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SHORT REPORT

Topical applications of an emollient reduce circulating pro-inflammatory cytokine levels in chronically aged humans: a pilot clinical study

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Abstract

Background While increased levels of circulating inflammatory cytokines in chronologically aged humans have been linked to the development of ageing-associated chronic disorders (e.g., cardiovascular disease, type II diabetes, osteoporosis and Alzheimer's disease), approaches that reduce circulating cytokines are not yet available. In chronologically aged mice, we recently demonstrated that epidermal dysfunction largely accounts for age-associated elevations in circulating cytokine levels, and that improving epidermal function reduced circulating cytokine levels.

Objective We performed a pilot study to determine whether improving epidermal function reduces circulating pro-inflammatory cytokine levels in aged humans.

Methods Thirty-three aged humans were topically treated twice-daily for 30 days, with ≈ 3 mL of an emollient, previously shown to improve epidermal function, while untreated, aged humans and a cohort of young volunteers served as controls. Changes in epidermal function and levels of three key, age-related, plasma cytokines (IL-1β, IL-6 and TNFα) were measured at baseline and after treatment, using Luminex 200™ system.

Results We also found significantly higher baseline levels of IL-1β, IL-6 and TNFα in aged vs. young humans ($P < 0.001$), as previously reported. Topical applications of the barrier repair emollient significantly enhanced epidermal permeability barrier function ($P < 0.01$) and stratum corneum hydration ($P < 0.05$). In parallel, circulating levels of IL-1β and IL-6 normalized, while TNFα levels declined substantially.

Conclusion The results of this preliminary study suggest that a larger clinical trial should be performed to confirm whether improving epidermal function also can reduce circulating pro-inflammatory cytokine levels in aged humans, while also possibly attenuating the downstream development of chronic inflammatory disorders in the aged humans.

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Conflicts of interest

PME and MQM serve as consultants to Neopharm, Ltd., South Korea. An invention disclosure has been filed with the UCSF Office of Innovation, Technology & Alliances for the concept of preventing/treating systemic disorders using strategies that improve epidermal function.

Funding sources

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Introduction

Aged humans exhibit chronic, subclinical systemic inflammation, commonly termed 'inflammaging', potentially linked to the downstream emergence of several age-associated chronic disorders (e.g., atherosclerosis, type II diabetes, osteoporosis, Alzheimer's disease).[1–3] While the aetiology of this age-associated, systemic inflammation remains uncertain, our recent studies have shown that both aged human and mouse skin display sustained abnormalities in epidermal permeability barrier homeostasis, stratum corneum (SC) hydration, and elevations in SC pH,[4–6] each of which has been shown to independently provoke cutaneous inflammation. We showed further that disruption of the epidermal permeability barrier provokes (i) an increase in cutaneous cytokine production; and (ii) an increase in serum cytokine levels, independent of hepatic or T-cell involvement.[7] Moreover, chronologically aged mice display elevations in both cutaneous and circulating levels of cytokines; and directly pertinent to this study, improving epidermal function in aged mice reduced cutaneous, as well as circulating levels of three key, age-associated cytokines.[7] In this proof-of-concept preliminary pilot study, we assessed whether improving epidermal function with an emollient, containing a mixture of lipids that mimics the components of normal SC, lowers circulating levels of these same pro-inflammatory cytokines in aged humans.

Materials and methods

Human subjects and treatment protocol

Based on the results of our prior study in aged humans, we performed N power calculations for required number of subjects, which showed that 25 subjects should suffice to detect significant reductions in circulating levels of IL-1β, IL-6 and TNFα. But because of an anticipated 20% case loss, we initially enrolled 30 + volunteers in both groups. Therefore, a total of 63 aged volunteers and 11 young volunteers (age 32.7 ± 0.9) were enrolled (Table 1). None of the subjects had a history of or visible signs of inflammatory skin diseases, or other known inflammatory disease. All stopped the use of topical skin care products, including soaps, for at least seven days prior to study entry. All of the aged subjects resided in the same assisted living facility, and alternately assigned to either the treated or untreated control group in order of registration (Fig. 1). Because a previous study had shown that topical applications of Atopalm® (Table S1), an over-the-counter, triple lipid formulation (Neopharm, #928 Tamnip-dong, Yuseong-gu, Daejeon, 305510, South Korea), improves epidermal function in humans,[8] we chose this formulation here. The entire skin surface of the treated, aged subjects was treated topically with ≈ 3 mL of this formulation twice-daily for one month, while the parallel, aged cohort remained untreated. To ensure consistency in the volume of cream applied, as well as the timing of each application, all topical applications were performed by designated, trained staff. The untreated, young control group were recruited from the residents of Dalian City. This pilot study was carried out during the early Spring (i.e., from 14 March to 14 April 2017), to prove the concept that improvements in epidermal function can lower circulating levels of cytokines. Because of the preliminary nature of this pilot study, we did compare the efficacy of Atopalm® to other products.

This human research protocol was approved by the Institutional Review Boards of Dalian Skin Disease Hospital, and registered with the Chinese Clinical Trials Registry (ChiCTR-RPC-16007717, 'Role of epidermal permeability barrier function in systemic inflammation'), dated 1 January 2016, in accordance with the Helsinki principles, and informed consent was obtained from participants prior to trial entry.

Measurement of plasma cytokines

Immediately prior to study entry and at the end of the study, blood samples were collected from both aged cohorts, and the young, control subjects for assessments of changes in circulating levels of the key, age-associated inflammatory markers. Cytokine levels were measured with Human Cytokine/Chemokine Magnetic Bead Panel (Item # HCYTOMAG-60k-05) (Millipore, Billerica, MA, USA), using Luminex 200^{tm} system (Austin, TX, USA).

Table 1 Demographic data of human subjects

Age group	Assigned treatment	Gender	Number	Age range (median)	Hydration	TEWL	pH
Young controls	Untreated	Females	9	29–38 (32)	32.73 ± 0.94	17.70 ± 1.16	5.47 ± 0.13
		Males	2				
Aged	Untreated	Females	23	58–93 (73)	28.63 ± 1.25	12.48 ± 0.91	5.61 ± 0.15
		Males	7				
	Treated	Females	21	58–95 (82)	26.24 ± 1.16	13.66 ± 0.78	5.65 ± 0.16
		Males	12				

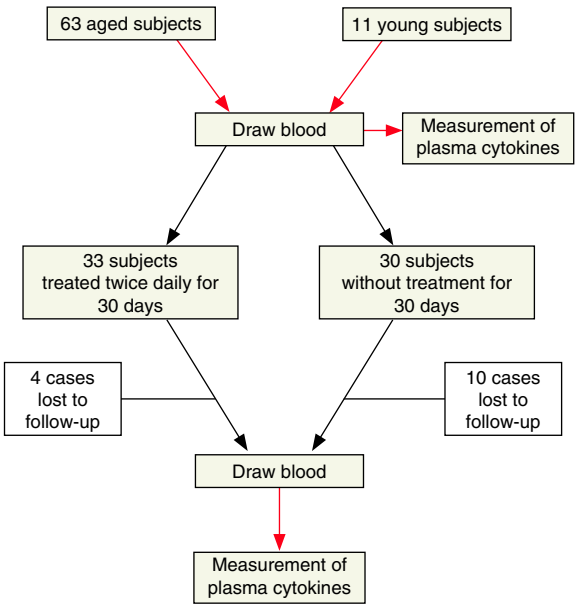

Figure 1 Flow chart of subject recruitment and treatment.

Measurements of epidermal function

A multifunctional skin physiology monitor (MPA5; Courage–Khazaka Electronic GmbH, Köln, Germany) was used to measure SC hydration (capacitance), skin surface pH and transepidermal water loss (TEWL) on the right forearm prior to and at the end of this study. Measurements were obtained in a room maintained at temperature of 18 ± 2°C and relative humidities of 60 ± 4%.

Subject exclusion

1 One young control subject was excluded because his baseline cytokine levels were >80 times higher than the next highest in the same cohort. The Q-test determined that exclusion was appropriate (Q = 0.99).
2 After treatment, IL-6 levels in one subject in the untreated, aged group were 3 times higher than the next highest level. The Q-test again determined that exclusion of this subject was appropriate (Q = 0.7138).

Statistics

All data are expressed as the mean ± SEM, using GraphPad Prism 4 software (San Diego, CA, USA) for statistical analyses. A paired t-test was used to determine the differences in epidermal function between pre- and post-treatment. Unpaired t-test with Welch's correction was used to determine the significances of differences between young and aged group, and between treated and untreated aged group. A one-way ANOVA with Dunnett's multiple comparison test was used to determine the significant differences between treated and untreated aged vs. young groups. In cases of missing data, last observation carried forward imputation method was also used to analyse the data. Because missing data in the present study were 'Missing at Random', and are usually ignorable, data were not analysed by multiple imputation; instead, a common 'complete case analysis' was used. Significances are indicated in figure legends.

Results

Topical applications of physiologic lipid-containing formulation improve epidermal functions

Aged humans display multiple functional abnormalities, including elevations in SC pH, decreased SC hydration and impaired epidermal permeability barrier homeostasis.[4–6] Our previous studies showed that topical applications of this formulation improved SC hydration and epidermal permeability barrier homeostasis in both humans and mice.[8,9] As shown in Fig. 2, 30 days of treatment with Atopalm® markedly increased SC hydration, while also significantly lowering surface pH vs. pretreatment levels. Although transepidermal water loss rates declined in both treated and untreated groups, a more significant reduction was observed in the treated group ($P < 0.01$, vs. pretreatment). These results indicate that this formulation improves key epidermal functions in aged humans.

Topical applications of physiologic lipid-containing formulation reduce circulating levels of IL-1β, IL-6 and TNFα in aged humans

Aged humans exhibit elevated circulating levels of several cytokines,[10] but of these cytokines, IL-1β, IL-6 and TNFα are considered most strongly associated with the development of

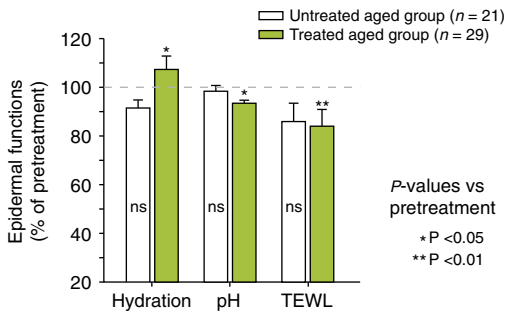

Figure 2 Topical treatments with physiologic lipid-containing formulation improve epidermal function in aged humans. Subjects and treatments were treated as described in Materials and Methods and Fig. 1. Data were expressed as per cent of pretreatment, setting pretreatment levels as 100%. Number of subject and *P* values are indicated in the figure.

age-associated chronic disorders.[11] Hence, we next compared basal levels (pretreatment) of these three cytokines in the plasma of aged vs. young humans (Table 1). As shown in Fig. 3a, basal levels of plasma IL-1β, IL-6 and TNFα were significantly elevated in chronologically aged individuals vs. young human controls (see also Ref.12). We next assessed whether repeated, topical applications of the physiologic lipid-containing formulation reduce circulating levels of IL-1β, IL-6 and TNFα in aged humans. After 30 days of twice-daily topical treatments, circulating levels of IL-1β and IL-6 decreased significantly in the treated aged cohort vs. the untreated aged controls (Fig. 3b). Surprisingly, these topical treatments reduced circulating levels of IL-1β and IL-6 to levels comparable to young controls (IL-1β: 13.8 ± 1.4 in treated aged vs. 10.5 ± 2.2 in young humans; IL-6: 5.7 ± 0.9 in treated aged vs. 5.1 ± 0.9 in young humans). Though levels of TNFα declined by over 40% in comparison with untreated aged humans, the difference did not attain statistical significance ($P = 0.1032$ vs. untreated aged controls). In consideration of missing data, we also analysed data using the 'last observation carried forward' method. The trend of changes in cytokine levels (Fig. S1) were similar to that described above. Together, these results suggest that improvements in epidermal function can largely normalize circulating levels of three pro-inflammatory cytokines that are strongly associated with chronologic ageing.

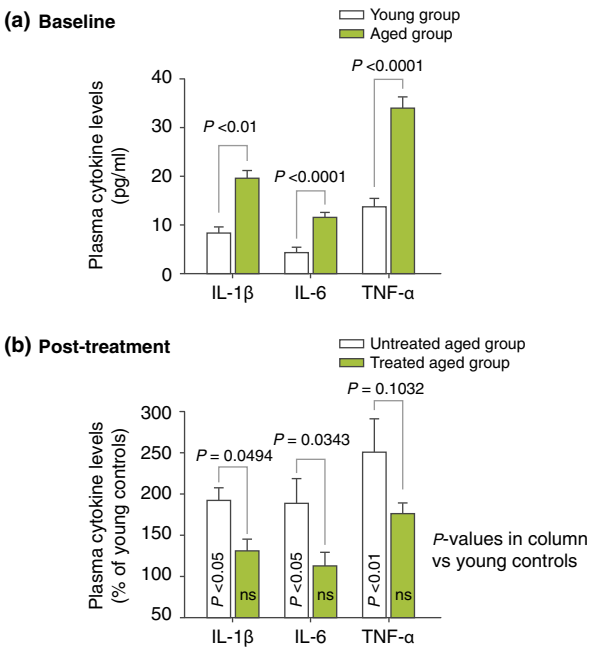

Figure 3 Circulating Levels of Inflammatory Cytokines in Chronically Aged Humans at Baseline and Post-Treatment. (a) Baseline levels of circulating inflammatory cytokines in young vs. aged humans. Significances are indicated in the figure. $N = 10$ for young group; $N = 57$ for aged group. (b) Changes in circulating levels of three inflammatory cytokines following topical applications of physiologic lipid-containing formulation. The significances are indicated in the figures ($N = 29$ in treated group; $N = 20$ in untreated controls [$N = 19$ for IL-6]).

Discussion

Among circulating cytokines known to be elevated in the elderly, IL-1β and IL-6 are considered of particular importance in the pathogenesis of age-associated disorders.[11] For example, serum levels of both IL-1β and IL-6 increase in several age-associated disorders, including cardiovascular disease, Alzheimer's disease and type II diabetes.[11] Moreover, elevated IL-6 levels have been linked to the development of other age-associated disorders, including lymphoma and osteoporosis,[12] and further associated with increased mortality in community-dwelling, aged adults.[13] Finally, very recent studies have demonstrated that canakinumab, an antibody directed against IL-1β, decreases cardiac events, independent of its effects on serum lipids.[14] Taken together, alleviation of systemic inflammation could in theory prevent and/or mitigate the development of certain age-associated chronic disorders, linked to 'inflammaging'.

Although a pathogenic role of 'inflammaging' in the development of systemic disorders in aged humans is widely acknowledged,[1–3,15,16] the possibility that the responsible cytokines could originate in the skin has not yet been explored. Yet, because of its sheer size, the skin is worthy of consideration as a potential contributor to systemic inflammation. In support of this potentially new paradigm, circulating levels of cytokines increase in inflammatory dermatoses that display prominent epidermal functional abnormalities, including atopic dermatitis and psoriasis, and both of these disorders are linked to the downstream development of chronic systemic disorders, such as atherosclerosis and type II diabetes. However, whether ageing causes inflammation or vice versa is not clear. At least in the skin, we postulated that ageing compromises epidermal functions, leading to productions of pro-inflammatory cytokines because a) aged skin displays multiple functional abnormalities, including compromised permeability barrier homeostasis, elevated SC pH and reductions in SC hydration, which all can increase pro-inflammatory cytokine production, and b) improvements in epidermal function can lower levels of pro-inflammatory cytokines in both the skin and circulation.[7] Despite the evidence from this pilot study, this hypothesis remains to be validated in future studies.

As early as age 50, chronologically aged humans exhibit multiple cutaneous functional abnormalities, which persist into advanced ageing,[4–6] paralleled by sustained elevations in several markers of cutaneous inflammation.[10] Because these elevations in cytokine expression could eventually provoke systemic inflammation, improvements in epidermal function could in theory prevent and/or mitigate systemic inflammation. Chronologically aged mice, with no clinical evidence of inflammation,

exhibit cutaneous functional abnormalities,[5,6] which are accompanied by elevated levels of certain cytokines in the skin and circulation.[7] Pertinently, disruption of epidermal permeability barrier function by repeated tape-stripping, and following acute exposure to erythemogenic UV-B provoke increases in the levels of cutaneous and circulating cytokines, including IL-1β, IL-6 and TNFα.[17,18]

Our recent studies showed that improving epidermal functions in chronologically aged mice skin normalizes not only cutaneous, but also serum levels of cytokines.[7] In this pilot study, we show that topical applications of a physiologic lipid-containing emollient, known to improve epidermal function in humans,[8] normalized circulating levels of key pro-inflammatory cytokines, closely associated with the development of chronic diseases in otherwise normal aged humans.

Yet, while the present pilot study provides evidence that topical applications of a physiologic lipid-containing emollient reduce circulating levels of certain cytokines in aged humans, the clinical significance of the present findings need to be addressed in proper controlled clinical trials. Whether this physiologic lipid-containing emollient also lowers levels of other inflammatory markers, such as amyloid A, also remains to be explored. Other remaining questions include whether other forms of topical therapy, which also improve epidermal functions, such as petrolatum or other emollients, are also effective; and what surface area of the body needs to be treated in order to lower circulating cytokine levels? Ultimately, it will be important to determine whether sustained improvements in epidermal functions can delay or attenuate the downstream development of chronic age-associated systemic disorders.

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Authors' contribution

LY, LH, ED, CY and CL performed the experiments and supervised the clinical studies; GW, TMM, KRF, LH, SJ and PME interpreted data; MQM originated the concept, designed experiments, analysed and interpreted data. TMM, PME and MQM wrote the manuscript.

References

1 Deleidi M, Jäggle M, Rubino G. Immune aging, dysmetabolism, and inflammation in neurological diseases. *Front Neurosci* 2015; **9**: 172.
2 Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* 2014; **69**: S4–S9.
3 Nadrowski P, Chudek J, Skrzypek M *et al.* Associations between cardiovascular disease risk factors and IL-6 and hsCRP levels in the elderly. *Exp Gerontol* 2016; **85**: 112–117.
4 Man MQ, Xin SJ, Song SP *et al.* Variation of skin surface pH, sebum content and stratum corneum hydration with age and gender in a large Chinese population. *Skin Pharmacol Physiol* 2009; **22**: 190–199.
5 Choi EH, Man MQ, Xu P *et al.* Stratum corneum acidification is impaired in moderately aged human and murine skin. *J Invest Dermatol* 2007; **127**: 2847–2856.
6 Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Invest* 1995; **95**: 2281–2290.
7 Hu L, Mauro TM, Dang E *et al.* Epidermal dysfunction leads to an age-associated increase in levels of serum inflammatory cytokines. *J Invest Dermatol* 2017; **137**: 1277–1285.
8 Jeong S, Lee SH, Park BD, Wu Y, Man G, Man MQ. Comparison of the efficacy of atopalm(®) multi-lamellar emulsion cream and physiogel(®) intensive cream in improving epidermal permeability barrier in sensitive skin. *Dermatol Ther (Heidelb)* 2016; **6**: 47–56.
9 Man MQM, Feingold KR, Thornfeldt CR, Elias PM. Optimization of physiological lipid mixtures for barrier repair. *J Invest Dermatol* 1996; **106**: 1096–1101.
10 Puzianowska-Kuźnicka M, Owczarz M, Wieczorowska-Tobis K *et al.* Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. *Immun Ageing* 2016; **13**: 21.
11 Dinarello CA, Donath MY, Mandrup-Poulsen T. Role of IL-1beta in type 2 diabetes. *Curr Opin Endocrinol Diabetes Obes* 2010; **17**: 314–321.
12 Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med* 2000; **51**: 245–270.
13 Lee JK, Bettencourt R, Brenner D, Le TA, Barrett-Connor E, Loomba R. Association between serum interleukin-6 concentrations and mortality in older adults: the Rancho Bernardo study. *PLoS ONE* 2012; **7**: e34218.
14 Ridker PM, Everett BM, Thuren T *et al.* Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017; **377**: 1119–1131.
15 Monti D, Ostan R, Borelli V, Castellani G, Franceschi C. Inflammaging and human longevity in the omics era. *Mech Ageing Dev* 2017; **165**: 129–138.
16 Fougère B, Boulanger E, Nourhashémi F, Guyonnet S, Cesari M. Chronic inflammation: accelerator of biological aging. *J Gerontol A Biol Sci Med Sci* 2017; **72**: 1218–1225.
17 Arican O, Aral M, Sasmaz S, Ciragil P. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators Inflamm* 2005; **2005**: 273–279.
18 Wolf P, Gruber-Wackernagel A, Rinner B *et al.* Phototherapeutic hardening modulates systemic cytokine levels in patients with polymorphic light eruption. *Photochem Photobiol Sci* 2013; **12**: 166–173.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Circulating Levels of Inflammatory Cytokines Analyzed by Last Observation Carried Forward Method.
Table S1 Ingredients of Atopalm®.