Acta Dermatovenerol Croat 2009;17(2):99-102 MEDICAL RESEARCH

Cutaneous Morphometric Parameters of Young FVB/N Mice Sustained in Aged Mice and in Calorically Restricted Transgenic αMUPA Mice

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SUMMARY Caloric restriction (CR) extends the lifespan of diverse animal species and is currently the only therapeutic intervention known to attenuate aging and increase longevity in laboratory animals. The effect of CR on intrinsic skin aging is not well understood. To study this issue, we took advantage of transgenic αMUPA mice that spontaneously eat less (20-30%) when fed *ad libitum* and live longer compared to their wild type (WT) FVB/N control mice. Herein we determined morphometric skin parameters in young (6-7 months) and aged (17-18 months) αMUPA and WT mice. In addition, we transplanted skin grafts excised from the aged or young αMUPA and WT mice into both types of young mice, to test whether the systemic environments of αMUPA and WT mice could affect the grafts differently. The results have shown that the mean epidermal thickness, number of hair follicles and number of dermal blood vessels were similar in all four groups regardless of age or mouse type. In addition, the post-graft specimens of all four groups exhibited increase in all parameters measured, in particular a remarkable 6-7 fold increase in epidermal thickness. However, no significant differences were detected in the post-graft samples between the four experimental groups. Our findings indicate that, at least in FVB/N mice, parameters measured in normal or grafted skin depend primarily on the intrinsic cutaneous capacity rather than on circulating factors as determined by age or reduced calories.

KEY WORDS: skin aging, epidermal thickness, dermal blood vessels, hair follicles, caloric restriction

INTRODUCTION

Data on the aging of rodent skin are still scarce and somewhat inconsistent. The Charles River hairless mouse strain, a murine model of cutaneous senescence, exhibited abnormalities in the epidermal barrier functions similar to those observed in humans (1). In addition, CBA mice have recently shown unique age-related histological modifications in the skin, including a prominent

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thinning of the epidermis as well as a reduced number of pilosebaceous units (2). However, epidermal stem cells of C57/B16 mice were resistant to cellular aging (3), and Fisher 344 rats exhibited an age-related increase in the epidermis, dermis and the fat layer (4), contrary to skin thinning that is characteristic of aged humans (5).

We previously observed that grafting aged human epidermis to immunodeficient mice resulted in a reversal of aging changes, including an increased proliferation index and the reappearance of the convoluted dermal/epidermal junction (5, 6). These findings suggest that systemic factors of young recipient mice could be involved in the beneficial influence detected in the old donor skin (7). The contribution of a young systemic environment to the rejuvenation of aged tissues in mice was also detected after heterochronic parabiosis, in which sharing of circulatory systems between old and young mice led to rejuvenated Notch signaling, cell proliferation and tissue regeneration capacity of the aged muscle (8).

Caloric restriction (CR), usually 20-40% reduction of *ad libitum* food consumption, extends the lifespan of diverse animal species and is currently the only therapeutic intervention known to attenuate aging and increase longevity in laboratory animals. In rodents, CR prevents or delays the onset of major age-related diseases, such as cancer and type 2 diabetes, and leads to changes in circulating hormones and growth factors, including reduced levels of IGF-1 and insulin (9). CR also modifies the transcriptional response of tissues towards a state that reverses or resists age-related changes, and is thought to promote susceptibility to apoptotic and autophagic cell death in mitotically competent tissues (10). Data on the effect of CR on rodent skin are still limited. In old Fisher 344 rats, CR delayed or prevented histomorphological changes resulting from intrinsic skin aging (4). In young C57BL/6J mice, CR reduced cell proliferation rates in a variety of tissue types including epidermis (11).

Whether CR can affect intrinsic skin aging through systemic or local factors is not well understood. To study this issue, we took advantage of transgenic αMUPA mice that carry as a transgene the cDNA encoding the urokinase-type plasminogen activator (uPA) (12). αMUPA mice spontaneously eat less (20-30%) when fed *ad libitum* and live longer compared to wild type (WT) FVB/N control mice (13). αMUPA mice exhibit additional similarities to calorically restricted mice, such as reduced body weight and lack of obesity, reduced levels of serum IGF-1 and glucose, enhanced capacity to conduct apoptosis in the liver, and reduced incidence of spontaneous tumors or carcinogen-induced pre-neoplastic lesions (14, 15). αMUPA mice also maintain a youthful appearance throughout most of their lifetime.

We herein determined morphometric skin parameters in young (6-7 months) and aged (17-18 months) αMUPA and WT mice. In addition, we performed skin transplantations of the young and aged αMUPA and WT mice into both types of young mice, to test whether the systemic environment of αMUPA mice could affect the grafts differently compared to that of the WT control mice.

MATERIAL AND METHODS

A total of 54 mice were included in this study, divided into the following four groups: 10 old WT FVB/N mice (mean body weight 38.9 ± 8.8 gram); 10 old αMUPA mice (mean body weight 26.8 ± 1.7 gram); 15 young WT mice (mean body weight 28.22 ± 0.3 gram); 19 young αMUPA mice (mean body weight 21.9 ± 1.54 gram). Unlike old WT mice, old αMUPA mice exhibited a young appearance of their fur. All of the mice were propagated and maintained at the Weizmann Institute Transgenic Mouse Facilities. Animal care and research protocols were in accordance with institutional guidelines and were approved by the Institutional Committee on Animal Use.

Skin specimens were obtained from the dorsal part of each mouse and were submitted for routine histological examination with hematoxylin and eosin. The histological assessment was performed by routine light microscope (6). Epidermal thickness was measured as described elsewhere (5, 6). Numbers of blood vessels were determined in the entire area of the dermis, and results were given as a mean number per mm^2. Terminal hair count was performed in a similar manner. Dermal cells were enumerated without specific staining and were also given as the mean number per mm^2. All measurements were conducted in a blind manner.

RESULTS

The results showed that the mean epidermal thickness was quite similar in all four groups regardless of age or mouse type, as follows: There was no difference between the epidermal thicknesses of old WT (45.6 ± 8 μm) and young WT mice (43 ± 6 μm) (Independent samples t-test, p=0.36). The mean epidermal thickness of old αMUPA mice (49

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± 4.5μm) was slightly however significantly higher than that of young αMUPA mice (43 ± 6 μm) (Independent samples t-test, p=0.01), but this 6-micron difference (95% confidence interval 1.56 - 10.44) is of no clinical implication. In addition, the mean numbers of hair follicles were similar in all four mouse groups: old WT mice (1.25± 0.36) versus young WT mice (1.35 ± 0.44) (Independent samples t-test, p=0.56); old αMUPA mice (1.48 ± 0.25) versus young αMUPA mice (1.76 ±0.53) (Independent samples t-test, p=0.13). The mean numbers of blood vessels or dermal cells were also similar in all groups.

To compare the influence of αMUPA and WT mice while serving as graft recipients, skin specimens from old and young WT and αMUPA mice were transplanted onto young WT and αMUPA mice. Transplantation was performed as previously described (6, 7), and the skin grafts were excised after four weeks. The post-graft specimens from all four groups exhibited a significant increase in epidermal thickness, dermal cells and dermal blood vessels, in agreement with previous results (6.7). For example, the mean epidermal thickness of old WT samples increased significantly from 42.8 ±5.9 μm before engraftment onto young WT mice to 280.7 ±141.6 μm after engraftment (Paired samples t-test, p=0.004) (Fig. 1). However, no significant difference was detected in the post grafts between the four experimental groups.

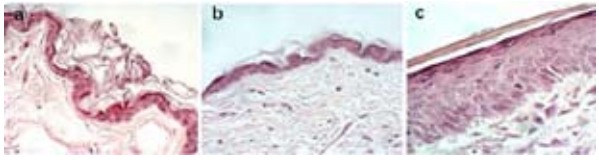

Figure 1. Photomicrograph of epidermal samples. Samples were obtained from a young WT mouse (a), an old WT mouse. (b), and a post-graft of an old WT mouse transplanted onto a young WT mouse (c). Note a marked increase in epidermal thickness in the post-graft. Magnification was x360 in a and c, and x128 in b.

DISCUSSION

This study shows that old WT FVB/N mice did not differ from young WT mice with respect to all morphometric parameters measured, suggesting that the skin of the FVB/N mouse strain is resistant to intrinsic aging. This conclusion is further supported by the findings showing that the post-graft samples of both old and young WT mice exhibited a similar impressive thickening compared to the normal skin samples. The latter result also indicates that the 35% increase in mean body weight of the old versus young WT group, and the ill appearance of the old fur, did not interfere with the proliferative capacity of the old skin grafts.

Given the resistance of the old WT skin to aging, it is not surprising that old αMUPA and old WT mice exhibited similar values for skin parameters. Furthermore, the findings that the old WT skin samples showed similar alterations irrespective of whether grafted onto αMUPA or WT mice, suggest that the systemic state induced by CR, at least in young αMUPA mice, did not interfere with the transplantation-imposed changes including the proliferative capacity. It is yet unknown, however, whether differences could be found after skin engraftment onto old WT versus old αMUPA mice.

Together, our findings on the normal and grafted skin are consistent with the view, that skin parameters in mice are determined primarily by an intrinsic cutaneous capacity that is independent of circulating factors as modified by age or reduced calories.

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