Age-related changes in the dorsal skin histology in Mini and Wistar rats

A. Ikawa¹, Y. Ishii², K. Suzuki¹, A. Yasoshima¹, N. Suzuki³, H. Nakayama¹, S. Takahashi³ and K. Doi¹

¹Department of Veterinary Pathology, ²Department of Biomedical Sciences, and ³Department of Cell Regulation, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

Summary. Mini rats (Jcl: WistarTGN(ARHGGEN)1Nts (MRs) are Wistar rat (WR)-derived transgenic rats in which the expression of growth hormone (GH) gene is suppressed by the presence of antisense transgene. The plasma GH level of this strain of rats is reduced to approximately 40% to 60% of that of WRs. In this study, to evaluate the influence of GH deficiency on the skin nature, age-related changes in the dorsal skin histology were compared between male MRs and WRs. Although there were no essential differences in the skin structures between the two strains, MRs had thinner skin with less collagens, more abundant subcutaneous adipose tissues and small-sized sebaceous glands compared with WRs. On the other hand, the hair cycle evaluated by the morphology and the depth of hair follicles was greatly different between them. Namely, two cycles of 4 weeks each were observed in both strains during the first 8 weeks after birth, but the cycle entered a long-lasting quiescence (telogen phase) in MRs while the 3rd cycle started in WRs afterwards. The lower level of serum insulin-like growth factor-1 (IGF-1) in MRs may be related to such a difference in hair cycle pattern, although the levels of IGF-1 and IGF-1 receptor mRNAs in the dorsal skin tissues were similar between MRs and WRs. MRs are considered to be a useful animal model for dermatopathy in patients suffering from GH deficiency and for grasping a clue to elucidate the exact effects of GH on the skin nature, especially on hair follicle development.

Key words: Mini rat, Wistar rat, Growth hormone-deficiency, Skin histology, Hair cycle

Introduction

Growth hormone (GH) is a 22.5kD polypeptide that exerts wide metabolic effects. It directly stimulates growth of cells of various types, especially hepatocytes, via GH receptor (GHR) (Nakajima, 1996). In addition, GH stimulates the expression of insulin-like growth factor-1 (IGF-1) gene via GHR in cells of a wide range such as hepatocytes, fibroblasts, keratinocytes, chondrocytes, osteoblasts, macrophages, lymphocytes and adipocytes (Zezulak and Green, 1986; Mertani and Morel, 1995; Nakajima, 1996). Then IGF-1 acts on target cells by an endocrine, paracrine or autocrine fashion (Su et al., 1999). Recently it has become clearer that the GH/IGF-1 axis plays a fundamental and essential role in regulating normal somatic growth throughout the fetal and childhood development (Kostyo and Isaksson, 1977; Green et al., 1985). GH has also been shown to have effects on skin nature and mechanical properties through stimulatory effects on various connective tissue components (Jorgensen, 1997). Clinical evidences of a role for GH in the skin growth have been obtained from patients suffering from GH abnormality such as pituitary gigantism, acromegaly and pituitary dwarfism, and it has been concluded that GH affects the skin and its appendages (Black et al., 1972). In addition, from the studies on recombinant human GH (rGH)-treated animals (Jorgensen, 1997) and GH-transgenic mice (Wanke et al., 1999), it was reported that GH affects the skin properties directly or through IGFs (Wolf et al., 1996). Recent histological studies have shown that GHRs and GH-binding protein (BP) are recognized in epidermis, epidermal appendages, dermal adipocytes, fibroblasts, vascular components and skeletal muscles (Lobie et al., 1990).

Mini rats: (Jcl: WistarTGN(ARHGGEN)1Nts) (MRs) are transgenic rats in which the expression of GH gene is suppressed by the presence of antisense RNA transgene. The plasma GH level of this strain of rats is reduced to approximately 40% to 60% of that of one of Wistar rats (WRs), the parental strain of MRs, and their body mass is half that of WRs after 8 weeks old. Matsumoto et al. (1993, 1995) suggested that MRs would be useful as an animal model for human pituitary dwarfism and for developing effective medicines for this disorder, because MRs show no abnormalities except for GH-deficiency.
In the field of experimental medicine, MRs have been extensively used to evaluate the influence of GH-deficiency on the hepatic responses to hepatotoxicants (Uetsuka et al., 1997a,b; Tani et al., 1999) and on the bone growth (Teranishi et al., 1999). However, there are few reports of the influence of GH-deficiency on the skin, which is one of the main targets of GH action.

In this study, to elucidate the effects of GH-deficiency on the skin nature, age-related changes in the dorsal skin histology were compared between GH-deficient MRs and normal WRs.

Materials and methods

Animals

Male MRs of 0 to 16 weeks old (4 animals at every week) were used. MRs were kindly given from Nisseeiken, Co., Tokyo, Japan. In addition, male WRs (Clea Japan, Shizuoka, Japan) of 0 to 13 weeks old (4 animals at every week) were also used. Among them, animals of 0 to 3 weeks old were obtained from pregnant rats raised in our animal facility. Animals were kept under controlled conditions (temperature, 23°C; relative humidity, 55±5%; lightening, 14h light-10h dark cycle) in an isolator caging system (Niki Shoji Co., Tokyo, Japan), and fed commercial pellets (MF, Oriental Yeast Co., Tokyo) and water ad libitum. After weighting body weights, 4 MRs each were sacrificed weekly by exsanguination under ether anesthesia from 0 to 16 weeks old. In addition, 4 WRs each were sacrificed in the same way from 0 to 13 weeks old. The present study was approved by the Laboratory Animal Use and Care Committee of the Graduate School of Agricultural and Life Sciences, The University of Tokyo.

Reverse transcription-polymerase chain reaction (RT-PCR)

The levels of IGF-1 and IGF-1 receptor (IGF-1R) mRNAs in the dorsal skin and liver were measured at 5 and 8 weeks old by the RT-PCR method. In brief, 2µg of total RNA extracted from the skin and liver of each rat was reverse transcribed to single strand cDNA using Superscript II reverse transcriptase (GIBCO BRL). Then, the resultant cDNA was amplified using Takara thermal cycler, using the following program: an initial pre-heating step at 94°C for 9 min, then repeated cycles of denaturing (94°C), annealing (58.5°C), and elongation (72°C) for 1 min at each step. The IGF-1 primers and PCR cycles are shown in Table 1.

For quantification, the PCR products were electrophoresed on a 2% agarose gel in Tris-borate EDTA buffer, stained with ethidium bromide (Sigma Chemical Co., St. Louis, MO), and analyzed using Quantity One v3.0.2 (pdi, New York, USA). The results were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression, and in turn normalized to the mean of the controls that was arbitrarily set as 1.0.

Enzyme immunoassay for serum IGF-1

The blood samples collected at 5 and 8 weeks old were tripled with Tris-EDTA (0.1M Tris, 5mM EDTA), and stored at -80°C until used. Rat IGF-1 EIA kit (DSL-10-2900) was used according to the manufacturer's protocol (Diagnostic Systems Laboratories, Inc. USA). In brief, the samples and standards were incubated with rat IGF-1-biotin conjugate solution and the rat IGF-1 antiserum for 1 hour at room temperature, and washed five times. Then, streptavidin-enzyme conjugate solution was added and incubated for 30 minutes at room temperature. The unbound streptavidin-HRPO was washed, followed by incubation with tetramethylbenzidine (TMB) Chromogenic Solution without exposure to direct sunlight. An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm.

Histology

The dorsal skin was obtained from each rat and fixed in 10% neutral-buffered formalin. Before the collection of skin samples, the dorsal skin was shaved with an electric shaver.

Four-µm paraffin sections parallel to the longitudinal axis of the body were stained with hematoxylin and eosin (HE) for histological examinations.

For electron microscopic examinations, small pieces of the dorsal skin were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4), postfixed in 1%
osmium tetroxide in the same buffer, and embedded in epoxy resin (Oken Shoji Co., Ltd., Tokyo). Ultrathin sections were double-stained with uranyl acetate and lead citrate, and observed under a JEM-1200EX electron microscope (JEOL Co., Ltd., Tokyo). Semi-thin sections (1 µm) stained with toluidine blue (TB) were also used for histological examinations.

Morphometry

Morphometrical examinations were carried out according to the reports of Iwamoto et al. (1998). The depth of hair follicles was measured on HE-stained sections using a micrometer under a light microscope (x 400) as shown in Fig. 1. Namely, 10 randomly chosen hair follicles/3 sections/rat were measured in all rats, and the mean was calculated for each rat. In addition, as shown in Fig. 1, the thicknesses of the whole skin (including epidermis, dermis and subcutaneous adipose tissue), epidermis and dermis (from the epidermal basement membrane to the border between dermis and subcutaneous adipose tissue) were measured at randomly chosen 10 points/3 sections/rat at 4, 8 and 12 weeks old when the hair cycle was in the telogen phase. The mean was calculated for each rat.

Statistical analysis

The data were expressed as mean ± standard deviation (SD) of four rats for each age group of each strain. The significance of differences between MRs and WRs was determined using Student’s t-test in cases of isovariance or Welch’s t-test in cases of unisovariance. P<0.05 was considered to be significant.

Results

Body weights

Body weights of MRs were lower than those of WRs throughout the experimental period, and the difference became larger after 8 weeks old (Fig. 2).

Expression of IGF-1 and IGF-1R mRNAs

In the skin, there were few differences in the levels of IGF-1 and IGF-1R mRNAs between WRs and MRs (Fig. 3). In the liver, the levels of IGF-1 and IGF-1R mRNAs were significantly lower in MRs than in WRs except for that of IGF-1R at 5 weeks old (Fig. 3).

Serum IGF-1 concentration

The concentration of serum IGF-1 was significantly lower in MRs than in WRs, especially at 8 weeks old (Fig. 4).

Histological findings

The epidermis became thinner after birth and reached a plateau at 4 weeks in both strains. It was thinner in MRs than WRs, especially at birth (Fig. 5).

Age-related increases in the thicknesses of the whole skin and dermis were observed in both strains (Fig. 6). Compared with WRs, MRs had thinner dermis with less collagen, whereas MRs had more abundant subcutaneous adipose tissues (Fig. 7). The percentage of the dermis in the whole skin was about 50% to 60% in MRs and about 80% to 85% in WRs, respectively. The size of sebaceous glands was generally smaller in MRs than in WRs (Fig. 8), although the size became larger with age in both strains.

Ultrastructural features of the dorsal skin were essentially similar between MRs and WRs.

The sequence of the hair cycle based on the morphology of hair follicles (Fig. 9) corresponded well to the change in the depth of hair follicles (Paus et al., 1999). Namely, hair follicles were deep in growth (anagen) phase, and they became shallower towards

![Fig.1. Points of measurements.](image1)

![Fig.2. Changes in body weights in MRs (gray square) and WRs (black diamond). Each value represents mean ±SD. **: p<0.01, Significantly different from WRs.](image2)
regression (catagen) phase. During their quiescence (telogen) phase, the depth of hair follicles was constantly shallow. Judging from the changes in the morphology and the depth of hair follicles, the sequence of hair cycle was almost similar between MRs and WRs during the first 8 weeks after birth, and 2 cycles of 4 weeks each were observed during this period (Fig. 10). After that, hair follicles of MRs were kept under telogen phase and no elongation of hair shafts was observed until 16 weeks old. On the other hand, the 3rd hair cycle started in WRs, though there were somewhat large individual differences observed in the depth of hair follicles.

Discussion

In the present study, age-related changes in the skin histology were compared between MRs and WRs. As a result, it was clarified that the deficiency of GH affected hair cycle, skin thickness and size of sebaceous glands.

GH and IGF-1 are reported to participate in the regulation of epidermal proliferation and/or differentiation (Ristow and Messmer., 1988; Gilhar et al., 1994; Eming et al., 1996; Wang et al., 1997; Wertheimer et al., 2000). In this study, MRs had significantly thinner epidermis than WRs at birth, and this suggests that GH may be also related with epidermal development in fetuses (Werther et al., 1993).
There is a lot of evidence indicating that GH may directly or indirectly regulate skin growth and collagen synthesis (Oyamada et al., 1990; Granot et al., 1991). For example, the skin thickness of patients with acromegaly was significantly increased associated with an increase in the amount of collagens (Black et al., 1972), and repeated administrations of recombinant human GH to GH-deficient adults resulted in stimulation of type I collagen synthesis in the skin (Kann et al., 1996). In experimental animals, biosynthetic human GH was able to induce a dose-dependent increase in collagen contents and mechanical strength of intact rat skin (Jorgensen et al., 1989; Oxlund et al., 1991), and increased skin thickness with increased dermal collagens was seen in dogs treated with porcine GH for 14 weeks (Prahala et al., 1998). The present results, that the thinner dermis of MRs contained less collagens compared with WRs, seem to support the effect of GH on collagen synthesis. On the other hand, abundant adipose tissues in the skin of GH-deficient MRs may indirectly indicate a certain effect of GH on lipolysis and/or anti-lipogenesis (Kristensen et al., 1998). In addition, sebaceous glands were hypoplastic in MRs. In this connection, it is reported that acromegalic skin is oily and sweaty, while patients with hypopituitarism show dry skin (Dawber et al., 1992). These findings may

![Fig. 7. Histology of the dorsal skin at 5 (anagen phase) and 8 weeks old (telogen phase). Compared with WR, MR has thinner dermis with fewer collagens and more abundant subcutaneous adipose tissues. HE stain, bar: 100 µm.](image-url)
suggest a certain effect of GH on the development of sebaceous glands.

Normal hair follicles undergo a cyclic process composed of growth (anagen), regression (catagen) and quiescence (telogen), known as the hair cycle, and this cycle is said to be highly synchronous in normal rodents (Wilson et al., 1994). In the present study, the sequence of hair cycle evaluated based on the morphology and the depth of hair follicles was similar between MRs and WRs during the first 8 weeks after birth, and two cycles were detected during this period. After that, hair follicles in MRs were kept under the telogen phase at least until 16 weeks old. On the other hand, the new hair cycle (the 3rd cycle) started in WRs. In this connection, our latest study showed that a new hair cycle started in MRs after the artificial hair plucking at 11 weeks old when the hair follicles were under telogen phase. This new hair cycle was transient (only one cycle), and thereafter the hair follicles were under telogen phase again at least until 5 months old. The detail of the study will be published elsewhere. From these findings, it is reasonable to consider that hair follicles of MRs do not permanently lose their cyclic process but they enter the long-lasting telogen phase after the 2nd cycle.

Fig. 8. Sebaceous glands of MR and WR at 12 weeks old (telogen phase). The size was smaller in MR. HE stain, bar: 50 µm.

Fig. 9. Hair follicles of MRs at 2 weeks old (A, anagen phase), 3 weeks old (B, catagen phase) and 8 weeks old (C, telogen phase). In B, some apoptotic bodies were observed. Toluidine blue-stained semi-thin section, bar: 20 µm.
There are some evidences that GH mediates hair growth. For example, some cases of hypopituitarism show reduced body hair (Dawber et al., 1992), and GH-responsive hair growth is occasionally observed in dogs with dermatopathy that may be caused by a lack of GH (Schmeitzel and Lothrop, 1990). Moreover, it has recently been shown that IGF-1 regulates hair follicles both as mitogen and as morphogen, especially during the shift from anagen to catagen phase (Philpott et al., 1994; Nixon et al., 1997; Rudman et al., 1997; Su et al., 1999). It is also established that IGF-1 has not only a growth stimulatory but also a chemotactic effect on outer root sheath cells (Fujie et al., 2001). In order to elucidate this point, the expression of IGF-1 and IGF-1R mRNAs in the skin was examined in the present study. As a result, there were no significant differences in the expressions of IGF-1 and IGF-1R mRNAs in the skin between the two strains, irrespective of the phase of hair cycle. On the other hand, their expressions in the liver, the main target organ of GH, were significantly lower in MRs than in WRs. In addition, the concentration of serum IGF-1 was also significantly lower in MRs than in WRs. Although it has been said that the serum IGF-1 has a little effect on the peripheral organs (Su et al., 1999), our results suggest a possibility that serum IGF-1 may have a certain effect on the development of hair follicles. To clarify the action of GH on the hair cycle exactly, further investigations on the localization of IGF-1 and IGF-1R in hair follicles in MRs and WRs and on the effect of GH- or IGF-1-administration on the hair cycle in MRs are needed.

In conclusion, it is considered that MRs may be a useful animal model for dermatopathy in patients suffering from GH-deficiency and for grasping a clue to elucidate the exact mechanisms of GH actions on the skin nature.

References


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