Paeonia Suffruticosa root bark extract synergizes with the α-MSH biomimetic peptide Greyverse™ to stimulate pigmentation in human hair follicles

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Abstract (Maximum of 250 words)

Background: Hair greying results from the loss of melanin pigment production in the hair follicle and reduced melanin deposition within the hair shafts. Individuals often suffer from psychological distress and therefore, the development of agents to prevent or reverse hair greying is highly demanded. Here, we analyze whether the αMSH biomimetic synthetic peptide Greyverse™ (Gv) together with a plant extract from *Paeonia suffruticosa* (PS), which possesses anti-oxidative effects in skin, synergistically or additively modulate melanogenesis in human HF melanocytes *ex vivo*.

Methods: We used microdissected human HFs and exposed them to Gv, PS, or Gv+PS *ex vivo.* Subsequently we assessed melanin content, and protein and enzymatic changes associated with pigmentation were analysed in the pigmentary unit or outer root sheath of HFs remaining in anagen VI by quantitative (immune-)histomorphometry.

Results: We show that PS and Gv co-treatment leads to an additive increase in melanin content ($p<0.05$), a synergic increase in Gp100 expression ($p<0.05$), an increased tyrosinase activity, and elevated αMSH and TYRP expression within the hair follicle pigmentary unit. Additionally, PS tendentially increased outer root sheath, αMSH expression, whilst Gv had little effect.

Conclusion: In conclusion, we provide evidence that hair pigmentation is promoted by a synergistic effects of PS and Gv, primarily through induction of melanogenesis in the HF.

Keywords: Plant extract, canities, hair treatment, α-MSH/MC1-R, pre-clinical assay

Introduction.

Premature greying of hair, also known as Canities, is an age-linked, progressive reduction of natural hair pigmentation through a loss of bulb melanocytes in hair follicles (HF) [1]. It can significantly alter the physical appearance of a person thereby negatively influencing their well-being, which drives the search and demand for new anti-greying agents. *Paeonia Suffruticosa* root bark extract (PS), has long been used in Chinese traditional medicine, since it is supposed to lower blood sugar and -pressure, and possess anti-inflammatory properties [2]. Additionally, PS has previously been shown to positively influence skin function, by exerting anti-oxidant effects, thereby protecting the skin from UVB-induced photo ageing, as demonstrated in mice [2, 3], and by stimulating cell viability of HaCaT keratinocytes [4]. However, the effect of PS on HF function, and particularly melanogenesis, has not been investigated yet. Interestingly, our preliminary studies revealed that PS, in combination with the alpha-melanocytes-stimulating hormone (αMSH) biomimetic synthetic peptide Greyverse™ (Gv), synergistically induces mitochondrial E3 ligase (MITOL) and melanogenesis in human melanocytes *in vitro*. αMSH is a known, pro-pigmentary hormone of the melanocortin family, which stimulates melanogenesis through its target receptor, MC1. In line, the biomimetic Gv has been established as promoter of HF melanogenesis with redox properties [5]. Therefore, in this study, we aimed at investigating whether PS and Gv, synergistically or additively, modulate melanogenesis in human HF melanocytes *ex vivo*.

Materials and Methods.

We organ cultured healthy human microdissected HFs *ex vivo* [6-7] for four days in serum-free medium, supplemented with PS [250ppm, 0.025%] and/or Gv [10,000ppm, 1%]. On day 3, the medium (including supplements) was changed. The HFs were snap-frozen in OCT and assessed for changes in melanin content of hair matrix melanocytes by Masson-Fontana staining. Protein expression and enzymatic changes associated to melanogenesis were examined by quantitative immune-histomorphometry. We quantified expression of tyrosinase-related protein 1 (TYRP), the pre-melanosome protein Gp100 and the activity of tyrosinase, which is the rate-limiting enzyme of melanin synthesis in the hair matrix (HM),

as well as αMSH expression in the outer root sheath (ORS) [8, 9]. All data are presented as mean ± SEM, representing a total of 9-14 anagen hair follicles from three donors. To statistically compare the different treatment groups, Kruskal Wallis- or Mann Whitney U-Tests were used.

Results.

In a first step, we analysed the melanin content in the pigmentary unit of anagen HFs under PS and Gv single-, and co-treatment. We only detected a tendential increase of melanin content under PS treatment alone, whereas application of Gv alone significantly enhanced it. This effect was further increased under co-treatment of Gv and PS. To unravel the underlying factors driving the enhanced melanin content, we examined tyrosinase activity, as rate-limiting enzyme of melanin synthesis, in the pigmentary unit of anagen HFs. Interestingly, treatment with PS alone and in combination with Gv tendentially increased tyrosinase activity, whereas application of Gv alone led to a significantly increased activity. Besides tyrosinase, also TYRP plays a crucial role in melanogenesis, by stabilizing tyrosinase and regulating its enzymatic activity [10]. Therefore, we also investigated expression of TYRP. However, treatment with PS and GV alone did not affect TYRP expression and the combination resulted only in a synergistic tendency, but not significant increase of TYRP expression. Next, we analysed expression of the pre-melanosome protein Gp100. Application of PS alone, as well as co-treatment of Gv and PS synergistically, resulted into significantly increased Gp100 expression within the pigmentary unit of anagen hair follicles. In a last step, we assessed αMSH expression in the HF ORS. However, we only detected a tendentially increase in αMSH expression under PS treatments, whilst Gv alone and the combination of PS and Gv had no effect.

Discussion.

We provide evidence that PS combined with Gv have synergic effects in promoting hair pigmentation. Gv is mainly acting on tyrosinase activity, which is in accordance with the literature, where it has been repeatedly shown that its α-MSH enhances tyrosinase activity [11]. Interestingly, PS seems to directly affect melanosomes, as demonstrated by an increased Gp100 expression. Thus, we are the first to provide evidence, that PS is involved in

melanosome maturation in HFs *ex vivo*. In line with our *ex vivo* results, in a clinical study with male volunteers, Gv decreased the overall whiteness of hair and darkened grey hair after three months by stimulation of melanosome biogenesis and eumelanin synthesis in plucked hairs [5]. Therefore, it is of interest to investigate whether PS in combination with Gv can further reduce the percentage of canities *in vivo.* Besides this, investigating the underlying mechanisms of PS and/or PS in combination with Gv on melanogenesis in HFs is of great interest, too. One approach could be to examine the effect of PS on oxidative damage in the HF, since various of the molecular compounds found in PS extract possess strong anti-oxidant effects [12] and it is widely accepted that physiological hair greying results, in part, from accumulation of oxidative damage generated during normal metabolism [13].

Conclusion.

Taken together our results demonstrate that the combinational treatment of PS and Gv deserves further pre-clinical and clinical exploration as formulation supporting the reversal of hair greying.

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Conflict of Interest Statement. D.B., M.vL., T.B., and M.B. are employees of Monasterium Laboratory GmbH, a contract research organization specializing in dermatology. A.T. works as cooperative researcher at , CellLab, NatureLab. Co., Ltd., Japan. A.R., S.K., A.A., N.T., and T.A. are employees of Research & Development Headquarters Self-Medication, Taisho Pharmaceutical Co., Ltd., Japan.

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