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Age-related features of bone regeneration (literature review)

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The number of elderly people is constantly increasing all over the world. They are most often the patients who need orthopedic surgeries like arthroplasty, osteosynthesis and others. It is known that the process of bone regeneration depends on the patient's age. However, certain characteristics of bone regeneration process depend on the age remain unclear, which is important for developing the best strategies for treatment of elderly patients. Objective. To identify age-related features of bone regeneration and to establish possible ways of influencing them in order to optimize the bone regeneration in elderly patients. Methods. Literature search was performed in the PubMed database. Inclusion criteria were original experimental and clinical studies in English. The search depth is accepted for 20 years. Results. It has been experimentally and clinically shown that bone tissue regeneration slows down with age, which is more pronounced in women. According to scientific information, this involves two signaling pathways — Notch and Wnt/β-Catenin, the activity of which is suppressed with age. However, the regulation of regeneration is a cascade of signaling pathways and macromolecules. The expression of growth factors after fracture changes at older age compared to a younger one. In particular, a decrease in the expression of TGFβ-1 was clinically revealed. In addition, in older patients after fracture, an increase in macrophage colony-stimulating factor and VEGF was recorded. It has been experimentally established that a combination of a slowdown in bone tissue regeneration with a decrease in the content of Indian Hedgehog, Sonic Hedgehog, BMP-2, 4, -7 proteins and MMP-9 in bone callus has been established. Among the ways to overcome the delayed bone regeneration in elderly patients can be the use of modern technologies of cell and gene therapy, inhibitors of macrophages, biologically active factors at certain stages of bone regeneration. For cell therapy, it is important to take into account the age of the cell donor because of the high probability of functional disorders in cells from older donors. Key words. Bone healing, aging, bone fracture, growth factor, mesenchymal stem cell.

Кількість людей похилого віку постійно збільшується у всьому світі. Саме вони найчастіше є пацієнтами, яким виконують ортопедичні операції — ендопротезування, остеосинтез тощо. Відомо, що процес регенерації кістки залежить від віку людини та, імовірно, уповільнюється з його збільшенням. Проте залишаються нез'ясованими певні особливості процесу відновлення кістки залежно від віку, що важливо для розроблення кращих стратегій лікування пацієнтів похилого віку. Мета. Виявити вікові особливості репаративного остеогенезу та встановити можливі шляхи впливу на них для оптимізації регенерації кістки в пацієнтів похилого віку. Методи. Пошук літератури виконано у базі даних PubMed. Критеріями включення були оригінальні експериментальні та клінічні дослідження англійською мовою. Глибина пошуку прийнята 20 років. Результати. Експериментально та клінічно показано, що регенерація кісткової тканини уповільнюється з віком і має більший прояв у жінок. За науковою інформацією, у цьому задіяні два сигнальних шляхи — Notch s Wnt/β-катенін, активність яких пригнічується з віком. Проте регуляція регенерації — це каскад сигнальних шляхів і макромолекул. Експресія факторів росту після перелому змінюється у старшому віці порівняно з молодшим. Зокрема, клінічно виявлено зниження експресії TGFβ-1. Крім того, у пацієнтів старшого віку після перелому зафіксовано підвищення макрофагального колонієстимулювального фактора і VEGF. Експериментально встановлено поєднання уповільнення регенерації кісткової тканини зі зниженням вмісту в кістковому мозолі білків Indian Hedgehog, Sonic Hedgehog, BMP-2, -4, -7 та желатинази MMP-9. Серед шляхів подолання сповільненої регенерації кістки в пацієнтів похилого віку може бути використання сучасних технологій клітинної та генної терапії, інгібіторів макрофагів, біологічно активних чинників на певних стадіях репаративного остеогенезу. Для клітинної терапії важливим є врахування віку донора клітин через високу ймовірність функціональних порушень у клітинах від донорів старшого віку.

Key words. Bone healing, aging, bone fracture, growth factor, mesenchymal stem cell

Introduction

Every year the percentage of elderly and senile people in the world population increases, especially in developed countries. According to the WHO, the number of people over 65 was 703 million in 2019, including 23 % more women than men [1]. In 2050, the total number of people over the age of 65 is likely to be 1.5 billion [1]. People of older age undergo a significant number of orthopedic operations, such as endoprosthetic replacement, osteosynthesis of bone fractures, etc. Today it is already known that the process of bone regeneration depends on a person's age and probably slows down with its increase [2, 3]. However, some age-specific features of bone regeneration remain unclear, which is important for developing better treatment strategies for elderly patients.

Bone regeneration is a complex process, the success of which is due to the coordinated action of many biological and physical factors. Biological factors include growth factors, mesenchymal stem cells (MSCs), poorly differentiated endosteum and periosteum cells, and actually bone tissue cells. Impairment/absence of any of them at any stage of the regenerative process can trigger such negative consequences as nonunion, formation of false joints, re-fracture, loosening of the components of endoprostheses.

Growth factors are signaling molecules that affect the proliferation, differentiation and apoptosis of bone-forming cells: bone morphogenetic proteins (Bone Morphogenetic Proteins — BMPs), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factors vascular endothelial growth factor (VEGF). The role of the latter is also associated with the stimulation of the formation of blood vessels, which are an integral part of reparative osteogenesis [4]. Cell biosynthesis of growth factors may vary with age, which will adversely affect reparative osteogenesis.

Poorly differentiated cells, in particular MSCs of the bone marrow, which migrate to the area of bone damage, are able to proliferate and differentiate in the osteogenic direction [5]. Their insufficient number or altered structural and functional features due to age can also adversely affect bone regeneration.

The purpose of the study: to identify age-related features of reparative osteogenesis and to establish possible ways of influencing them for possible optimization of bone regeneration in elderly patients.

Material and methods

The literature search was performed in the PubMed database using the Mesh keywords for the following search queries: («Bone Regeneration / analysis» OR «Bone Regeneration / etiology» OR «Bone Regeneration / immunology» OR «Bone Regeneration / physiology» OR «Fracture Healing / etiology» OR «Fracture Healing / immunology» OR «Fracture Healing / physiology») AND («Age Factors» OR «Aging») and «Osteoblasts» OR «Osteoclasts») AND («Age Factors» OR «Aging»). The inclusion criteria were original experimental and clinical studies in English. The search depth is 20 years.

Results and their discussion

A total of 63 studies were selected for analysis. Some of them state the relationship between the age of the subject (animal or human) and the result of bone regeneration for a certain period of observation. We have put them together in the next section.

Slowing of bone regeneration with age

Female Sprague-Dawley rats at 26 weeks of age were found to form complete fusion of diaphyseal closed fracture with intramedullary fixator two-fold later (8–10 vs. 4 weeks) as compared by radiography with rats at 6 weeks of age [6]. Regeneration of bilateral mandibular condyle fracture in male 36-week-old Sprague-Dawley rats was also slow compared to 3-week-old [7]. Similarly, male Wistar rats aged 2 and 18 months with a bilateral defect in the mandible after 3 and 6 weeks, a smaller amount of new bone was found in the defect of older rats [8]. Mice line C57BL / 6 aged 25 months were found to have a smaller size of the callus compared to 5 months in 20 days after fracture of the tibia [9].

The peculiarity of bone regeneration with age was the suppression of bone formation in the bone marrow canal of the tibia or femur after bone marrow removal in heterozygous 7.5-month-old mice, 7.5-month-old mutant *Runx*2 mice (two-fold shortened *Runx*2 synthesis), which was not observed in 2.5 month-old mice [10]. Healing of a critical skull defect with a diameter of 3; 4; 5 mm in male CD-1 mice aged 6 or 60 days in 8 weeks also differed: in younger mice, radiographic defects of all sizes were more complete (≈ 36 %) compared to older ones (≈ 5 %) [11]. One explanation for this can be an increased expression of sclerostin in the parietal bone of 60-day-old mice found by M. Kwan et al. [12], the biological role of which is defined as a negative regulator of osteoblastogenesis, which inhibits the ability of MSCs to osteogenic differentiation, compared to 6-day-old mice. Contrary to this, D. Joiner et al. [13] did not find a difference in the amount of regenerate depending on the age of male Sprague-Dawley rats aged 6 and 12 months in 12 weeks after performing a cortical defect in the femur. However, in the regeneration

of older rats, the density of osteocytes was lower and mineralization was higher compared to younger ones.

It has been shown that slowing of bone regeneration is more common in women than in men, i. e. sex can be considered as one of the risk factors. In particular, in female SAMP6 mice aged 10 months with accelerated aging and osteoporosis, remodeling of the callus at week 5 after femoral fracture was slower compared to mice from the SAMR1 age-resistant line [14]. At the same time, no such difference was found for male SAMP6 mice of the same age. Interestingly, the experiment performed by M. Egermann et al. [15] found no difference in bone regeneration after histological examination and micro-CT in female SAMP6 mice, but at 5 months of age, compared with SAMR1 control mice in 4 and 6 weeks after plate fixation osteotomy. In 12-month-old female Sprague-Dawley rats, a regeneration delay was recorded at 6 weeks after femoral osteotomy with fixation compared to males [16]. In contrast, D. M. Pien et al. [17] in an experiment on Wistar rats aged 1 and 3 months determined greater bone volume and the percentage of osteointegration around the titanium implant in the tibia in older male rats one month after implantation, and there was no difference in females of different ages.

It has been established that bone regeneration is affected by a decrease in estrogen levels with age. In rats after ovariectomy, there was a delay in the formation of bone tissue in the alveolar cavity after tooth extraction compared with intact animals [18].

In clinical conditions, it was noted that the frequency of secondary displacement after fracture of the distal radial bone was approximately 1.5 times higher in patients older than 65 years compared with persons aged 18–44 years in the range up to 8 weeks after application of a plaster cast [3]. In fractures of the talus in children, the frequency of fractures with displacement and the frequency of complications also increases with age [19]. The number of non-unions after fracture of the humeral diaphysis also increased in patients older than 55 years compared with younger ones [2].

Biologically active factors

Delayed fracture healing with age is associated with decreased mRNA expression of genes associated with mitochondrial oxidative function and nerve cell function [20, 21]. In female 6-week-old rats, the expression level of mRNA genes responsible for electron transfer and the tricarboxylic acid cycle was 2-fold higher than in 52-week-old rats in 6 weeks after closed femoral fracture [20]. Besides, an experiment on mice after fracture of the femur found age-dependent different expression of mRNA genes that are responsible for the functioning of nerve cells [21].

The results of several studies indicate the effect of age on the expression of growth factors and pro-inflammatory cytokines after fracture. C. G. Eriksen et al. [22] found that in cultured osteoblasts derived from individuals aged 73–85 years, OPG and TGF-β1 mRNA expression was lower and interleukin-6 (IL-6) mRNA was higher compared to individuals aged 21–27 years. In a clinical study, serum TGF-β1 levels in patients older than 50 years after fracture of long bones were lower than in younger individuals in 24 weeks after surgery, and lower in women than in men [23]. It was shown that within 6 months after fracture of long bones, the average serum content of macrophage colony-stimulating factor and VEGF was higher in the elderly and women [24]. R. A. Meyer et al. [25] in an experimental study found that slower regeneration of the diaphyseal defect of the femur of female Sprague-Dawley rats aged 12 months was accompanied by lower mRNA expression of Indian Hedgehog and BMP-2 proteins compared to younger rats at 6 weeks of age. B. Yue et al. [26] also confirmed a decrease in BMP-2 in the cortical layer of the femur of male Wistar rats with age (age 1; 9; 24 months). Decreased expression of BMP-2, -4, -7, FGF-2 and its FGFR-1 receptor was found in the callus of 60-day-old mice 2 weeks after reproduction of a critical skull defect compared to younger animals 6 days of age [27]. Hedgehog is a signaling pathway involved in early bone mineralization in young C57BL / 6 mice after tibial fracture [28]. Sonic hedgehog protein functions differently in 5- and 60-week-old mice after rib fracture [29]. In older mice, inhibition of Sonic hedgehog protein expression was observed compared to younger mice. In addition, in older mice, this pathway stimulated the formation of osteoclasts, and osteoblasts in young ones [29].

Another part of the mechanism of slowing down bone regeneration with age is the suppression of Notch signals transmitted by mesenchymal cells. In culture of mesenchymal cells obtained in C57BL / 6 mice aged 5 and 25 months, P. L. Mutyaba et al. [30] found inhibition of Notch basal signaling activity in older mice. The expression of various proteins that regulate vascularization at the fracture site also varies with age. In particular, in the callus of mice aged 1; 6; 18 months on the third day after tibial fracture, the expression of hypoxia-inducible factor-1α (HIF1α) and VEGF transcripts was found only in 1-month-old mice [31].

The expression of matrix metalloproteinases (MMPs) gelatinase (MMP-9) and collagenase (MMP- 13) one week after fracture was higher in younger mice, and 3 weeks later, in 18-month-old animals compared to other age groups. Adequate levels of matrix metalloproteinases are important at the stage of remodeling cartilage regenerate into bone. The density of blood vessels in the area of the fracture a week later was higher in 1-month-old rats [31]. In another study, Sprague-Dawley rats aged 12 weeks showed higher MMP-9 expression in bone marrow a week after femoral osteotomy with fixation compared to 52-week-old mice, and MMP-13 expression did not differ between age groups [32]. This reflects the change in MMP biosynthesis with age.

It has been determined that one of the subtypes of prostaglandin E2 receptors, which plays an important role in bone formation and maintenance of bone homeostasis throughout life, namely EP4, is involved in bone regeneration. The authors showed a smaller callus volume in 2 weeks after femoral fracture in older mice aged 15–16 months with no EP4 receptor and incomplete fracture fusion was observed in 4 weeks after the fracture compared to wild-type mice. It was determined that the violation of bone formation was the result of inhibition of osteoblastogenesis and, accordingly, reduced mineralization [33].

Ecto-5'-nucleotidase (CD73), which activates osteoblasts and is a regulator of skeletal growth, has been shown to regenerate a defect in the cortical layer of the tibia in older mice aged 46–52 weeks. Inhibition of bone matrix formation in knockout CD73 mice has been established [34].

The Wnt / β-catenin (β-catenin) signaling pathway is also involved in fracture regeneration. In an experiment on mice with the Catnblox gene (ex3) aged 6 and 12 months after activation of the Wnt / β-catenin signaling pathway by tamoxifen, the regeneration of the perforated defect in the metaphysis of the femur was faster, with better vascularization and osteogenic differentiation compared to wild-type mice of both age groups [35]. Also due to the activation of the Wnt / β-catenin signaling pathway, the level of MMP-9 and VEGF in the regenerate was increased, which, according to other experimental studies, decreases during bone regeneration with age [24, 32, 33].

Bone tissue cells

The functional state of bone cells (osteoblasts, osteocytes and osteoclasts), which also changes with age, is essential for bone regeneration. Osteoblasts are formed from the MSCs of the periosteum, endosteum, bone marrow, but with age the differentiation of the latter occurs more often in adipocytes than in osteoblasts [36, 37]. Other cells also affect osteoblast differentiation. Experimental studies have shown that megakaryocytes also stimulate osteoblast differentiation, but this ability decreases with age [38].

A decrease in the number of osteocytes during aging was found in C57BL / 6 mice aged 22 months compared with 5 month-old ones. This causes disruption of the connections in the lacunar-tubular system and worsens the response of osteocytes to external stimuli [39]. In addition, a decrease in the mechanical sensitivity of osteocytes with age in 22-month-old mice compared with 5-month-old mice has been demonstrated [40]. One of the reasons for this phenomenon is probably the slow recovery of plasma membrane disorders that occur to trigger the transmission of a mechanical stimulus [41]. In osteocytes in the cortical layer of the bones of mice aged 24 months under aging conditions, the expression of kappa-B nuclear factor activator receptor ligand (RANKL) increases, which activates osteoclast differentiation from monocytes / macrophages and leads to loss of bone mass in the cortical layer; no increase in osteoclasts was observed in the cancellous bone, but bone loss also occurred [42]. The probable reason for this is the aging of osteoclasts and suppression of their function [43]. An experimental study in mice confirmed the role of aging osteocytes in increasing the porosity of the cortical layer. In particular, mice after removal of proapoptotic proteins Bak and Bax increased the life expectancy of osteoblasts and osteocytes, which at the 22nd month of life triggered an increase in the mass of cancellous bone tissue in the femur. But in the cortex the opposite results were recorded — the mass decreased due to the formation of large cavities. The authors of this study attribute this fact to the aging of osteocytes, which was accompanied by the activation of the resorptive function of osteoclasts by increased expression of RANKL and VEGF by osteocytes. Increased porosity of the cortical layer of bone can cause non-vertebral fractures in patients [44].

The protein composition of the bone matrix of the cortical layer of bone, which changes with age, affects the activity of osteoclasts. During the cultivation of human monocytes on the cortical bone of cows aged 8 months and 9 years higher osteoclast activity was recorded in a sample from an older animal aged 9 years, and the content of α/β-CTX in the matrix was 3 times higher compared to the younger (8 months) sample [45]. In vitro, it has been found that the ability of osteoclasts obtained in healthy elderly women to

fuse increases with age, which increases their resorption activity and leads to bone mass loss [46].

Another mechanism that adversely affects bone is mitochondrial dysfunction. In mice, osteoclast activity increased with age due to mitochondrial dysfunction caused by mutations in somatic mitochondrial DNA, which inhibited bone matrix mineralization by osteoblasts. Age-related bone loss was significantly greater in mice with activated mitochondrial DNA mutators compared to wild-type [47]. Also osteoclasts of old mice aged 18–24 months synthesized sclerostin in vitro, which inhibits the mineralization of the bone matrix in contrast to the osteoclasts of young mice aged 6 weeks or 12 months [48].

Optimization of bone regeneration

It has been suggested that a positive IL-1 genotype may promote fracture regeneration. O. I. Weiss et al. [49] did not find better results of probing depth and quality of clinical attachment after periodontal treatment with bone grafts in 13 people with positive genotype IL-1 compared to 31 people with negative genotype IL-1.

Mesenchymal stem cells and biologically active factors

The use of cell therapy is one of the promising areas for optimizing reparative osteogenesis. The age of the donor cells for cultivation can affect their regenerative potential. It was determined that in cultured cells from older rabbits [50] and mice [51] the rate of proliferation, osteogenic [51] and chondrogenic potential [50] was reduced compared to younger donor animals. Ovariectomized mice injected into the femur with cultured adipose tissue cells pre-induced to differentiate into osteoblasts from 1- or 10-month-old donor mice were shown to have a significant increase in bone mineral density after 4 months in the case when the cell donor was younger [51]. In contrast, T. Morihara et al. [50] found no difference between the healing of osteochondral defect at week 12 in the femur of male rabbits aged 8–10 months or 4–5 years under conditions of transplantation of chondrogenic cells from donors of different ages (8–10 months or 4–5 years). The clinical study did not establish a dependence on the age of osteogenic differentiation of MSCs obtained from the bone marrow of 20 patients who underwent total femoral arthroplasty [52]. This may be due to the small number of participants, 15 of whom were over 50 years old. However, it has been experimentally established that MSCs in the bone marrow of 6-week-old and 9-month-old rats respond equally to platelet-released supernatant (PRS), increasing mitogenic activity and chemotactic motility [53]. This confirms the possibility of successful use of growth factors to stimulate the proliferation and migration of MSCs in the elderly after fracture. Also in rats older than 2 years and in osteoporosis, the use of recombinant human platelet-derived growth factor-BB with tricalcium phosphate / collagen injection matrix helped to increase the torsional strength of callus at week 5 after tibial fracture compared to treatment without shingles. [54]. The use of titanium implants treated with recombinant human TGFβ-2 contributed to the formation of callus in the humerus of Beagle dogs aged 1–2 and 10–12 years. [55]. However, in older Beagle dogs, the formation of thinner bone trabeculae with a wider osteoid was recorded at 4 weeks post-implantation compared to younger dogs [55].

Gene therapy is another approach to treating bone dysregeneration. The use of MSCs with the transduced BMP-2 protein gene, which decreases with age, together with tricalcium phosphate ceramics [25, 26] accelerated the regeneration of a segmental femoral defect in male Wistar rats aged 24 months compared with the group without the use of cells with transduced BMP-2 [26].

Macrophages play an important role in fracture regeneration in old age. J. A. S. Shantz et al. [56] found that male C57BL / 6L mice (78 weeks of age) increased bone marrow volume and enhanced bone formation after a closed unstable tibial fracture when using pexidartinib (Pexidartinib, PLX3397), which blocks activation of macrophages, compared with young mice (age 12 weeks) with PLX3397 treatment, no increase in callus was recorded compared with control mice [56]. Presumably, blocking a certain type of macrophage, namely type M1, which is proinflammatory and can adversely affect bone regeneration, is crucial in optimizing bone regeneration with age. In a recent study older mice aged 24 months were found to have an increased expression of M1 macrophage genes at the fracture site, enlarged cartilage area and decreased bone area compared with 3-month-old mice 10 days after closed tibial fracture [57]. Administration of the PLX3397 inhibitor to old mice resulted in an increase in bone area in 10 and 21 days post-fracture compared to untreated animals. In young mice, the use of PLX3397 did not show a pronounced effect [57]. Another confirmation of the different effects of macrophages on bone regeneration with age was obtained in chimeric 12-month-old mice transplanted with bone marrow from 4-week-old animals who were found to have larger callus size and faster bone formation compared to older mice where the bone marrow donor was of their age [58]. In a similar experiment with younger

mice, such a dependence was not recorded. In this experiment, osteoblasts and chondrocytes were recipient's cells and proinflammatory cells were donor's cells [58]. Another study also confirms the important role of pro-inflammatory cells in bone regeneration with age. In older 12-month-old mice transplanted into a critical MSC skull defect from young or old donors derived from human muscle and transduced with Lenti-BMP2 / green fluorescent protein, the amount of bone tissue was lower than in younger 9-month-old animals, which was accompanied by a high level of RANKL in the blood, activating the differentiation of osteoclasts from macrophages [59].

Type M2 macrophages are anti-inflammatory and stimulate angiogenesis through the synthesis of IL-10, IL-1 receptor type α and TGF-β. With age, the number of such macrophages decreases, which, according to a study in female Sprague Dawley rats aged 3 and 12 months, leads to a slowdown in regeneration after osteotomy of the femur in older rats [60]. In contrast, transplantation of CD14 macrophage precursors improves bone regeneration in 12-month-old rats [60].

Apolipoprotein E (ApoE) may be one of the therapeutic targets for the treatment of dysregeneration with age. Its level increases in the blood with age, which inhibits bone formation due to exposure to osteoblasts [61]. ApoE -/- 24-month-old mice showed better bone formation and bone mineralization after tibial fracture compared to wild-type mice [61].

The use of allo- and xenoimplants is one of the methods for the treatment of dysregeneration. However, the effectiveness of this method is influenced by the age of the donor and recipient. Lyophilized demineralized xenoimplants obtained from older human donors and implanted into muscle in mice showed a lower ability to stimulate new bone formation compared to xenoimplants from younger donors, which might be associated with lower content of morphogenetic proteins at older age [62]. The use of xenogeneic demineralized matrix in an experiment on male Wistar rats aged 3 and 18 months contributed to regeneration of the femoral condyle defect on the 45th day in both age groups compared with the unfilled defect, but the area of bone regeneration was higher in younger rats compared to older ones [63].

Conclusions

Thus, it has been experimentally and clinically shown that bone regeneration slows down with age, which is more pronounced in women. According to scientific information, this involves two signaling pathways – Notch and Wnt / β-catenin, whose activity is suppressed with age. However, the regulation of regeneration is a cascade of signaling pathways and macromolecules. The expression of growth factors after fracture changes in older age compared to younger. In particular, a decrease in TGFβ-1 expression was clinically detected. In addition, an increase in macrophage colony-stimulating factor and VEGF was observed in elderly patients after fracture. The combination of slowing down bone regeneration with a decrease in the content of Indian Hedgehog, Sonic Hedgehog, BMP-2, -4, -7 and MMP-9 gelatinase proteins in bone marrow has been experimentally established. The ways to overcome delayed bone regeneration in elderly patients may include the use of modern technologies of cell and gene therapy, macrophage inhibitors, biologically active factors at certain stages of reparative osteogenesis. For cell therapy, it is important to take into account the age of the cell donor due to the high probability of functional disorders in the cells from older donors.

Conflict of interest. The authors declare the absence of conflict of interest.

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AGE-RELATED FEATURES OF BONE REGENERATION (LITERATURE REVIEW)

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