



BIOPHYSICAL AND BIOCHEMICAL MODULATORS ASSOCIATED WITH HEALING OF THE OSSEOUS TISSUE

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ABSTRACT

Introduction: Fracture is a bone loss that can be discovered by pathological factors, congenital diseases, avitaminosis or even surgeries, and the cicatricial process as recovery of tissue stability. We have the possibility of assisting in the healing of the bone tissue of biochemical and biophysical modulators, from the use of hydroxyapatite, chitosane, ultrasound and cryotherapy. Objective: This literature review aimed to discuss the characteristics of bone tissue, its regeneration and the main biochemical and biophysical treatments without the use of auxiliary agents in the cicatricial process. Methodology: A research of data on PubMed, SciELO, LILACS, Journal of Cell Science and the Ministério da saúde was done through the keywords “Bone healing”, “Modulators” and its components in Portuguese and English, in the last 10 years. 92 articles were found, with 43 articles selected. Results: According to the studies of literary bases, the biochemical and biochemical ones were constituted and are great auxiliaries in the cicatricial process of the bone tissue. Conclusion: Evidence in the literature has shown that modulators can reduce the time of regeneration of bone tissue.

Keywords: Bone Histogenesis, Healing, Cicatricial Modulators

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How to cite this article:

Mateus Porfírio Rodrigues, Ingrid Larissa da Silva Laurindo, Adalgiza Suellen da Silva Prado, José Carlos Nogueira Nóbrega Júnior, Jéssica Andréia Pereira Barbosa, Marllon Alex Nascimento Santana
.BIOPHYSICAL AND BIOCHEMICAL MODULATORS ASSOCIATED WITH HEALING OF THE OSSEOUS TISSUE. American Journal of Orthopedic Research and Reviews, 2020, 3:11

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INTRODUCTION

Bones are organs formed by a rigid and multifunctional tissue [1], that have a high capacity for repair and regeneration, so that both processes are involved in a complex integration of cells, extracellular matrix and growth factors such as: endochondral and intramembranous ossification. The repair process will consist of restoring the continuity of damaged tissues without necessarily increasing bone volume, while in the regeneration involves the differentiation of new cells and the formation of a new bone tissue [2]. In the development process, the endochondral ossification will be responsible for the growth of the long and short bones and the intramembranous for the flat bones [1].

In Brazil, according to the Ministério da Saúde, between 2007 and 2016, the major incidents that causes of bone fractures in the country were traffic accidents and osteoporosis [3]. However, this developmental process can be restored through bone healing which consists of a complex biological process that follows specific patterns of regeneration and involves changes in the expression of thousands of genes [4].

Although there are many studies to fully understand the processes of bone regeneration, these studies are focused on an understanding of fracture healing, which can be classified directly or indirectly and can integrate both intramembranous and endochondral bone formation. The most common consolidation is the indirect one, since it is a process that is surrounded by a sequence of steps that will involve local and systemic factors interacting with several types of cells, which are recruited for the lesion and for circulation [2], being important for this process is a good blood supply and revascularization [5].

Studies have reported as a possibility in the treatment of restoration and repair of bone tissue the biochemical (MB) and biophysical modulators (MBF), through the use of hydroxyapatite and chitosan, which stand out

due to their biocompatibility and biofunctionality, respectively, and the ultrasound and cryotherapy, allowing angiogenesis and osteogenesis at the site [6,7].

Thus, this review aims to discuss the characteristics of bone tissue, its regeneration and the main biochemical and biophysical modulators used in order to assist the healing process.

METHODOLOGY

There were included in this review studies presenting the process of remodeling, consolidation and regeneration of bone tissue in humans. The exclusion criterion was studies addressing other types of healing. The search was performed independently in the following databases: PubMed, SciELO, LILACS, Journal of Cell Science and Ministério da saúde. The article search was conducted in September 2018 and used the following terms for the search: "Bone healing", "Modulators" and its components in Portuguese and English, in the last 10 years.

The evaluation of the titles of all articles identified in the search strategy was carried out independently by the researchers. All abstracts that provided the necessary information about the inclusion and exclusion criteria were selected for evaluation of the full text. In the second phase, the reviewers individually assessed the full texts of the selected articles following the criteria of eligibility. Adding up all the articles found in the search, we counted 92 articles. Of these, 43 articles were selected, which were read in full and obeyed the criteria adopted for the composition of the review.

HISTOGENESE BONE

Bone tissue is one of the types of connective tissue that has support, protection and locomotion functions [8]. Its basic constitution comes from an organic matrix composed of collagen, in abundance of type I, proteoglycans and adhesive glycoproteins, where are deposited the inorganic components calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), magnesium, citrate,

sodium, potassium and bicarbonate. In addition, this tissue is composed of cells such as osteoblasts, osteocytes and osteoclasts [9]. According to [10], osteoblasts present as polarized cells of mesenchymal origin, with spherical nucleus and basophilic cytoplasm, producing organic matrix of the bone and its mineralization. The osteoblasts present as polarized cells of mesenchymal origin, with spherical nucleus and basophilic cytoplasm, producing organic matrix of the bone and its mineralization. Osteocytes are those osteoblasts that in proportion to secrete the matrix are trapped in gaps, demonstrating a gradual decrease in the amount of their synthesis and secretion organelles as the golgi complex and endoplasmic reticulum, characterizing a poor metabolic activity, however substantial for bone homeostasis [11]. Defined as giant cells and multinucleated, osteoclasts are characterized by secreting hydrochloric acid that dissolves the bone mineral matrix and proteases that degrade the organic portion [12]. The structural unit of compact bone tissue is called osteon or Haversian system, a cylindrical unit lamellar bone matrix involving the channels of Havers, where this Haversian system runs parallel to the long axis of the bone and transports small veins and arteries. The Volkmann canal is perpendicular to the long axis of the bone and is directed to the blood circulation and to the nerves of the periosteum through the Havers channel [13].

The formation of the bone occurs in two ways, by endochondral ossification and/or by intramembranous ossification, both of which are responsible for certain types of bones, where the endochondral predominates in long and short bones, whereas the intramembranous predominates in flats bones [14]. The intramembranous ossification occurs within conjunctive membranes due to a differentiation of mesenchymal cells into osteoblasts [1]. According to [15], an ossification center is formed, which is surrounded by osteoblasts for the deposition of the bone, in which a bone sheet

forms due to a network of trabeculae that have fused and thickened, and the surface of the formed tissue becomes the periosteum. The successive layers of periosteal bone are formed by the osteoblasts until the structure reached will be definitive, resulting in the ossification of the surrounding fibrous tissue. In the endochondral ossification, it starts from the hyaline cartilage that is formed by the solidification of the mesenchyme. The condensed cells of this region differentiate into chondrocytes, cartilaginous cells, in addition, occurs the chondrocyte hypertrophy, decreased of cartilaginous matrix, mineralization and death of these cells. In the formed cavities extend the capillaries and mesenchymal cells, undifferentiated from adjacent connective tissue. These cells will differentiate into osteoblasts, and will deposit bone matrix over the remnant of calcified cartilage [16].

In the long bone, it is initially due to the intramembranous ossification of the perichondrium, which will involve the diaphysis to form the bone collar. In sequence, cartilaginous cells surrounded by the collar will hypertrophy, undergo apoptosis and the cartilage matrix is mineralized. The blood vessels of the periosteum will penetrate the calcified cartilage to transport osteoprogenitor cells that then proliferate and differentiate into osteoblasts. Thus, the osteoblasts constitute layers in the walls of the mineralized cartilage and synthesize the bone matrix that will be mineralized. This process is then defined as the primary ossification center, which rapidly grows until the whole diaphysis is occupied and for the formation of the red bone marrow. The osteoclasts in the center of the cartilage make the absorption of the matrix formed to occur to the formation of the spinal canal, in which the participation of the blood cells allows the formation of this marrow. In the secondary center of ossification occurs later on all the ends of the bone. In these centers the bone evolution is radial and when the osseous tissue is formed, the cartilaginous tissue remains only as articular

cartilage and epiphyseal disc. From this stage of development, the epiphyseal disc that lies between the epiphysis and the diaphysis is responsible of long bone growth [1].

INCIDENTS ASSOCIATED WITH AGENTS CAUSING FRACTURES IN BRAZIL

The fracture is a bone discontinuation caused by an excess of load responsible for the decrease of resistance or due to fatigue of the bone components. There are two main types of fractures: closed (internal fracture, without allowing the fracture to contact the surface of the skin) or open (exposed fracture, which allows the fracture to contact the surface of the skin) [17; 18]. Among its causative agents can be highlighted by pathological issues, traffic accidents, vitamin deficiencies, hormonal imbalance, genetic diseases and mineral deficits [19; 20; 21].

Research in Brazil has shown that the main causes of fractures in the country are osteoporosis and traffic accidents. As the traffic accidents, the main cause of injuries between the years 2007 to 2016, representing the percentage of 38.2% of the fractures, being 22.5% found in the lower limbs and 15.7% in the upper limbs. As for osteoporosis, it is a progressive disease that causes loss of bone mass and consequently leaves the bone susceptible to fractures. The highest index of this pathology is found in women over 50 years and represents about 30% of fractures in the country [3].

Another worrying factor is that in most of these cases surgical procedures are necessary for the repair of fractures, which in some ways present high costs and can often interfere with the wound healing process [22].

BONE HEALING

Healing is a complex biological process dependent on the combination of several factors, involving resident cells, molecular and hormonal factors, aiming at the restoration of the functional and anatomical continuity of the tissue. There are two ways in which bone tissue

can be consolidated, the primary consolidation that is characterized by the non-formation of the bone callus, as well as the death of the osteogenic and endothelial cells of the capillaries due to the discontinuity of the circulation. In the regions of the Haversian canals, where there are viable cells will occur mitoses, cell growth and neovascularization. The newly formed osteoblasts have the role of initiating the reorganization of the Haversian canals [4].

The secondary consolidation process is complex and can be separated in three phases: the inflammatory, proliferative phase and remodeling. The inflammatory phase is initiated after injury, represented by the origin of a blood clot at the injury site and the formation of an edema. Then, the acute phase of inflammation is established through the intense mobilization of neutrophils, macrophages, mast cells, lymphocytes, platelets, fibroblasts, endothelial cells, besides growth factors (PDGF and TGF- β 1) and the release of interleukin 1 (IL-1) and interleukin 6 (IL-6) [23; 24; 25]. At this stage, the action of non-steroidal anti-inflammatory drugs consists of the inhibition of cyclooxygenase 2, promoting the decrease of prostaglandin production, leading to the regression of inflammation [26;27].

With the appearance of macrophages, there will be a large production of proteolytic enzymes, responsible for the phagocytic actions and also for performing other important functions, such as the formation and migration of fibroblasts and the stimulation of vascular neoformation [28; 29]. The proliferation phase begins with the influence of the mesenchymal cells found in the endosteum and periosteum, which will later be differentiated into fibroblasts, the main type III collagen synthesizer, which constitutes the fibrous callus that surrounds the injured area. With the formation of the fibrous callus, the endothelial cells of damaged vessels will form new networks of capillaries that soon invade the region of the clot together with fibroblasts and osteoblasts to initiate the process of

intramembranous and endochondral ossification [30].

During this phase, fibroblasts and mesenchymal cells present differentiate into chondroblasts capable of synthesizing elements of the extracellular cartilaginous matrix (collagen type II, hyaluronic acid, glycoproteins and proteoglycans), characterizing the soft callus. These same chondroblasts undergo another stage of cell differentiation, giving shape to the chondrocytes that will cause cartilage hypertrophy, granting little stability until the callus cartilage is gradually mineralized [31; 32].

The mineralization occurs as a consequence of vascularization that forms within the cartilaginous callus, leading to chondrocyte apoptosis and cartilaginous degradation, which are necessary to allow blood vessel growth to occur in the new tissue to be formed [2]. Since this structural pattern is achieved, the vascularization process is regulated primarily by two molecular pathways, one angiopoietin-dependent pathway and other dependent of vascular endothelial growth factor pathway (VEGF) [32]. Thus, the extracellular matrix of the fracture callus becomes calcified and the healing methods activated only by macrophages, as well as the macrophage colony stimulating factor (M-CSF), Receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG) and tumor necrosis factor alpha (TNF- α), initiates reabsorption of this mineralized cartilage [33; 34].

In the course of this process, M-CSF, RANKL and OPG also participate in the convocation of osteogenic cells coming from the blood supply, with the functions of reabsorption of the cartilaginous matrix performed by osteoclasts that will be gradually replaced by osteoid (organic matrix) synthesized by osteoblasts. Another function associated with this type of cell is the production of a protein called osteocalcin, which is inserted into the bone matrix, contributing to the mineralization [34].

The remodeling phase begins after the composition of the hard callus, referring to the

replacement of the trabecular bone by bone marrow, which may be called the Harvesian remodeling. This process will promote reconstitution of the spinal canal and modification of the scar tissue formed during consolidation, making the bone look the same as the original. The osteoclasts are the most requested cell type during this stage, because of the reabsorption of the mineralized callus that together with the osteoblasts will have the important role of restructure the bone tissue [35]. However, in some cases, healing may have segmental failures due to the great loss of bone mass or difficulty in its regeneration, being one of the main themes of studies in the search for effective methods to accelerate osteogenesis or even more appropriately assist the cicatricial process [9].

BIOCHEMICAL AND BIOPHYSICAL MODULATORS INVOLVED IN THE TREATMENT OF BONE HEALING

Hydroxyapatite is a mineral and natural constituent present in the mass of bones. The synthetic version of this mineral is widely used as a substitute for human bone in implants and prostheses because of its biocompatibility and osseointegration properties [7]. Among the calcium phosphate cements most used in bone surgeries, hydroxyapatite has a great prominence due to its similarity in the physico-chemical formulation with the bone, thus allowing a substrate for the osteoconduction process [6]. Its biocompatibility is represented by the acceptance of the biomaterial by the body and due to its biofunctionality, thus making it possible to exercise its desired functions [7].

Another possible resource, which can be used, because it has a high capacity for tissue repair, chitosan is a biocompatible, non-toxic and biodegradable compound capable of promoting bone neoformation due to its stimulation in the release of cytokines, allowing angiogenesis and osteogenesis [6]. Due to its characteristics, because it is considered a cationic polysaccharide which has a structure similar to glycosaminoglycans, the main components of

the extracellular matrix of cartilages and bones, chitosan acts by attracting calcium ions, thus favoring the process of bone healing [36]. According to [6] studies carried out with bone-implant and bone marrow-associated interface, they demonstrated the performance of this polysaccharide promoting the activation of osteoblasts and osteoclasts, large amounts of fibroblasts, connective tissue, intense vascular neoformation, advanced wound healing and presence of differentiating mesenchymal cells. Thus, as chitosan is characterized as a biomaterial osteoinducer, accelerating and maximizing bone repair, with great osteogenic potential [36].

Among the possibilities of treatment, a possible non-pharmacological approach is through the use of therapeutic Ultrasound. This type of device uses the ultrasonic energy to produce mechanical vibrations in the body generated by a transducer, these vibrations can vary the frequency according to the need of the patient and promote the instability of the cellular environment causing on-the-spot biological changes such as: promote the renewal of the calcium cycle, regenerate tissue, stimulate the release of macrophages, assist in protein production, increase joint mobility, decrease edema and even reduce the pain of the region [37]. The amount of heat generated by the device is dependent on factors such as the emission (continuous / pulsed) regime, intensity, frequency and duration of treatment [38]. The therapeutic Ultrasound can have two effects: thermal and non-thermal. The thermal effects are promoted due to the application of continuous mode in the affected place, not having the interruption of the ultrasonic wave, causing the elevation of the tissue temperature and causing several cellular alterations. The non-thermal ones happen due to the use of the pulsed mode, in which there are successive interruptions of the transmission of the ultrasonic wave, prevailing the mechanical effect, caused by the agitation of the molecules [37].

The cryotherapy is also a widely used feature in the initial stages of the healing process, and this can be defined as a therapeutic procedure that uses the application of cold in varied conditions for therapeutic purposes [39]. This feature can be used in different forms of application: cold compress, gel massage, cold gel compresses, cold chemical compresses, among others [40]. Ice acts on cardinal signs of inflammation (pain, edema, hyperemia, increased temperature and decreased function), and its application may present different forms, depending on the objective to be achieved and the lesion installed [41]. The use of cryotherapy promotes vasoconstriction by the direct action of cold in the blood vessels. In addition, it causes analgesia due to reduced muscle spindle activity, peripheral nerve conduction velocity and reduction of reflex muscle activity [42; 39; 43].

CONCLUSION

Through the findings in the literature, we could verify that the bone tissue after the injury has a high healing capacity, which involves processes of cellular regeneration and tissue repair, being able to be aided by biochemical and biophysical modulators that act both in analgesia controlling pain, edema or inflammation, and in the process of cell proliferation, always seeking success in the healing effect.

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