Effect of aging on fracture healing

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Abstract

The article is dedicated to the investigation of bone regeneration during ageing. The aim of the study was to find out the influence of aging to the fracture healing process. The study was carried out on 60 Vistar female rats. Animals were divided into three groups depending on age (4 month, 12 months and 24 months old). In all cases perforated defect (fracture) of the distal femur metaphysis was performed. The results were compared among juvenile, middle-aged, and elderly rats during repair of a femur fracture. The animals were derived from experiment on the 3rd, 5th, 10th and 30th days after operation. Broken femurs were subjected to histological examination. It was established that regardless of the age of the animals fracture healing process proceeds from the general scheme and in the late stages callus takes the form of the mature bone but the stage-temporal parameters of regeneration in senile animals showed slowing of fracture healing process.

Key words:
fracture healing, rat, regeneration, age

Introduction

Skeletal fracture healing is a significant clinical problem. Most fractures are still treated with closed reduction and non-rigid fixation and heal normally. There are, however, risk factors such as avascularity, instability, large fracture gaps or concomitant infections that lead to poor fracture healing [1]. Non-unions and delayed healing are still prevalent complications in fracture and bone defect healing [2].

Bone healing is well understood, although there are many circumstances that need to be investigated and could impact clinical treatment. As one example, the influence of ageing to the fracture healing process. Due to the progressive aging of the world population especially important to the study of age-related aspects of the various pathological conditions including the processes of reparative osteogenesis in bone fractures [3]. The lifetime risk
for hip fractures is 17% in Caucasian women and 6% in Caucasian men from age 50 years onward [4].

Approximately 1.5 million fractures occur annually in the United States and entail health care costs of about $13.8 billion, with $8.7 billion attributed to the age-related hip fractures [5]. Significantly, with the increase in life expectancy, these estimates will have to be revised dramatically upwards. The number of men and women older than 65 years is predicted to increase from 32 million in 1990 to 69 million in 2050, and 15 million people will be 85 or older. However, in neither of the post-fracture patient managements, the biology of the aged skeletal system has been adequately considered. A rational therapeutic strategy for the aged, mostly osteoporotic patient must be guided by biology. With increasing age not only the fracture incidence is increased. In post-fracture management, the overall length of hospital stay positively correlates with patient age and older patients may be transferred to a skilled nursing facility and could be discharged earlier from the hospital than juvenile individuals [6].

Age is poorly understood factor influencing the course of fracture healing. In fact, there are few data on age differences of bone fractures repair [7]. According to modern concepts these differences may be due to a decrease in the activity of genes in cells of regenerate with age [8]. However more recent studies have found no clear differences in the expression of genes involved in bone tissue regeneration in animals of different ages.

Whereas bone fractures are a significant clinical problem, particularly in osteoporosis and other age related diseases of skeleton, there has been a tremendous interest in research of age effect on callus formation.

The aim of our examination was to study age peculiarities of fracture healing.

Methods

Research is constantly ongoing to understand and improve skeletal healing. As a result, there is a need for models to study these healing processes. Variety of surgical techniques and fixators reported in nonhuman primates, dogs, rabbits, guinea pigs and rats.

These models are useful but don't always provide basic insight into the mechanisms of bone healing. When rigid fixation is used, there is mechanical stability in the regenerating bone during every healing phase. Therefore, the biomechanical properties of the structure depend on the fixation and the healing tissue. Load sharing between the fixator and bone can result in stress shielding, and prevent the transmission of biomechanical stresses and strains that are conducive to bone formation. As a result, drill hole defects have been used in the cortex of long bones and calvariae of rabbits and rodents [9–13].

The study was conducted on 60 adult female rats of the “Vistar”, contained in standard vivarium conditions of the Institute of Gerontology of NAMS of Ukraine, led by professor V. Povoroznyuk – Head of the department of clinical physiology and pathology of the musculoskeletal system.

Rats were divided into 3 groups of 20 animals each: Group A (juvenile) – rats aged 4 months; Group B (middle-aged) – rats aged 12 months; Group C (elderly) – rats aged 24 months. In all animals manual perforated defect (fracture) of distal femur metapophysis with a bradawl was performed. All manipulations were carried out under inhalation ether anesthesia.

The animals were derived from experiment on the 3rd, 5th, 10th and 30th days by an overdose of ether. Taken material (broken femur) was subjected to histological examination.

Histological study was performed under the direction of professor N. Dedukh, the Chief of Laboratory of connective tissue morphology, Sytenko Institute of Spine and joint Pathology, NAMS of Ukraine, Kharkiv. Histological sections were made at Sannomiya microtome “Reichert”, stained with hematoxylin and eosin. Colored sections were analyzed under a microscope MICROS, and polarized light (Polmy–A). Preparations photographed with a digital camera Canon EOS-300D under the microscope “AxioStar Plus”.

All procedures were approved by the Ethics Committee at the Institute of Gerontology of NAMS of Ukraine. Rats were allowed to move freely after recovering from anesthesia. Analgesic (Cetorolac, 0.1 mg/kg) was administered immediately after surgery.

Results

At the stage of inflammation (1–3 days after surgery) in all groups of animals regenerate at the fracture site was attended by the remnants of the hematoma, which occupied a major area of defect. In elderly animals this area was wider than in other ones. Pool of cells (neutrophils, macrophages and lymphocytes) was highest in juvenile animals. In addition to neutrophils, macrophages and lymphocytes in group A field of fibroblasts was identified, while in other groups we saw small number of fibroblasts, which were located mainly around the forming vessels. In group C an increased density of neutrophils was observed compared with animals of A and B groups (Fig. 1).
In assessing the formation of the vessels we paid attention to prevalence of vascular capillary type in juvenile animals, while in animals of middle and old age sinusoid type capillary was dominated.

Thus, at the inflammation stage (1–3 days after traumatic lesions) in animals of all ages standard microscopic picture of inflammation was observed while rats aged 24 month showed increased number of neutrophils and low density of fibroblasts around the vessels of sinusoidal type, indicating a delay in cell differentiation and the formation of the vascular bed compared with younger animals (Fig. 2).

At the stage of callus formation and the beginning of its mineralization (the 10th day after fracture) in group A regenerate was represented by fibroreticular osteogenic tissue with a high density of osteoblasts. In some places osteoblasts formed osteoid (non-mineralized bone) with a high density of fibroblasts. In group B the density of osteoblasts was lower and in group C we saw only single osteoblasts (Fig. 3).

At the stage of mineralization and remodeling of bone regenerate (30 days after fracture) in juvenile animals a full reclaim submitted lamellar bone tissue in the cortex and cancellous bone was formed. In middle-aged animals the the cortex was represented by a network of lamellar structured trabecular bone, but small pockets of fibroreticular tissue remained. In elderly animals we noticed signs of osteoporosis, thinning of bone trabeculae in regenerate and mature bone as well, the centers of lysis and homogenization occurred in the bone regenerate. Large pockets of fibroreticular tissue in regenerate remained (Fig. 4).

Thus, 3 days and 5 days after traumatic injury there were no any difference between animal groups in number of inflammatory cells. On day 30, however, a full reclaim formed bone plate type took place only in juvenile animals. In middle-aged animals along with areas of lamellar bone lesions sections of fibroreticular tissue were present. In elderly animals osteoporotic bone breach of maternal and secondary disturbances of bone regenerate formation – pockets of lysis, bone density reduction, the formation of homogenization areas were expressed.

These data suggest that regardless of the age repairation goes according to general scheme but stage-timing recovery is shifted.
Discussion

Most bone fractures can be spontaneously repaired; however, each year in the United States, approximately 10% of fractures show delayed union or nonunion, resulting in enormous medical costs and lost productivity [14, 15].

The effect of age seems to invert the effect of mechanical properties of the callus, which was not correlated to callus size. Optimization of mechanics alone seems to be not sufficient. The underlying mechanisms and causes of the age-related influences and their clinical counterparts need to be further investigated.

For a long bone fracture with only moderate inherent stability, the process involves an inflammatory phase, a reparative phase and then a final remodeling phase [3]. The inflammatory phase consists of a hematoma formation, immune response and progenitor cell recruitment to the fracture. The reparative phase consists of revascularization and bone formation by both intramembranous and endochondral pathways, preceding remodeling to restore the normal bone structure. Sites that are more mechanically stable and naturally in compression may predominantly heal with primary bone in an intramembranous pathway [16].

The physiological process of aging is highly complex at the molecular, cellular, and systemic levels. The cumulative effects of this process may lead to cognitive and functional degenerative outcomes. Clinical observation suggests that the majority of age-related events are initially constructive, optimal and conducive to maximized survivability. However, through subtle changes and as a consequence of poorly understood mechanisms, the fate of the organism stumble toward a degenerating finality. ‘The aging process’ and osteoporosis are two compelling degenerating changes that have a profound human impact. We recognize that not all elderly patients are osteoporotic. Further, an osteoporotic individual may not be elderly. However, it is generally accepted that if we live long enough, we will become osteoporotic [17]. In large part due to population demographics and to some extent as a
Consequence of the greater amount of physical activities available for the elderly, there is a compelling concern about the steady increase in the number of fractures each year. Consequently, the financial burden of health care becomes more daunting each year, and there is a commensurate increase in morbidity and mortality [18].

There are age-related changes in bone repair. Juveniles heal much more rapidly than adults, but the effect of age on fracture healing in adults remains controversial [19, 20]. Study in mice has demonstrated that 4-week juvenile mice heal fractures more quickly than 6-month adults, and that healing capacity continues to decline as animals grow beyond middle-age. We didn’t see such differences between juvenile and middle-aged rats. Multiple factors could contribute to the age-related changes in fracture healing, such as decreased number and/or function of stem cells [21, 22], structural and cellular changes in periosteum, decreased chondrogenic potential of periosteum, and changes in the local signaling milieu at fracture site. However, the effect of age on vascularization during fracture healing has not been well determined [23]. We agree with Lu et al. (2008) that the area of new born vessel net in the place of fracture is much reacher in juvenile rats. But the quantitative difference between juvenile and middle-aged animals was not appreciable. We noticed only some differences on qualitative level dealing with the type of blood vessels (capillary vs synusoids).

Meyer et al. reported that 6-week-old rats regain normal bone biomechanics at 4 weeks after a fracture, 26-week-old rats require 10 weeks, and 1-year-old rats require more than 6 months [24]. We did not observed an experimental animals for so long time. But in our cases callus formation was observed in every group. And quality of callus was consequent to the state of mature bone before fracture. Age-related changes in mice include a delay in the onset of the periosteal reaction, delays in cell differentiation, decreased bone formation, delayed angiogenic invasion of cartilage, a protracted period of endochondral ossification, deceased bone formation and impaired bone remodeling. Disrupted regulation of osteogenic differentiation, which is highly associated with blood vessel formation, is likely contributory to impaired fracture healing [25]. We assume that these morphologic and biomechanical aspects from rodent models, may also account responsible for the compromised bone regeneration capacity of the elderly patient. Underlying these age related changes at the cellular and molecular level will help to guide design and development of a rational therapeutic protocol.

Conclusion

In conclusion, regardless of age fracture healing process in the fracture area occurs in the overall scheme, but phase-timing shifted towards slower reparative processes in elderly. In the later stages of regeneration bone looks like a mature with regard to its original state before fracture, i.e. osteoporotic in elderly animals like their bone in paratraumatic zone during first 3 days after fracture. Callus formation was observed in every group. And quality of callus was consequent to the state of mature bone before fracture. The experimental data should be considered in clinical practice for the treatment of bone fractures in patients of different age groups.

References


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Santrauka


Gyvūnų rezultatai nagrinėti eksperimento ant 3, 5, 10 ir 30-ą dienomis po operacijos. Lūžę šlaunikauliai buvo iširti histologiškai.

Buvo nustatyta, kad nepriklausomai nuo gyvūnų amžiaus lūžio gijimo procesas prasideda pagal bendrąją schemą ir vėlesniuose kaulinio kaliuso susidarymo etapuose išgyja brandaus kaulo formą, tačiau šie stadijos priklauso nuo senėjimo įtakos.

Reikšminiai žodžiai:
kaulų lūžių gijimas, žiūrės, regeneracija, amžius