3A1*, *LMNB1*, *SIRT1*, and *NRF2*, which are reportedly decreased upon senescence (see [Table 1](#) for statistical details). DMG-Na treatment markedly and in the case of *MMP1*, *MMP3*, *IL1B*, *MKI67*, *MTCO1*, *COL1A1*, *COL3A1*, *LMNB1*, and *SIRT1* statistically significantly prevented the effect of H_2O_2 to up- or downregulate the expressions of the assessed

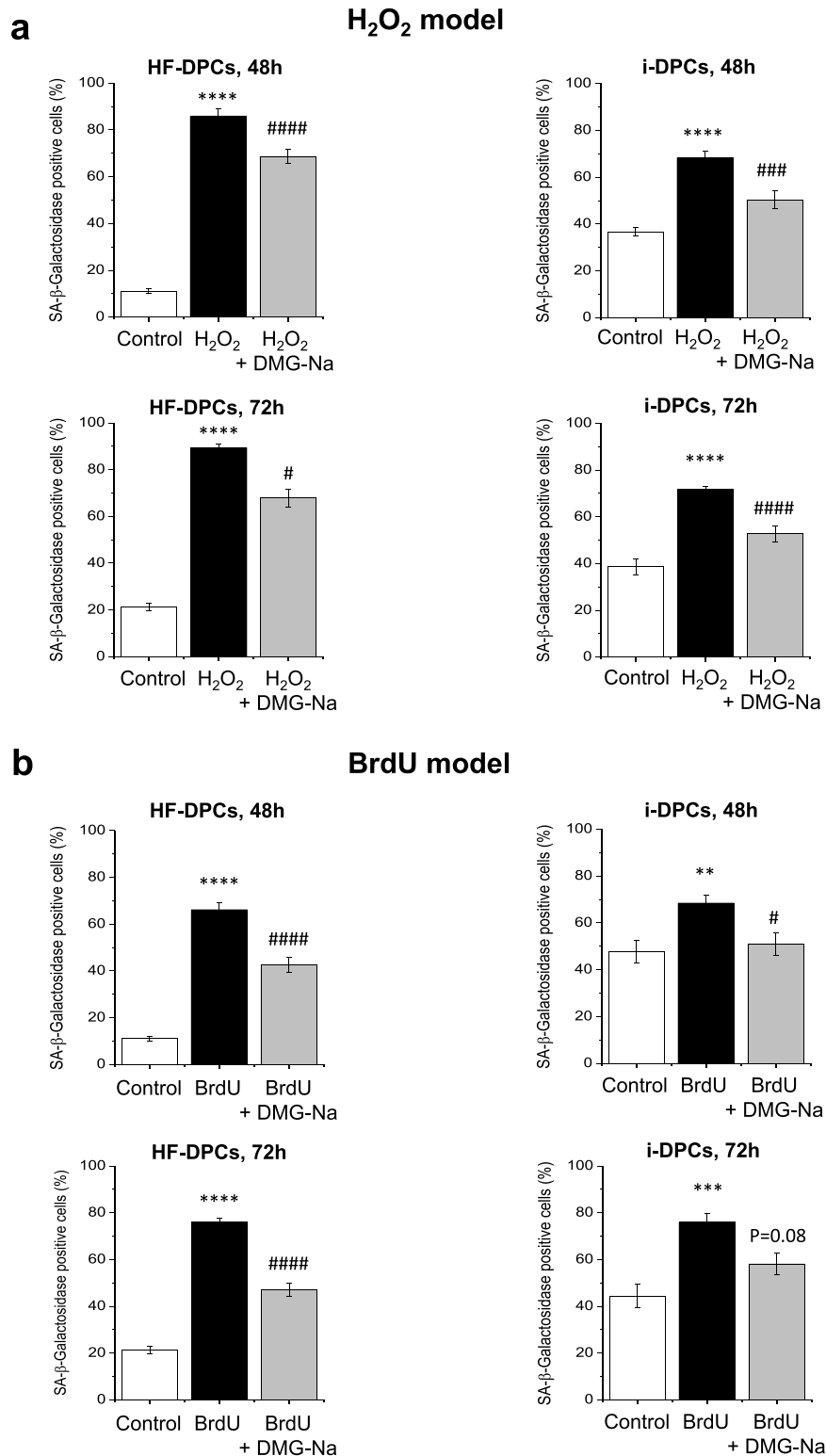


Fig. 1. DMG-Na prevents the effect of H₂O₂ and BrdU to upregulate the number of SA-β-Gal cells in HF-DPCs and i-DPCs in vitro. Statistical analysis of the percentage (%) of SA-β-Gal positive cells in the (a) H₂O₂ and (b) BrdU models in HF-DPCs and i-DPCs measured after 48 and 72 h of treatment. Graphs show pooled data (mean ± SEM) of n = 3–4 independent experiments (12–15 independent visual fields) on both cell types. For P-values, * marks the statistical difference compared with the control (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001) whereas # marks the statistical difference measured in the H₂O₂ vs-H₂O₂ + DMG-Na (a) and BrdU vs. BrdU + DMG-Na (b) comparisons (# P < 0.05, ## P < 0.01, ### P < 0.001, #### P < 0.0001).

senescence-associated markers further corroborating that DMG-Na may indeed exert anti-senescence actions.

Comparable results were observed in the BrdU model across both cell types (Table 2). As anticipated, BrdU treatment led to an

upregulation of *MMP1*, *MMP3*, *P21* (*CDKN1A*), *P16INK4A* (*CDKN2A*), *TNFA*, *IL1B*, and *IL6*, alongside a corresponding downregulation of *MKI67*, *COL1A1*, *COL3A1*, and *LMNB1* after 24 and/or 48 h of exposure in both HF-DPCs and i-DPCs, indicative of senescence induction

Table 1Effects of DMG-Na on multiple senescence markers in the H₂O₂ model on HF-DPCs and i-DPCs in vitro (qRT-PCR assessment).

Genes	HF-DPCs				i-DPCs			
	24 h		48 h		24 h		48 h	
	H ₂ O ₂ (FC)	H ₂ O ₂ +DMG-Na (FC)	H ₂ O ₂ (FC)	H ₂ O ₂ +DMG-Na (FC)	H ₂ O ₂ (FC)	H ₂ O ₂ +DMG-Na (FC)	H ₂ O ₂ (FC)	H ₂ O ₂ +DMG-Na (FC)
MMP1	1.22↑*	1.04 (pr,[#])	4.09↑	3.53 (pr,t)	8.63↑	7.91 (pr,t)	12.04↑**	4.98 (pr,[#])
MMP3	2.29↑****	1.88 (pr,[#])	2.13↑*	1.60 (pr,t)	9.97↑**	10.42	8.55↑	4.14 (pr,t)
P21	2.19↑**	1.75 (pr,t)	2.71↑**	2.21 (pr,t)	4.28↑**	2.46 (pr,t)	12.24↑**	3.93 (pr,[#])
IL1B	3.43↑****	2.04 (pr,^{####})	2.72↑**	1.11 (pr,^{##})	1.78↑	1.42 (pr,t)	4.74↑	3.64 (pr,t)
IL6	3.53↑	1.39 (pr,t)	1.31↑	-1.06 (pr,t)	-1.07	1.17	2.78↑	1.8 (pr,t)
MKI67	-18.07↓****	12.49 (pr,t)	-29.17↓****	-21.77 (pr,t)	-40.29↓****	-16.61 (pr,[#])	-149.44↓****	-42.62 (pr,^{####})
MTCO1	-1.44↓***	1.31	-3.09↓*	-1.99 (pr,t)	-2.75↓***	-1.11 (pr,[#])	-8.07↓****	-1.61 (pr,[#])
COL1A1	-2.75↓****	-1.17 (pr,^{####})	-5.29↓****	-4.01 (pr,t)	-3.81↓****	-2.22 (pr,^{##})	-3.15↓****	-2.04 (pr,[#])
COL3A1	-3.43↓****	1.29 (pr,^{##})	-16.57↓****	-12.87 (pr,t)	-3.98↓****	-2.31 (pr,^{##})	-3.43↓****	-1.73 (pr,[#])
LMNB1	-8.82↓****	-7.12 (pr,t)	-14.70↓****	-11.53 (pr,t)	-16.11↓****	-5.15 (pr,^{####})	-32.14↓****	-17.26 (pr,^{##})
SIRT1	-1.24	-1.02	-1.98↓	-1.50 (pr,t)	-1.69↓**	1.15 (pr,^{##})	-1.02	1.05
NRF2	-1.27↓	1.01 (pr,t)	-1.35↓	-1.28	-1.41↓*	-1.08 (pr,t)	-1.05	-1.01

The table presents pooled fold change (FC) values of 4 independent experiments.

For the comparison of the H₂O₂ group compared with Control

- ↑ represents upregulation. Upregulation is defined if the expression of the given gene was statistically significantly higher and/or at least by 0.25 FC value higher in the respective H₂O₂ group compared with Control (FC = 1)
 - ↓ represents downregulation. Downregulation is defined if the expression of the given gene was statistically significantly lower and/or at least by 0.25 FC value lower in the respective H₂O₂ group compared with Control (FC = 1)
 - * marks statistically significant difference; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$
- For the comparison of the H₂O₂+DMG-Na and the H₂O₂ groups
- ‘pr’ represents prevention, i.e. DMG-Na prevents the effect of H₂O₂
 - when prevention is statistically significantly proven, # marks statistically significant difference; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$
 - when prevention proves to be statistically not significant but the value in the H₂O₂+DMG-Na groups (when compared with the H₂O₂ group) is at least by 0.25 FC value lower (in the case of the genes that were upregulated by the inducer) or higher (in the case of the genes that were downregulated by the inducer), prevention is considered tendential and marked with a letter ‘t’
 - to highlight the effect of DMG-Na (i.e. prevention of the effect of the inducer), **prevention is shown in bold fonts**, irrespective of whether it is statistically significant or tendential

(see Table 2 for statistical details). Notably, co-treatment with DMG-Na mitigated the BrdU-induced modulation of several senescence-associated markers, reaching statistically significant prevention for MMP1, MMP3, P16INK4A (CDKN2A), TNFA, IL1B, IL6, COL1A1, COL3A1, and LMNB1. However, the overall anti-senescence efficacy of DMG-Na appeared to be modestly reduced in the BrdU model compared with the H₂O₂ challenge and was slightly more pronounced in HF-DPCs than in i-DPCs.

The robust anti-senescence effects of DMG-Na observed in both cell types in both models were further substantiated using complementary methodologies that quantified selected senescence-associated markers at the protein level (the rationale for the selection of markers and the technologies can be found in the Supplementary text). Specifically, immunocytochemical analysis revealed that DMG-Na treatment significantly counteracted the BrdU-induced downregulation of the proliferation marker Ki67 in both HF-DPCs and i-DPCs (Fig. 2a and b). Moreover, ELISA analysis demonstrated that DMG-Na treatment significantly (in i-DPCs) or tendentially (in HF-DPCs) inhibited the BrdU-induced increase in IL-6 secretion, a key senescence-associated secretory phenotype (SASP) factor [13,14,25] (Fig. 2c). Furthermore, Western blot analysis of both cell types (Fig. 3) showed that H₂O₂ treatment significantly reduced lamin B1 protein levels while it increased the expression of p21 and MMP3. Notably, co-treatment with DMG-Na mitigated these effects; however, due to notable inter-experimental variability, the protective effect of DMG-Na was primarily tendential and reached statistical significance only for MMP3 in HF-DPCs.

Due the shortage of the number of cells of the primary HF-DPC cultures, we were able to carry out only prototypic experiments for

Ki67 in the H₂O₂ model and for Lamin B1 and p21 in the BrdU model. These experiments revealed that DMG-Na treatment prevented the H₂O₂-induced suppression of Ki67 (immunocytochemistry, Supplementary Figure 3) as well as the BrdU-induced downregulation of Lamin B1 and upregulation of p21 (Western blotting Supplementary Figure 4) in both HF-DPCs and i-DPCs.

Collectively, these findings – derived from multiple complementary methodologies applied to *in vitro* cultured primary and immortalized DPCs – unprecedentedly demonstrate that DMG-Na exhibits potent anti-senescence activity against both oxidative and non-oxidative pro-aging stimuli.

3.2. Formulation containing N,N-Dimethylglycine sodium salt (DMG-Na) improves hair loss in vivo

To evaluate the efficacy of DMG-Na in human subjects, two randomized, double-blind, placebo-controlled, parallel-group, sex-stratified clinical studies were conducted involving a total of 308 participants (154 males and 154 females) presenting with pattern hair loss or hair thinning. Human subjects provided written, informed consent prior to the study. The design of the studies for non-invasive surveys were approved by the internal revision committee of the Sapienza University of Rome, Italy. In each study, 77 participants received either a verum tonic containing 0.5% DMG-Na and 0.2% caffeine or placebo (identical scalp care properties without bioactives), applied daily over a 6-month period. In these studies, male participants ranged in age from 18 to 65 years, with mean ages of 38.8 and 36.8 years in the verum and placebo groups, respectively. Female participants were aged 30–65 years, with corresponding

Table 2

Effects of DMG-Na on multiple senescence markers in the BrdU model on HF-DPCs and i-DPCs in vitro (qRT-PCR assessment).

Genes	HF-DPCs				i-DPCs			
	24 h		48 h		24 h		48 h	
	BrdU (FC)	BrdU+DMG-Na (FC)	BrdU (FC)	BrdU+DMG-Na (FC)	BrdU (FC)	BrdU+DMG-Na (FC)	BrdU (FC)	BrdU+DMG-Na (FC)
MMP1	1.414†	-1.29 (pr,t)	1.22†*	-1.08 (pr,##)	1.14	1.22	2.54†	2.75
MMP3	1.4†	-1.59 (pr,t)	1.74†**	1.15 (pr,#)	1.79†	1.04 (pr,t)	2.14†	1.61 (pr,t)
P21	2.30†	1.13 (pr,t)	1.70†****	1.33 (pr,##)	1.07	1.09	1.66†	1.51
P16INK4A	2.29†	-1.01 (pr,t)	1.71†**	1.04 (pr,##)	1.05	1.10	1.31†	1.11
TNFA	3.46†	2.41 (pr,t)	7.11†**	3.85 (pr,#)	5.53†****	2.11 (pr,###)	26.5†	19.8 (pr,t)
IL1B	1.05	-1.71	1.14	1.02	1.21†	1.37	4.99†**	2.21 (pr,#)
IL6	1.11	-1.12	2.31†	1.05 (pr,t)	1.67†**	-1.33 (pr,##)	3.73†**	1.44 (pr,#)
MKI67	-1.13	-1.03	-2.95↓****	-2.69 (pr,t)	-1.38↓	-1.18	-2.51↓*	-1.19 (pr,t)
COL1A1	-1.41↓	-1.07 (pr,t)	-1.11	1.01	-2.65↓**	-1.49 (pr,#)	1.21	1.05
COL3A1	-1.36↓*	-1.10 (pr,t)	-1.62↓*	-1.3 (pr,t)	-1.88↓**	-1.06 (pr,#)	-1.70↓	1.75
LMNB1	-1.09	1.21	-2.78↓****	-2.75	-1.21	-1.13	-2.52↓***	-1.52 (pr,#)

The table presents pooled fold change (FC) values of 4 independent experiments.

For the comparison of the BrdU group compared with Control

– † represents upregulation. Upregulation is defined if the expression of the given gene was statistically significantly higher and/or at least by 0.25 FC value higher in the respective BrdU group compared with Control (FC = 1)

– ↓ represents downregulation. Downregulation is defined if the expression of the given gene was statistically significantly lower and/or at least by 0.25 FC value lower in the respective BrdU group compared with Control (FC = 1)

– marks statistically significant difference; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001

For the comparison of the BrdU+DMG-Na and the BrdU groups

– ‘pr’ represents prevention, i.e. DMG-Na prevents the effect of BrdU

– when prevention is statistically significantly proven, # marks statistically significant difference; # P < 0.05, ## P < 0.01, ### P < 0.001, #### P < 0.0001

– when prevention proves to be statistically not significant but the value in the BrdU+DMG-Na groups (when compared to the BrdU group) is at least by 0.25 FC value lower (in the case of the genes that were upregulated by the inducer) or higher (in the case of the genes that were downregulated by the inducer), prevention is considered tendential and marked with a letter ‘t’

– to highlight the effect of DMG-Na (i.e. prevention of the effect of the inducer), prevention is shown in bold fonts, irrespective of whether it is statistically significant or tendential

mean ages of 51.3 (verum) and 51.9 (placebo). The degree of hair loss severity at Baseline is detailed in [Supplementary Tables 2 and 3](#).

As already mentioned above, the current FPHL and MPHL studies represent an extension of our prior, recently published human trial [20], albeit with notable differences. Specifically, in the prior human study, we assessed the effects of a shampoo (i.e. a rinse-off formulation) in a smaller cohort involving a smaller number of male subjects. In the current trials, however, we evaluated a novel topical ethanol-based leave-on formulation and, importantly, included a substantially larger and more diverse cohort of both men and women with pattern hair loss.

In both current studies, the primary efficacy endpoint was the change in the number of hairs pulled at 3 and 6 months in the Hair Pull Test relative to Baseline. Notably, participants using the verum tonic demonstrated a significantly greater reduction in hair shedding at both time points compared with those receiving placebo (both $P < 0.001$; exact Mann–Whitney test), in both the male ([Fig. 4a](#)) and female ([Fig. 4b](#)) cohorts. Additionally, the exact Friedman test revealed a significant reduction in hair shedding among male participants using the verum tonic (16.3 ± 1.1 at Baseline vs. 13.1 ± 2.1 at 6 months; $P < 0.001$), while a slight but statistically significant increase was observed in the placebo group (16.3 ± 1.2 at Baseline vs. 17.1 ± 2.2 at 6 months; $P = 0.008$). Similarly, in the female cohort, the number of pulled hairs significantly decreased following verum tonic application (16.6 ± 1.0 at Baseline vs. 13.0 ± 2.5 at 6 months; $P < 0.001$), in contrast to a significant increase seen with placebo treatment (16.4 ± 1.0 at Baseline vs. 17.1 ± 1.8 at 6 months; $P = 0.001$) ([Supplementary Figure 5a](#), [Supplementary Table 4](#)). Notably, covariance analysis

indicated no significant sex-related effect on treatment response after 6 months ($P = 0.24$), indicating that the efficacy of the intervention was sex independent.

Phototrichograms and trichoscopic analyses were conducted on a subset of 60 selected participants (15 per group per sex). Detailed quantitative outcomes are presented in [Supplementary Tables 5–7](#). The analyses demonstrated that treatment with the verum tonic significantly reduced hair loss and promoted hair growth compared with placebo. In both male and female participants, significant improvements were observed in hair density ([Fig. 4c and d](#)) and the proportion of anagen hairs ([Fig. 4e and f](#)), alongside a reduction in the percentage of telogen hairs ([Supplementary Figure 6a and b](#)) after 3 and 6 months of treatment (3 months vs. Baseline and 6 months vs. Baseline; $P < 0.001$, t -test). Specifically, mean hair density increased by 49.1 hairs/cm² in males and 58.0 hairs/cm² in females following verum application ([Supplementary Figure 5b](#), [Supplementary Table 5](#)). Although Baseline values showed a minor but significant difference between sexes ($P = 0.021$), covariance analysis confirmed no statistically significant sex-based difference in treatment efficacy at 6 months when comparing hair density changes between verum and placebo groups ($P = 0.68$). Representative phototrichogram images of the verum groups, both in the female and male arms of the study, can be found in [Supplementary Figure 7](#).

Collectively, these results provide unprecedented evidence supporting the therapeutic potential of DMG-Na as an efficacious topical agent for the management of male and female pattern hair loss.

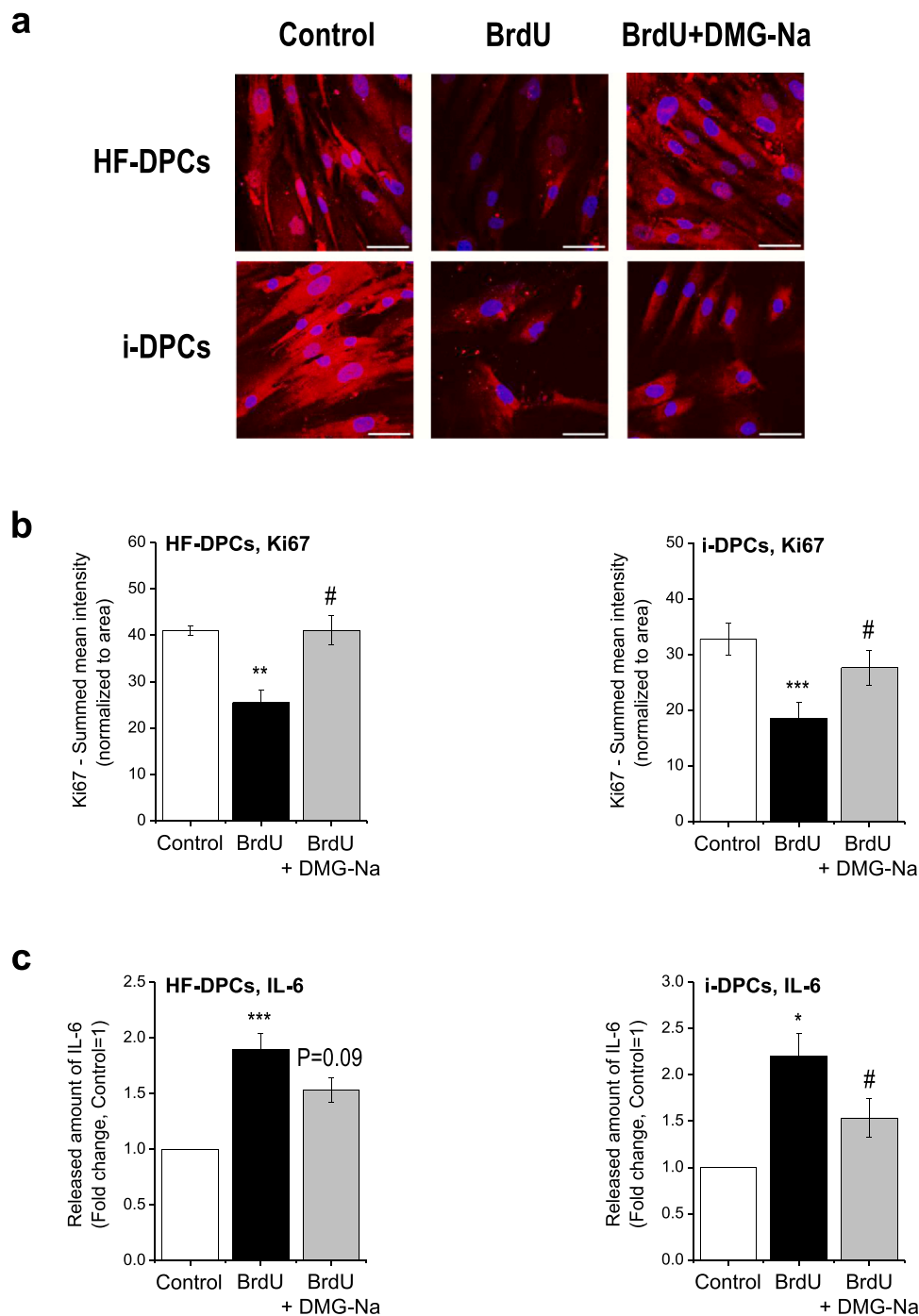


Fig. 2. DMG-Na prevents the effect of BrdU to downregulate the expression of Ki67 and to upregulate the secretion of IL-6 in HF-DPCs and i-DPCs *in vitro* (a, b) Immunofluorescence assessment of Ki67 in the BrdU model in HF-DPCs and i-DPCs measured after 48 h of treatment. (a) Representative images of Ki67 immunofluorescence labeling (Ki67: purple, DAPI: blue). Scale bar = 50 μ M. (b) Statistical analysis of Ki67 immunofluorescence intensity. (c) Statistical analysis of ELISA determination of IL-6 secretion in the BrdU model in HF-DPCs and i-DPCs measured after 48 h of treatment. (b, c) Graphs show pooled data (mean $\pm$ SEM) of $n = 3$ –4 independent experiments (12–15 independent visual fields for the Ki67 labeling) on both cell types. For P-values, * marks the statistical difference compared with the control (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) whereas # marks the statistical difference measured in the BrdU vs. BrdU + DMG-Na comparisons (# $P < 0.05$).

4. Discussion

In the *in vitro* arm of our current work, we provided compelling evidence that DMG-Na exerts robust anti-senescence effects on human HF-derived DP cells. Indeed, DMG-Na, in most cases statistically significantly, prevented the increase of SA- β -Gal positive cells and altered the expression of multiple senescence-associated markers in response to pro-senescence stimuli. Therefore, albeit further studies may be required to support this, DMG-Na appeared to exert

universal (i.e. independent from the nature of the stressor) anti-senescence actions. Namely, DMG-Na effectively prevented the pro-senescence effects of both the oxidative challenge of H_2O_2 (mediated by the generation of reactive oxygen species, which in turn, results in the activation of the DNA damage response pathway, telomere shortening, protein and lipid oxidation, lipofuscin accumulation, mitochondrial dysfunction, etc.) [26], and the non-oxidative stimuli BrdU (which by incorporating into the DNA, destabilizes nucleosomes and disrupts chromatin structure, thereby leading to a DNA

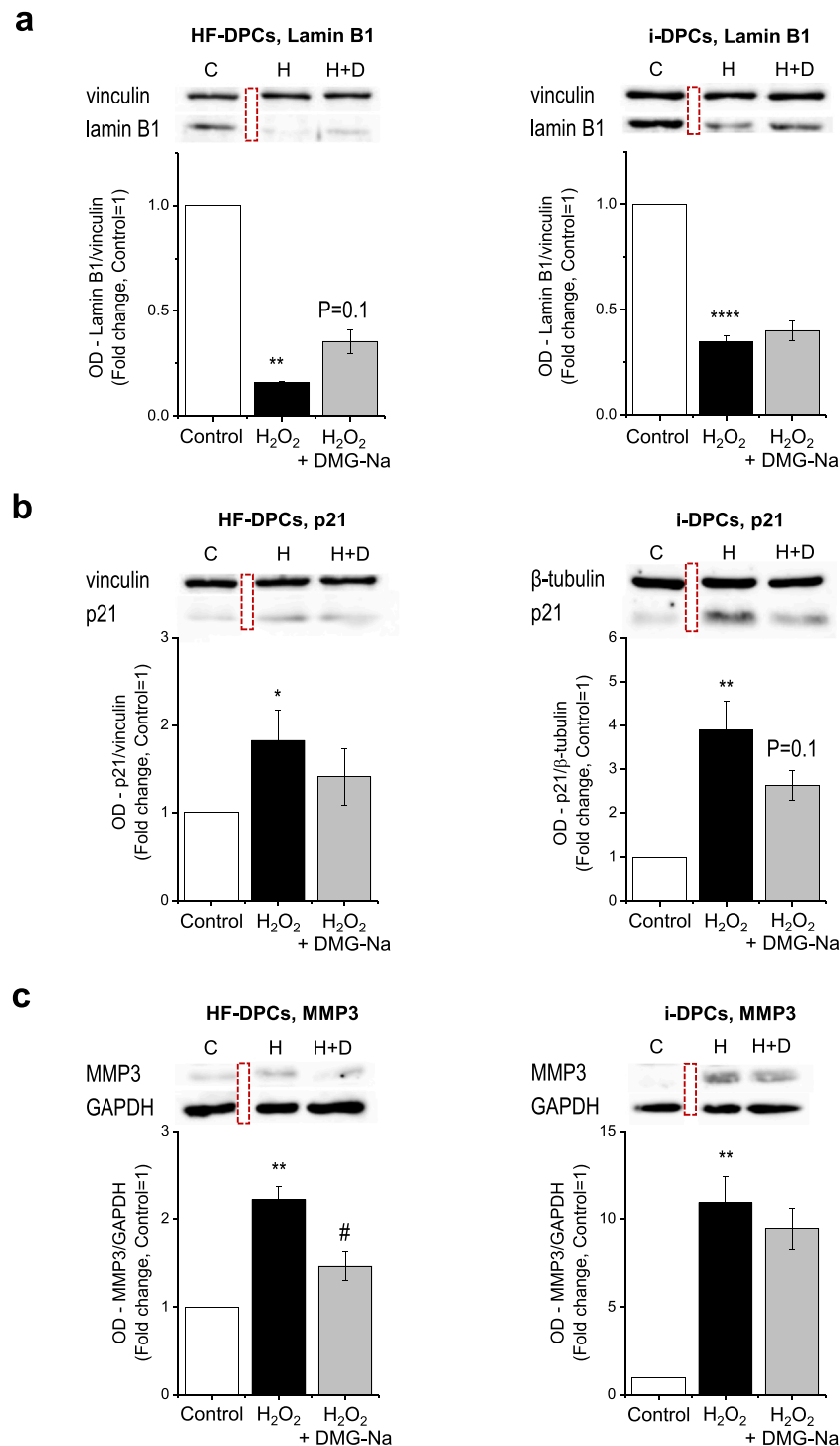


Fig. 3. DMG-Na prevents the effect of H₂O₂ to downregulate the expression of lamin B1 and to upregulate the expression of p21 and MMP3 in HF-DPCs and i-DPCs *in vitro*. Western blot measurement in the H₂O₂ model in HF-DPCs and i-DPCs measured after 48 h of treatment. Representative Western blot immunolabeling and statistical analysis of optical density measurements on immunoblots of protein expressions of (a) lamin B1, (b) p21 and (c) MMP3 as well as the respective housekeeping proteins, used as loading controls, vinculin, β-tubulin and GAPDH. Graphs show pooled data (mean ± SEM) of n = 3–4 independent experiments on both cell types. For P-values, * marks the statistical difference compared with control ('C' in the immunoblots) (* P < 0.05, ** P < 0.01, **** P < 0.0001) whereas # marks the statistical difference measured in the H₂O₂ ('H' in the immunoblots) vs-H₂O₂ + DMG-Na ('H+D' in the immunoblots) comparisons (# P < 0.05). The separating boxes in the immunoblots indicate that the blots were spliced.

damage response and cell cycle arrest) [27]. While our experiments were initially conducted with i-DPCs and subsequently validated with HF-DPCs, being aware of the technical difficulties in obtaining and culturing primary HF-DPCs as well as in light of the recently published data showing that primary HF-DPCs rapidly (within 5 passages) enter into senescence upon *in vitro* culturing [14], our data argue for that i-DPCs can be used as proper surrogates to assess DP

cell functions, at least for exploring the anti-senescence activity of candidate hair molecules.

Our current findings show that DMG-Na significantly prevented the effects of pro-senescence stimuli to downregulate the expression of the proliferation marker Ki67 in DPCs. This is in perfect agreement of our previous data from human epidermal keratinocytes, where DMG-Na markedly promoted cellular proliferation and migration

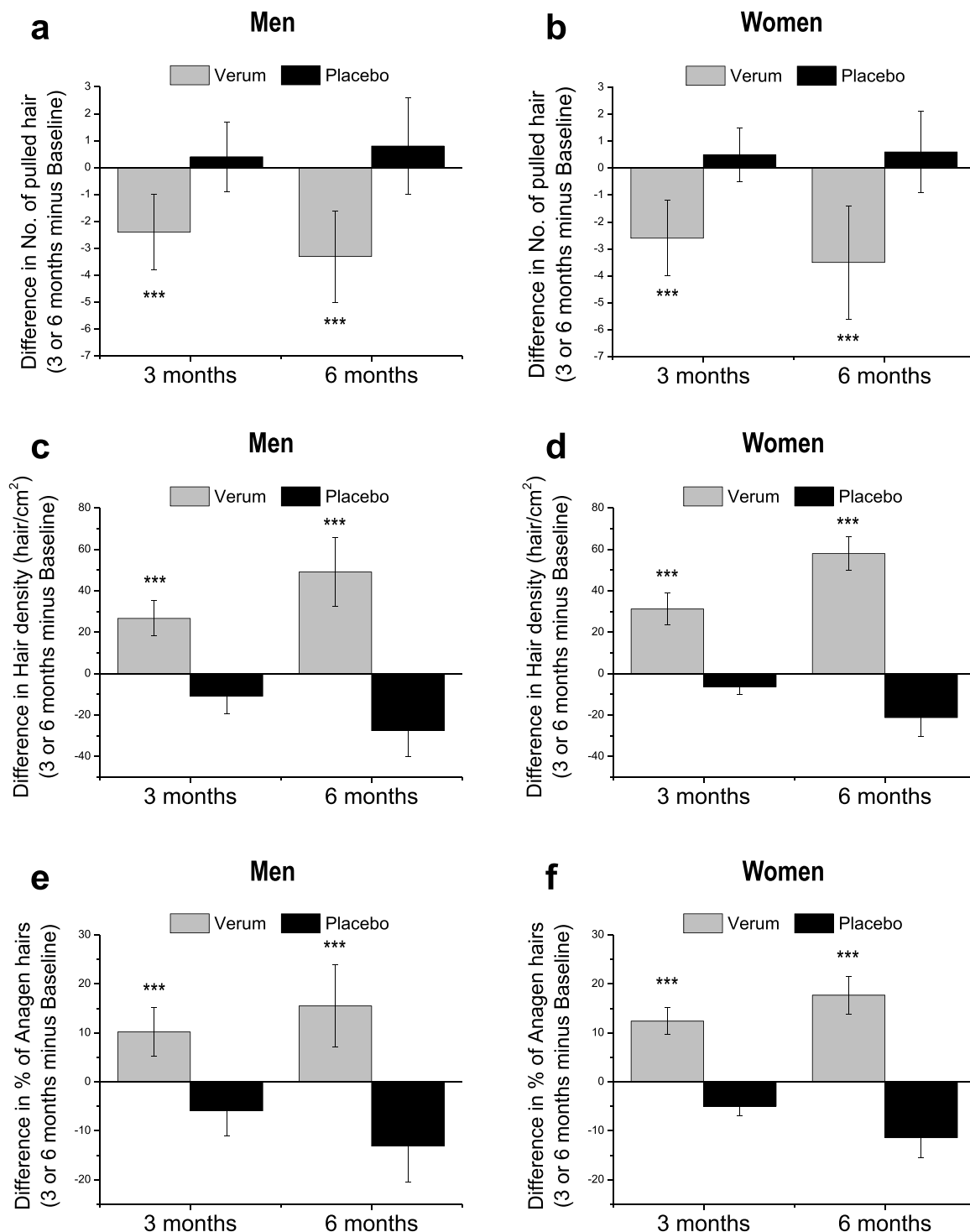


Fig. 4. Change in hair loss parameters after Verum and Placebo tonic application in vivo (Hair pull test, Hair density and Percentage of Anagen hairs) (a, b) Difference in the number of pulled hairs between values measured after 3 or 6 months of treatment and at Baseline in (a) men (n = 77 subjects for each group) and (b) women (n = 77 subjects for each group). (c, d) Difference in the hair density (hair/cm²) between values measured after 3 or 6 months of treatment and at Baseline in (c) men (n = 15 subjects for each group) and (d) women (n = 15 subjects for each group). (e, f) Difference in the percentage of anagen hairs between values measured after 3 or 6 months of treatment and at Baseline in (e) men (n = 15 subjects for each group) and (f) women (n = 15 subjects for each group). For all panels, graphs show pooled data (mean ± SEM) of values measured on multiple individuals (for details, see [Supplementary Materials](#) and Methods). For P-values, * marks the statistical difference measured in the Verum vs. Placebo comparisons (***P < 0.0001) at 3 and 6 months of treatment.

[18]. Furthermore DMG-Na prevented the upregulation of pro-catabolic SASP molecules as e.g., IL-1 β , IL-6, and TNF- α [28,29], and downregulation of pro-anagen molecules as e.g., sirtuins [30]. The effect of DMG-Na in preventing the senescence-induced upregulation of IL-6 is especially intriguing as this SASP was shown to play a central role in blocking telogen to anagen transition of HF, a key

pathogenetic event in the development of hair loss [13]. Previous data from human epidermal keratinocytes showed that DMG-Na also upregulated the expression of growth factors [18] among which VEGF is of particular interest since it was extensively shown to prolong anagen and hence promote hair growth [31], and also exhibits anti-aging actions [32]. Moreover, in previous investigations

we also found that DMG-Na induced the release of NO from HDMECs [19] which is also regarded as a hair growth-promoting molecule [33]. Taken together, DMG-Na appears to target several key markers and molecules at the cellular level, inducing pro-proliferative effects that are crucial for optimal hair growth conditions.

The above mentioned pro-catagen SASP cytokines (i.e. IL-1 β , IL-6, and TNF- α) also play key roles in various skin inflammatory processes [34] which also contribute to pathological hair loss, when the scalp is affected. With this regard it is noteworthy that, in various inflammation models of human epidermal keratinocytes, DMG-Na exerted profound anti-inflammatory effects [18]. Moreover, DMG-Na also prevented the effect of UVB irradiation to boost the production of reactive oxygen species *in vitro* [18]; this anti-oxidant effect of DMG-Na could be exploited in such conditions when hair loss is induced by pro-oxidative stimuli.

In addition to the above discussed *in vitro* results, topical application of a gel containing 1% DMG-Na to male and female subjects enhanced skin blood flow (most probably via engaging endothelium and NO-mediated vasodilation) thereby resulting in an increased nutrition supply to all skin compartments. Recently, Deng et al. [35] identified microvascular abnormalities in the DP of balding scalp compared with non-balding scalp of patients with AGA, indicating malnutrition of affected hair follicles. Therefore, the vasodilatory and associated pro-nutrition effects of topical DMG-Na certainly also contribute to its beneficial action to promote and maintain physiological hair growth.

In the *in vivo* arm of our current study, we provided data that DMG-Na serves as a highly effective candidate for the topical management of hair loss (as indicated by findings from our aforementioned smaller human trial, [20]). Indeed, the DMG-Na containing verum formulation (when compared to placebo) statistically ($P < 0.001$ for all parameters) significantly decreased the number of pulled hairs and the % of telogen hairs as well as increased hair density and the % of anagen hairs after both 3 and 6 months of treatment. Of remarkable importance, there were no statistically detectable differences between the effects of DMG-Na on male and female patients suggesting that DMG-Na could be equally used for the successful topical therapy of hair loss seen in both FPHL and MPHL patients. It should be also noted that, the topical formulation with 0.5% DMG-Na used in the current human *in vivo* study also contained 0.2% caffeine, another bioactive for topical treatments [36]. While we acknowledge that the precise contributions of these two agents to the anti-hair loss efficacy of the tested topical formulations remain undefined, further *in vivo* investigations are necessary to clarify their distinct roles and relative impact on the treatment outcomes.

The synthesis of the above *in vitro* and *in vivo* data strongly argues that the anti-senescence actions of DMG-Na identified on cultured human DPCs may contribute to its robust anti-hair loss effects in FPHL and MPHL patients. Therefore, our current study is unprecedented to show direct effects of DMG-Na on cells derived from the controlling unit of the HFs (thereby “targeting the root of the problem”).

As detailed in the Introduction, male and female pattern hair loss is a multifactorial condition that necessitates a comprehensive target approach. Taking all of our current and previous results into account, especially in light of the aforementioned “universal need” for multi-acting agents, topically applied DMG-Na is proposed to prevent hair loss and fuel physiological hair growth by directly or indirectly acting on various cutaneous cell populations. These effects may result in a complex amalgamation of several biological mechanisms, including (i) promoting nutritional skin blood supply, (ii) cell proliferation, (iii) growth factor production, as well as (iv) HF anagen prolongation and telogen to anagen transition; (v) preventing premature and/or pathological senescence as well as catagen “shift”; and (vi), as shown in the current study, inhibiting

pro-inflammatory and pro-oxidative processes. Therefore, we believe and our data strongly suggest that DMG-Na is an effective active for the future management of hair conditions characterized by impaired cellular growth, repair, and dysregulated oxidative metabolic status.

CRediT authorship contribution statement

Conceptualization: JM, JMV, ESzW, LC, AR, AB; Methodology: GB, AL, EH, MVT, AlaL, ÁL; Writing-Original Draft Preparation: JM, GB, TB; Writing-Review and Editing: JM, AL, GB, JMV, ESzW, LC, AR, TB; Visualization: AL, GB, TB; Supervision: TB, JMV, ESzW, AR, JM; Project Administration: GB, EH.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Johanna Mihály reports financial support was provided by Dr Kurt Wolf GmbH & Co KG. Jorn Michael Volker reports financial support was provided by Dr Kurt Wolf GmbH & Co KG. Erik Schulze zur Wiesche reports financial support was provided by Dr Kurt Wolf GmbH & Co KG. Erik Schulze zur Wiesche reports financial support was provided by Dr. August Wolff GmbH and Co KG Arzneimittel. Lorenzo ALA reports a relationship with Lilly, Dercos, Cantabria, Italy that includes: funding grants. Attila Bacsi reports a relationship with Hungarian Research Grants - the National Research Development and Innovation Office that includes: funding grants. Jorn Michael Volker has patent issued to Dr. Kurt Wolff GmbH and Co. KG. Erik Schulze zur Wiesche has patent issued to Dr. Kurt Wolff GmbH and Co. KG. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdermsci.2026.06.002.

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