ORIGINAL ARTICLE

THE CONTENT OF METALLOPROTEINASE-2 AND METALLOPROTEINASE-9 IN THE SKIN OF RATS OF DIFFERENT AGES AFTER CLOSURE OF THE WOUND BED

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ABSTRACT

The aim: The aim of the study was to determine the content of metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) in the skin of rats of different ages after closure of the wound bed.

Materials and methods: The studies were performed on 40 white nonlinear male rats, 20 of which were 3 months old and 20 – 12 months. In each group 10 rats were control and in 10 others facelift operations were performed and cut wounds on the anterior abdominal wall were simulated. On the day of complete healing, the animals were killed, and the skin was cut in the areas of the former wound bed. In control rats, the skin was excised in the same places. The content of MMPs was determined in the skin by enzyme-linked immunosorbent assay.

Results: In rats aged 3 months after re-epithelialization of the wound bed, the content of MMP-2 was 17,1% higher compared to control rats but the level of MMP-9 didn't change. In control rats aged 12 months, the levels of MMP-2 and MMP-9 in the skin were 22,9% and 34,4% lower compared to control rats at 3 months of age. In rats 12 months of age after re-epithelialization of the wound bed, the content of MMP-2 and MMP-9 were 92,6% and 102,5% higher compared to control rats.

Conclusions: We suggested that the violation of homeostasis between MMPs in rats 12 months of age disrupts wound healing and promotes the formation of pathological scars.

KEY WORDS: wounds; skin; metalloproteinases

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INTRODUCTION

According to the literature, the number of elderly people is growing in economically developed countries and there is an increase in the number of age-related pathologies including long-term non-healing wounds [1]. This was confirmed by experimental animal studies, which showed a 20-60% delay in wound healing in the old compared with young [2]. But the biochemical and molecular mechanisms of this phenomenon remain unclear, as noted in the literature [3]. The urgency of the problem of wound healing in the elderly has increased due to the growing number of wounded in the war declared by Russia against Ukraine.

Morphological and structural changes in the skin with aging are well described. Natural skin aging is characterized by laxity, fine wrinkling, a thinning epidermis and as a result there is a progressive atrophy of the dermis [4-6]. Changes in the epidermis caused by aging include a decrease in the number of Langerhans cells, which leads to the suppression of local immunity [7]. Changes in the number of melanocytes and flattening of the dermo-epidermal junction are also expected [1]. With aging, there is a decrease in the proliferation of keratinocytes against the background of a significant increase in the duration of their migration from the basal layer to the skin surface. Aging skin has fewer fibroblasts, macrophages and mast cells [1]. It reduces the number of blood vessels, changes the extracellular matrix composition, which registers much less collagen and glycosaminoglycans [8]. In aging skin, not only the production of collagen decreases, but also its degradation increases, which leads to an overall decrease in the amount of collagen [9, 10].

Morphological changes in the skin, depending on age, are the result of disruption of molecular and biochemical processes in the cells of the dermis, which are insufficiently studied, as we mentioned above. We believe that these disturbances, among various factors, are also related to age-related changes in the content of matrix metalloproteinases (MMPs) in the skin. MMPs belong to the family of Zn2+- and Ca2+-dependent endopeptidases involved in connective tissue remodeling by destroying its organic components at physiological pH values. MMPs got their name for their ability to specifically hydrolyze the main proteins of the extracellular matrix.

Age-related morphological changes in the skin undoubtedly have an effect on wound healing. This leads to delayed re-epithelialization, delayed wound healing, delayed collagen deposition and remodeling and development of chronic wounds [11, 12].

It is well known that in chronic wounds the levels of such MMPs as collagenase and gelatinases A (MMP-2) and B (MMP-9)

are increased in comparison with acute wounds [13, 14]. However, the dependence of MMPs content in the wound bed after its re-epithelialization on age remains an open question. Among the large number of MMPs, we focused on MMP-2 and MMP-9, as they play an important role in maintaining angiogenic balance, and angiogenesis, the process of forming a new capillary network, is an important stage in wound healing [15, 16].

THE AIM

The aim of the study was to determine the content of metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) in the skin of rats of different ages after closure of the wound bed.

MATERIALS AND METHODS

All experiments were carried out according to the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), approved by First National Congress for Bioethics (September 2001), and approved by the the Ethical Committee of Educational and Scientific Center "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.

The investigations were performed on 40 male white nonlinear rats which included 20 3-month-old rats and 20 12-month-old rats. age group of animals was randomly divided into two subgroups. The first subgroup served as a control (animals were without surgery). In rats of the second subgroup, we performed a facelift operation and simulated cut wounds on the anterior abdominal wall (5 cm x 0.5 cm) [17]. The duration of wound healing in rats of different age groups was recorded. On the day of complete healing, the animals were killed. Next, we cut the skin from the former wound bed in the animals of the second subgroups and in the animals of the control subgroups cut healthy skin in the relevant areas. Skin placed in an Eppendorf by adding a physiological solution and kept frozen. Subsequently, the skin was homogenized in the cold with saline. The obtained homogenate was filtered through four layers of nylon mesh and used to determine the contents of MMP-2 and MMP-9. The content of MMP-2 and MMP-9 was determined in the skin by enzyme-linked immunosorbent assay [18]. Values were expressed as optical density / mg of protein. Total proteins were determined by Bradford's method [19].

The obtained results were subjected to statistical processing using the «Statistica 8.0» software package. Initially, the results were tested for normal distribution using a test Shapiro-Wilk test. Because the data were distributed normally, we used Student's t-test for independent samples. Mean and standard deviation (SD) were calculated for each group.

RESULTS

It was found that in control rats there was no difference in the content of MMP-2 and MMP-9 in the skin in different parts of the body (head and anterior abdominal wall). There was also no difference in the content of the studied indicators after re-epithelialization of the wound bed on the head and anterior abdominal wall. This made it possible to obtain more biomaterial after the experiment.

As can be seen from figure 1, in rats 3 months of age after complete re-epithelialization of the wound bed, the content of MMP-2 was 17,1% ($p<0,05$) higher compared to control rats of the same age (Fig. 1).

In rats 3 months of age after complete re-epithelialization of the wound bed, the content of MMP-9 was the same as in the rats of the control group (Fig. 1).

In control rats aged 12 months, the levels of MMP-2 and MMP-9 in the skin were respectively 22,9% ($p<0,05$) and 34,4% ($p<0,05$) lower compared to control rats 3- x months old (Fig. 2).

At first glance, the data obtained seem to contradict the known facts that the content of metalloproteinases in the skin with aging is increased. But we used 12-month-old rats in our experiments, which refers to early adulthood, not old age. And during life fluctuations of the maintenance of enzymes in skin are possible.

In rats 12 months of age after complete re-epithelialization of the wound bed, the levels of MMP-2 and MMP-9 were respectively 92,6% ($p<0,01$) and 102,5% ($p<0,01$) higher compared to control rats of the same age (Fig. 3).

DISCUSSION

In contrast to the early phases of wound healing, there is limited information in the available literature regarding age-dependent changes in the complete healing phase [1].

The obtained results suggest that a slight increase in the content of MMP-2 and the absence of changes in the content of MMP-9 in the wound bed of 3-month-old rats after complete wound healing does not lead to a significant decrease in collagen content. It should be emphasized that in control rats 3 months of age the content of MMP-2 and, especially, MMP-9 is negligible. This is consistent with the literature that because MMPs degrade the endoplasmic reticulum, in healthy tissue these enzymes are contained in trace amounts, with the exception of MMP-7, which is constitutively synthesized by epithelial cells [20].

It is known that the content of metalloproteinases, such as collagenase and gelatinases A and B, in the substrate of chronic wounds is increased compared to acute wounds [13, 14].
Since in 12-month-old rats the content of MMP-2 and MMP-9 in the wound bed after its complete re-epithelialization was significantly higher than in 3-month-old rats, we concluded that age is a risk factor for chronic wound healing. This is consistent with our data that in 12-month-old rats, wound healing is slower [21]. Our results obtained in rats are consistent with human observations [12]. This work shows the activation of MMP-2 and MMP-9 and the decrease in the content of MMP-1 and MMP-2 inhibitor in the wound bed 84 days after skin injury in healthy elderly people. This imbalance between MMPs and their natural inhibitors leads to increased proteolytic activity and reduced collagen accumulation in people and animals.

So, proteolysis can play a significant role in delayed wound healing in older animals and age is a risk factor for the chronicity of the wound process.

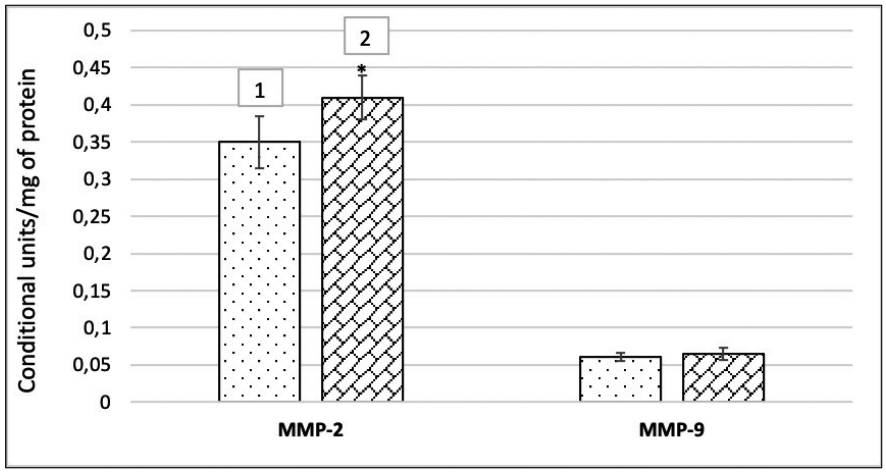

Fig. 1. The content of MMP-2 and MMP-9 in the skin of 3-month-old rats after complete reepithelialization of the wound:
1 – control, 2 – experiment;
* – p<0,05.

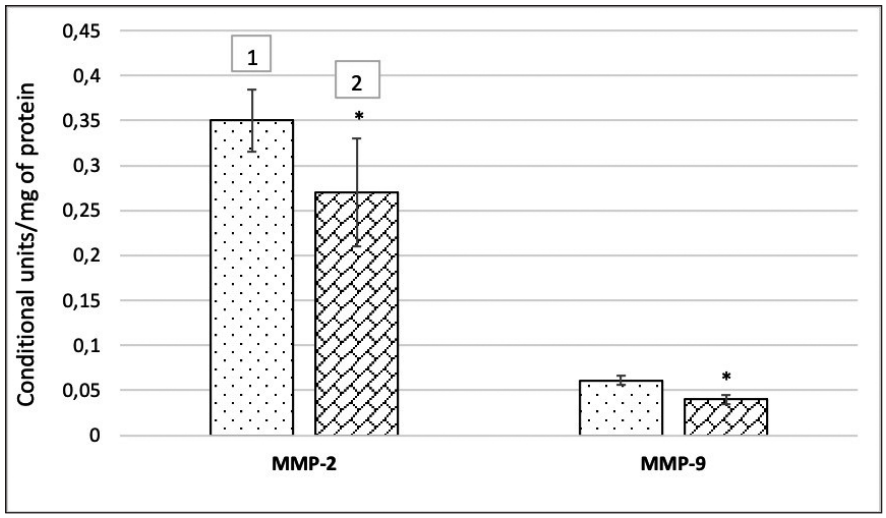

Fig. 2. The content of MMP-2 and MMP-9 in the skin of intact rats aged 3 (1) and 12 (2) months:
* – p<0,05.

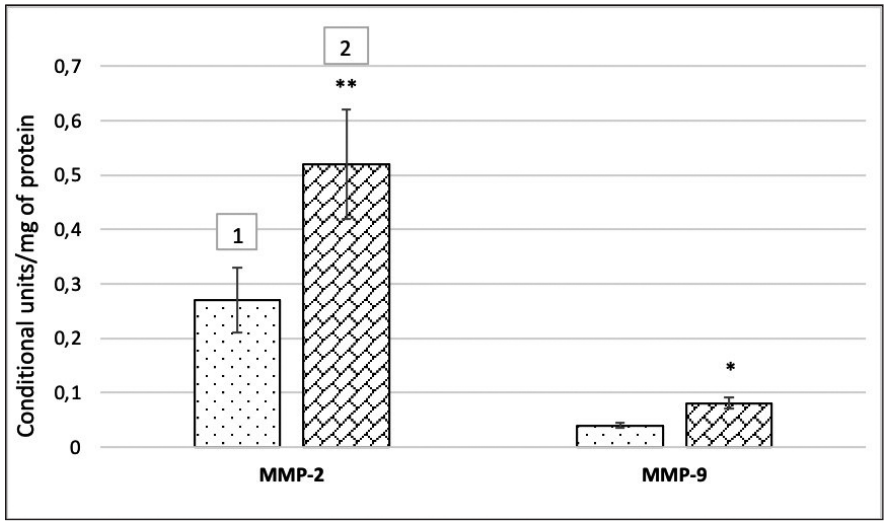

Fig. 3. The content of MMP-2 and MMP-9 in the skin of 12-month-old rats after complete reepithelialization of the wound:
1 – control, 2 – experiment;
* – p<0,05, ** – p<0,01

CONCLUSIONS

1. In the skin of rats 3 months of age after complete re-epithelialization of the wound bed, the content of MMP-2 was 17,1% (p<0,05) higher compared to control rats of the same age.
2. In the skin of control rats at 12 months of age, the level of MMP-2 in the skin was 22,9% (p<0,05) lower compared to control rats at 3 months of age.
3. In rats 12 months of age after complete re-epithelialization of the wound bed, the content of MMP-2 was 92,6% (p<0,01) higher compared to control rats of the same age.
4. The level of MMP-9 in the skin of 3-month-old rats after complete closure of the wound bed was the same as in control rats of the same age.
5. In control rats at 12 months of age, the level of MMP-9 in the skin was 34,4% (p<0,05) lower compared to control rats at 3 months of age.
6. In rats 12 months of age after complete re-epithelial-

ization of the wound bed, the content of MMP-9 was 102,5% ($p<0,01$) higher compared to control rats of the same age.

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Conflict of interest:
The Authors declare no conflict of interest.

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