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Hyaluronic acid as an active agent to accelerate bone regeneration aftertooth extraction: a literature review

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ABSTRACT

Tooth extraction is a dental treatment that is performed frequently in dentistry. This procedure will stimulate a sophisticated healing process involving a variety of biological factors although it takes a long time to complete. Three phases occur in this process i.e. the inflammatory phase, the proliferation phase, and the remodeling phase which aim to restore the tissue function. Several interventions can be used to accelerate bone formation after tooth extraction. Recently, hyaluronic acid (HA) has been commonly used in dentistry due to their essential physiological effects for the periodontal connective tissue, gingiva, and alveolar bone. Hyaluronic acidis a natural non-sulfate glycosaminoglycans compound that has high molecular weight consisting of D-glucuronic acid and N-acetylglucosamine. Hyaluronic acidis also a component of the extracellular matrix that plays an important role in morphogenesis and tissue healing. The mechanism of action of HA works in two ways, that is passive and active mechanism. The passive mechanism is depend on physical and chemical properties of HA that can change the molecular weight and concentration properties. The active mechanism of HA works by stimulating signal transduction pathway initiated by ligand binding with its receptors through autocrine or paracrine processes. The administration of HA can accelerate bone formation due to it can enhance bone morphogenetic protein (BMP) which belongs to the TGF-β superfamily that has high osteogenic capacity. The HA works through a passive mechanism that depends on its molecular weight and an active mechanism by increasing BMP activity.

ABSTRAK

Pencabutan gigi merupakan perawatan gigi yang sering dilakukan di bidang kedokteran gigi. Prosedur ini akan merangsang proses penyembuhan yang melibatkan berbagai factor biologis meskipun membutuhkan waktu lama. Proses ini terdiri atas tiga fase, fase inflamasi, fase proliferasi, dan fase remodeling yang bertujuan untuk mengembalikan fungsi jaringan. Beberapa intervensi dapat digunakan untuk mempercepat pembentukan tulang setelah pencabutan gigi. Beberapa tahun terakhir asam hialuronat (AH) biasa digunakan dalam kedokteran gigi karena memiliki efek fisiologi spenting untuk perawatan kelainan pada jaringan periodontal, gingiva, dan tulang alveolar. Asam hialuronat merupakan senyawa glikosa minoglikan non sulfat alami yang memiliki berat molekul tinggi yang terdiri dari asam D-glukuronat dan N-asetilglukosamin. Asam hialuronat juga merupakan komponen matrik sekstra seluler yang berperan penting dalam morfogenesis dan penyembuhan jaringan. Mekanisme kerja AH bekerja dalam dua cara yaitu mekanisme pasif dan mekanisme aktif. Mekanisme pasif ini bergantung pada sifat fisika dan kimia AH yang dapat mengubah berat molekul dan sifat konsentrasinya. Mekanisme aktif AH bekerja dengan menstimulasi jalur transduksi sinyal yang diprakarsai oleh pengikatan ligan dengan reseptornya melalui proses auto krin atau parakrin. Pemberian AH dapat mempercepat pembentukan tulang karena dapat meningkatkan protein morfogenetik tulang (PMT) yang termasuk dalam super famili TGF-β yang memiliki kapasitas osteogenik tinggi. Asam hialuronat bekerja melalui mekanisme pasif yang bergantung pada berat molekul dan mekanisme aktif dengan meningkatkan aktivitas PMT.

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INTRODUCTION

Tooth extraction is a dental treatment that is performed frequently in dentistry. This procedure will stimulate a sophisticated healing process involving a variety of biological factors and takes a long time to complete. Three phases occur in this process, the inflammatory phase, the proliferation phase, and the remodeling phase, which aim to restore the tissue function. However, this process can lead to anatomical and morphological changes of the hard and soft tissues in the extraction site and complicate subsequent dental treatment.[1-3] Therefore, it is necessary to take immediate action to accelerate this process and prevent these changes.[3] FIGURE 1 showed the bone resorption on alveolar bone that can occur after tooth extraction. Notice the gingival notch originally located on palatal of anterior teeth slightly shifted to anterior after teeth extraction indicating that the decrease of alveolar bone level.[4]

FIGURE 1. Bone resorption post tooth extraction.[4]

Several methods can be used to accelerate bone formation after tooth extraction. One is by performing tooth extraction by mitigating trauma to avoid the risk of fracture to the teeth and alveolar bone.[5] Preventing the detachment of platelets from the extraction site to the oral cavity by suturing and adding collagen or polylactide sponge to avoid infection and delay the healing process.[6] Application of bone graft or soft tissue graft material into the alveolar socket after tooth extraction can maintain alveolar bone volume and prevent bone deformation due to resorption.[7]

In recent years hyaluronic acid (HA) has been commonly used in dentistry because of their essential physiological effects for the periodontal connective tissue, gingiva, and alveolar bone. Hyaluronic acid was discovered and isolated from the vitreous body of a cow's eye by Karl Meyer and John Palmer in 1934. The name of hyaluronic acid is taken from the Greek word *hyalos* which means glass and uronic acid.[8] In dentistry, HA is available in various preparations and concentrations such as sprays, mouthwash gel. Sprays and mouthwash contain 0.01% of HA concentration, while gel has a higher concentration of 0.2% and 0.8%. In low concentrations of 0.2%, the HA has been commonly used to treat the oral lesion, the gingival margin after scaling and root planning treatments, or as a mouthwash.[9] Severe lesions, such as deep periodontal pocket treatment in periodontitis patients and wound healing post-tooth extraction, HA is often applied by injection with higher concentrations of 0.8%. Application with this method is more effective in the oral cavity because it can prevent the dissolution of material into the oral cavity.[10] In this review, we reported the role of HA as an active agent to accelerate bone regeneration after tooth extraction.

DISCUSSION

The existence and physiological role of HA

Hyaluronic acids are a natural non-sulfate glycosaminoglycans compound which have high molecular weight consisting of D-glucuronic acid and N-acetylglucosamine. Theseare also a component of the extracellular matrix which plays an important role in morphogenesis and tissue healing. Hyaluronic acid is a linear polysaccharide from the extracellular matrix which is often found in connective tissue, synovial fluid, embryonic mesenchymal cells, vitreous humor, skin, and other tissues or organs in the body. Hyaluronic acid has good biophysical properties such as high viscosity and elasticity. Other properties such as antibacterial, anti-fungal, anti-inflammatory, osteogenesis and progyogenetic properties are also seen in this compound. Furthermore, HA is also involved in several important biological processes such as mediation and signal transduction, regulating cell adhesion and proliferation, and cell differentiation.[11]

The mechanism of action of HA works in two ways, that is passive and active mechanism. The passive mechanism is depend on physical and chemical properties of HA that can change the molecular weight and concentration properties.[12] Hyaluronic acid with high molecular weight has an ability to form stronger bonds than HA with low molecular weight. Increasing the molecular weight and concentration will increase the viscosity and viscoelasticity of HA. Hyaluronic acid with high molecular weight is thought to have anti-inflammatory effects to accelerate the healing process.[13] The active mechanism of HA works by stimulating signal transduction pathway initiated by ligand binding with its receptors through autocrine or paracrine processes.[14] Hyaluronic acid is estimated has an ability to bond with transmembrane receptors on the surface of endothelial cells.[15] This component is able to increase and stabilize ligand bonds with its receptors, and activate growth factors that will control the processes of proliferation, differentiation, migration, and extracellular matrix protein synthesis. The growth factor that plays a important role in wound healing is transforming growth factor-beta (TGF-β).[16] One of the members of the TGF-β super family is bone morphogenetics protein (BMP). The signal transduction process from BMP occurs due to the presence of ligand bonds with the type I and II serine/threonine kinase receptors which can activate the transcription factors SMAD 1/5/8 through the phosphorylation process by protein kinase groups. Furthermore, the SMAD protein complex with SMAD4 mediators will translocate into the nucleus which can regulate the transcription of the target gene. The signal transduction process through these receptors is slow and takes a long time.[17]

Physical and chemical structure of hyaluronic acid

Hyaluronic acid is a natural polymer belong to the heteropolysaccharide glycosaminoglycan (GAG) family that can diffuse into several cells such as epithelial cells, connective tissue, and nerve fibers. In the human body, the amount of HA is estimated at 15 g of 70 kg of adult body weight. Hyaluronic acid spread in various cells and can form a pericellular membrane on the extracellular matrix (ECM) connective tissue. Nearly 50% of total HA was located on the skin layer of dermis and epidermis. In the synovial fluid and vitreous body, which is composed of ECM, it contains hyaluronan of 3-4 mg/mL and 0.1 mg/mL, respectively. The excretion process of hyaluronan into the extracellular fluid does not need a long time, about 5 g/day, and regulated by enzymatic and non-enzymatic processes.[12]

All glycosaminoglycan molecules, such as chondroitin sulfate, dermaten sulfate, keratin sulfate, heparin sulfate, and heparin are composed of disaccharide units of amino sugars (N-acetyl-

galactosamine or N-acetyl-glucosamine) and uronic sugars (glucuronic acid, iduronic acid or galactose). However, HA is slightly different from other GAG molecules because it does not contain sulfate molecules and neither synthesize from the enzymes of the Golgi apparatus. HA is produced on the inside of the plasma membrane and is not covalently bound to its core protein. HA molecules can reach a very high molecular weight (108 Da), while other GAG molecules have relatively smaller molecular weights (<5 x 104 Da). FIGURE 2 showed the main chain structure of

HA consists of repeating disaccharide units (N-acetyl-galactosamine and N-acetyl-glucosamine) connected by β-1.4-glycosidic bonds. In a physiological solution, the HA carboxyl group will be negatively charged and can be balanced by binding with positive ions such as Na^+, K^+, Ca^{+2}, and Mg^{+2} to form hyaluronic salts. This salt is very hydrophilic on water molecules that will create a barrier surrounding it. In water-based solutions, HA is hydrophobic that can facilitate the aggregation process in the polymerization reaction.[18]
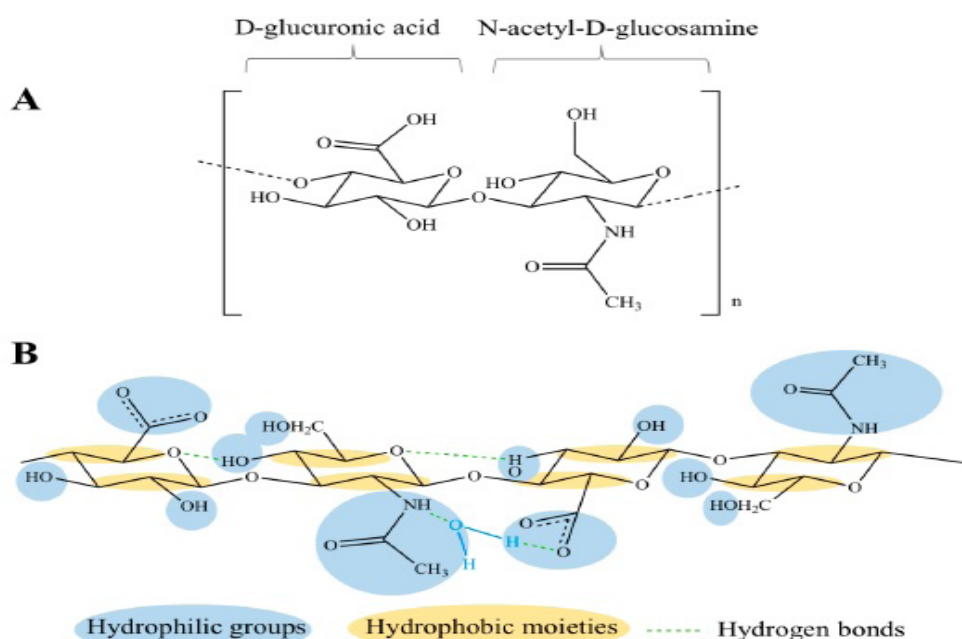

FIGURE 2. Chemical structure of Hyaluronic acid; B. Hydrophilic and hydrophobic properties of HA through hydrogen bond.[8]

The bond strength of the HA molecule highly depends on its molecular weight. HA with a high molecular weight (> 106 Da) will form stronger bonds at low concentrations of HA (1μg/mL). By increasing the molecular weight and its concentration, the bonds formed by the HA molecule will be more stable. That will also increase the viscosity and viscoelasticity of the solution. Because of the polyelectrolyte properties of hyaluronic acid, so that its rheological properties are also affected by its ion's molecule, pH, and temperature. Increasing these factors will significantly decrease the

polymer bonds formed by HA. HA is also very sensitive to changes in pH. At pH below 4 or above 11, HA will be broken down by hydrolysis enzymes. This situation will be more visible in alkaline conditions because of the breakdown of the hydrogens bonds that form the HA chain.[13]

Synthesis and degradation of hyaluronic acid

Hyaluronic acid synthesis in the human body occurs through enzymatic processes. HA is produced and secreted by fibroblast cells, keratinocytes, and chondrocytes. This synthesis is carried

out by hyaluronan synthase enzymes such as HAS1, HAS2, and HAS3, which are enzymes found in the protein membrane. In the meantime, the degradation of HA from the body can also be done through enzymatic or non-enzymatic processes. The enzymatic process involving several enzymes that are very essential such as hyaluronidase, b-D-glucuronidase, and b-N-acetyl-hexosaminidase. These enzymes are generally found in the serum and interstitial space. The role of hyaluronidase is to break down high molecular HA into smaller molecules, while b-D-glucuronidase and b-N-acetyl-hexosaminidase are important to release sugar bonds at their terminal ends. While, the non-enzymatic processes are highly affected by the environment's thermal, chemical, and mechanical properties. All hyaluronidase enzymes found in the human body have a function to degrade HA molecules into smaller molecules. HYAL1 is a lysosomal enzyme that will break down HA into tetrasaccharides. HYAL2 which is attached to the plasma membrane can also degrade HA with high molecular weight into smaller parts. Other forms of enzymes are HYAL3, HYAL4, PHYAL1, and SPAM, where the mechanism of action is not well known

yet. HA secretion can be carried out through the lymphatic system. First, HA with high molecular weight is partially degraded before being released into the tissue matrix and then entering the lymphatic system. Most of the breakdown of HA molecules occurs in the lymph nodes and then enters the peripheral blood vessels and into the blood circulation. The remaining HA is removed by the liver and kidneys.[8]

A study shows that HA concentrations vary in the human body. Most commonly found in the placenta as much as $4g\ kg^{-1}$, $2\text{-}4\ g\ L^{-1}$ in synovial fluid, $0.2\ g\ kg^{-1}$ in the dermis layer, $10\ mg\ L^{-1}$ in the thorax lymph nodes, and $0.1\text{-}0.001\ mg\ L^{-1}$ in normal serum. Hyaluronic acid catabolism pathway showed in FIGURE 3 usually takes place in a matter of days depending on its location in the body. In cartilage tissue, the half-life ranges from 1-3 weeks, whereas in the epidermis it ranges from 1-2 days. The half-life in blood is shorter than the others, which is about 2-5 min. Besides being carried out by enzymatic and non-enzymatic mechanisms, the catabolic process of HA can also be released from the tissue matrix, drainage to the blood vessels, and excretion through the lymph vessels, liver, and kidneys.[19]
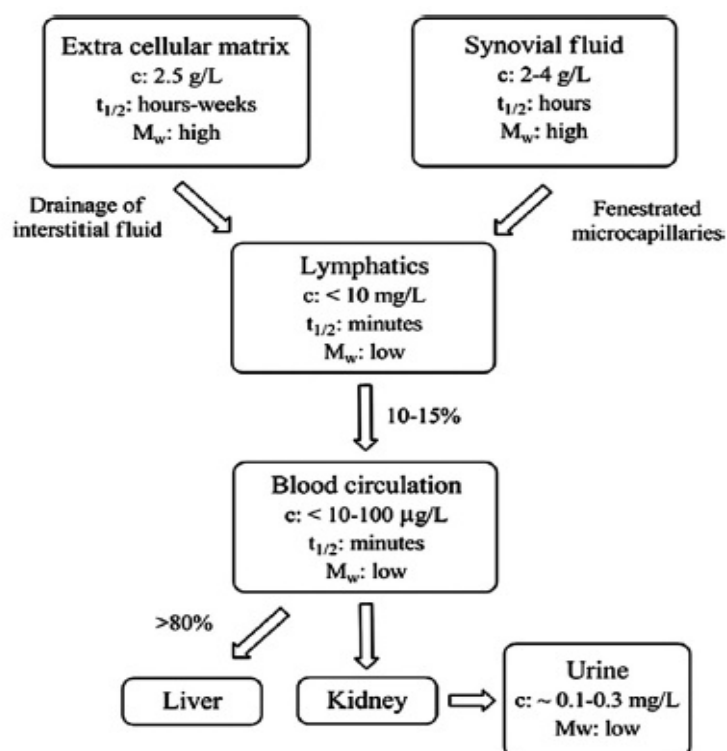

FIGURE 3. Catabolism pathway of HA in the human body.[13]

To understand the pharmacokinetic and pharmacodynamic effects of HA on the human body, a study was conducted by administrating HA through intravenous infusion on healthy human subjects. The subjects were infused with a single dose of HA and increasing the dose gradually. The dosages used were 1.5 mg/kg, 3.0 mg/kg, 6.0 mg/kg, 12 mg/kg, where the total volume of 250 mL. Hyaluronic acid was dissolved in 0.9% sodium chloride solution for 120 min. Blood samples were taken before and after HA administration. In this study, it was found that HA serum levels increased after administration and peaked at 2 h afterward. Subsequently, there was a periodic decreased and reached the lowest rate at all doses on day 3.[20] That study also observed the relationship between HA serum levels with different doses on the ability to bind to mononuclear peripheral blood cells such as T lymphocytes, B lymphocytes, and monocytes. These peripheral blood cells are thought to express HA receptors such as CD44 and RHAMM. In this study, the results showed that at the beginning after administration there was an increase in binding with monocytes along with an increase in serum HA levels. However, when there was a decrease in serum HA levels, there was a difference in the ability to bind to the monocytes. Administration with a high dose of 12 mg/kg or a low dose of 1-3 mg/kg increases the ability of monocytes to bind to HA and corresponds to serum HA levels in the blood. Monocytes will bind more HA at high serum levels and bind less HA at low serum levels. The HA binding ability appeared to be the same at baseline until the next 14 h for all doses. However, at a dose of 3 mg/kg (period 2), there was an increase to a maximum at 14-74 h after administration. The same thing happened at the doses of 6 mg/kg (period 3) and 12 mg/kg (period 4).[20]

Biological properties of HA

Hyaluronic acid has several important functions in the extracellular matrix, because of its ability to bind to cells and other biological components through specific and non-specific bonds. Several molecules and receptors that interact with HA will be involved in the signal transduction process, such as aggrecan, versican, and, neurocan molecules; and receptors such as CD44, RHAMM, TSG6, GHAP, ICAM-1, and LYVE-1. CD44 is a surface receptor that is found on many cell types, so it is currently considered the main receptor for HA. In the early stages of wound healing, HA plays a role in increasing the release of pro-inflammatory cytokines such as TNF-α, IL-1b, and IL-8 through a mechanism mediated by the CD44 receptor. The inflammatory process can also be prolonged by the release of TSG-6, an HA-binding protein, which is stimulated by TNF-α. HA also affects the attachment of lymphocytes activated by cytokines to the endothelial lining. Cell proliferation is a very important phase in wound healing. Hyaluronic acidisindispensable for the process of releasing fibroblasts from the extracellular matrix and mitosis. In the early stages of wound healing, HA will increase the migration of cells, because the newly formed extracellular matrix provides an ideal environment for migration through the CD44 and RHAMM receptors.[8] The interactions between HA and its receptors are regulated in cells by cytokines. Activation of the HA signal depends on the simultaneous interaction between the CD44 and HA molecules. The higher the HA molecular weight, the more attachment areas for the CD44 receptor.[11]

Mechanism of HA to accelerate bone regeneration after tooth extraction

The healing process of alveolar bone right after tooth extraction begins with the emergence of various kinds of cells, such as polymorphonuclear cells (PMN), lymphocytes, and macrophages as inflammatory cells. Growth factors will immediately activate and begin to control the process of proliferation, differentiation, migration, and extracellular matrix protein synthesis.

The growth factor that plays a role in wound healing is transforming growth factor-beta1 (TGF-β1). Bone morphogenetic protein 2 (BMP-2) is a family of TGF-β1, which plays a role in bone formation. Here HA is thought to have the ability to increase BMP-2 activity by increasing the recruitment of type-II receptors, which are part of the sub-unit of the BMP-type I receptor complex.[21-24]

The HA molecule works through two mechanisms, active and passive mechanism. The passive mechanism is related to its physic properties, chemical properties, and molecular weight. Here, HA can increase tissue hydration, maintain osmotic pressure, and form a strong and stable ECM structure so that cells, collagen, elastin fibers, and other matrix structures can be maintained.[12] The active mechanism of HA undergoes through the signal transduction process by binding to its receptors. HA receptors can be divided into HA binding proteoglycans (extracellular or matrix hyaladherins) and HA cell surface receptors (cellular hyaladherins). Hyaluronic acid has two mechanisms of molecular interaction with hyaladherins that are autocrine and paracrine. An autocrine mechanism occurs when a ligand interacts with its receptors on the same cells, leading to changes in those particular cells. Whereas, the paracrine mechanism occurs when a ligand interacts with a receptor from nearby cells and altering those cell behaviors.[14] Hyaluronic acid with high molecular weight can bind to several surface receptors and proteoglycans simultaneously. The structure formed by this interaction will combine with the variety of protein components that compose the ECM. Therefore, HA acts as a framework that will stabilize ECM not only through a passive mechanism, but through binding with various extracellular hyaladherins, such as aggrecan (found in cartilages), neurocan and brevican (found in the central nervous system), and versican (found in various soft tissues).[13,25] Interaction of HA and its receptors occurs through 3 biological processes, namely: signal transduction, pericellular coat formation, and receptor-mediated internalization. The primary receptor for HA is CD44, which is a multifunctional transmembrane glycoprotein receptor found in various isoforms and distributed in all human cells. These receptors specifically can internalize HA. However, the CD44 receptor binds not only to HA but also to various growth factors, cytokines, and ECM proteins, for example, fibronectin.[26,24] Because of this ability, this bond is estimated to act as a docking agent for the matrix protein MMP9 or as a reservoir for growth factors.[13]

Interaction between HA and CD44 will activate the tyrosine kinase receptor pathway as shown in FIGURE 4, which is an enzyme that can transfer phosphate groups from ATP to proteins contained in cells. The bond between the ligand and the receptor will trigger a series of molecular events that culminate in gene transcription. HA-CD44 interaction will increase the activity of tyrosine kinase HER2 and non-receptor kinase Src. Src will phosphorylate cortactin (cortical actin-binding protein), which will then bind to the cell membrane. Cortactin is a protein in the cytoplasm that functions to increase polymerization and rearrangement of cytoskeleton actin, especially actin in the periphery of the cell. This interaction is also able to activate RHOA and Rac1 and trigger bonds between CD44 and Tiam1 and Vav2. Hyaluronic acid also increases CD44 binding with cytoskeletal proteins such as ankyrin and ERM proteins. The interaction between HA-CD44 is estimated to improve communication between various intracellular signaling pathways. For example, between Rho / Ras signaling and receptor-linked HER2/non-receptor linked c-Src tyrosine kinase, which will increase cell activity such as adhesion, proliferation, migration, and invasion such as the growth of tumor cells.[27]
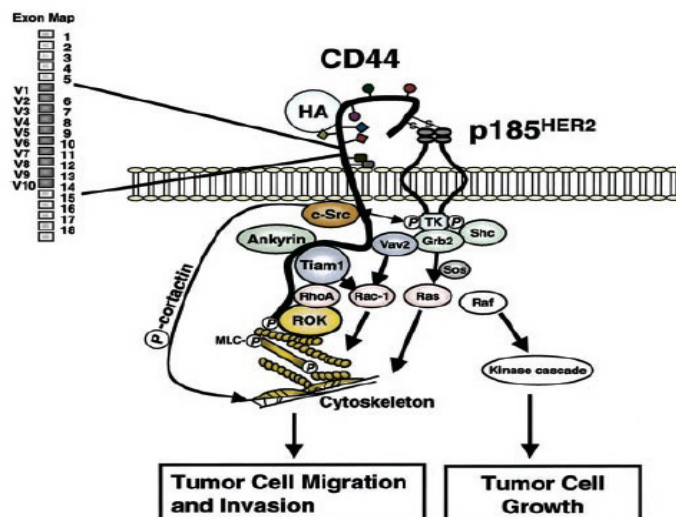
FIGURE 4. Signaling pathway HA-CD44.[27]

Another receptor considered as the primary receptor for HA is RHAMM. RHAMM (the receptor for HA-mediated cell motility) or CD168 is a HA receptor that is present in various isoforms and is present not only on the cell membrane but also in the cytoplasm. The interaction between HA-RHAMM showed in FIGURE 5 will increase the activity of cell migration, formation, and strengthening of the mitotic spindle. This interaction is also involved in the inflammatory process and wound healing by triggering various signal cascades to control macrophage and fibroblast cells.[13] HA-RHAMM interactions can also activate the tyrosine and serine/threonine kinase pathways via activation of Ras and Src. The RHAMM surface receptor can modify the ability of the PDGF receptor to activate Erk kinase, a kinase that functions to regulate cell motility, while the intracellular RHAMM receptors can encode some docking kinases and recognize the location of the protein translation. Intracellular RHAMM is also related to the cytoskeleton, more precisely to the mitotic microtubule spindles. Thus, RHAMM has a role to activate protein tyrosine kinase by endothelial cells because of the binding to HA.[27]
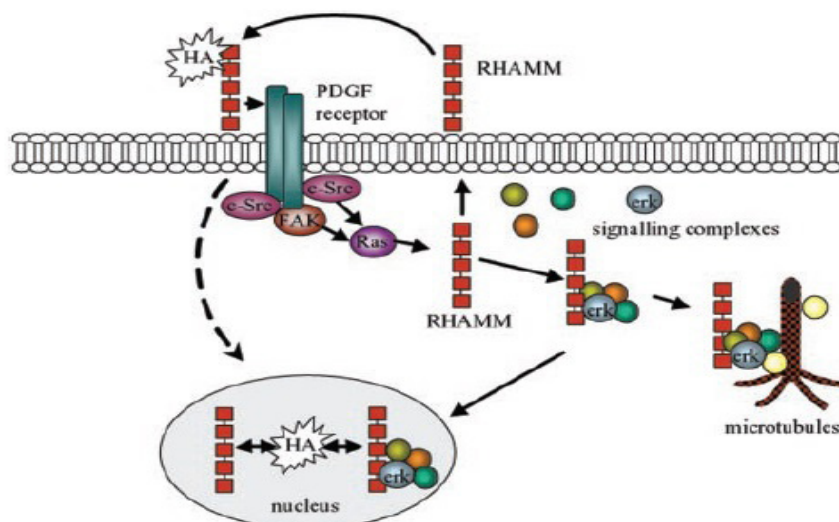

FIGURE 5. Signaling pathway of HA-RHAMM.[27]

Several receptors are capable of binding to HA. For example, HARE (hyaluronan receptor for endocytosis) interacts with HA and other glycosaminoglycan molecules. HARE can break down and remove HA from the blood circulation. Next, LYVE1 (lymphatic vessel endothelial hyaluronan receptor 1) is a HA receptor seen in lymph vessels and macrophages. These receptors play a role in regulating tissue hydration and biomechanical properties. LYVE1 will also form bonds with growth factors, prostaglandins, and other tissue mediators that regulate lymphangiogenesis and intracellular adhesions. TLRs (toll-like receptors) are also receptors capable of binding HA. The interaction between HA and TLRs can recognize bacterial lipopolysaccharides and lipoproteins and activate the immune system.[28]

The mechanism by which HA accelerate bone formation through BMP induction is not very clear. Some studies estimated that the action of BMP depends on the concentration and its combination with various components in the extracellular matrix. This is considered to have a role in increasing the differentiation of mesenchymal cells into osteoblasts. Hyaluronic acidis the compound most commonly found in the extracellular matrix components that functions are essential in maintaining matrix stability and tissue hydration, as well as regulating various cell functions by binding to surface receptors to enhance the signal transduction process.[29] FIGURE 6 shows the signal transduction pathway of BMP involving several type of growth factors. TGF-β groups, such as BMP-2 and activins, are considered to have an ability to activate the SMAD pathway. SMAD is a pathway that transduces the extracellular ligand of TGF-β from the surface receptor to the nucleus where transcription occurs. The ligand-binding with the surface receptor serine/threonine kinase will result in phosphorylation of SMAD 1, SMAD2, SMAD3, and SMAD5 to stimulate the differentiation of osteoblasts. The BMP transduction process begins with when the ligand forms a bond types II receptors, which are specific transmembrane receptors. Next, type I receptors activated because of those bonding previously formed. That will result in molecular phosphorylation so that molecular complexes start to enter the cytoplasm and activate the SMAD pathway. Inside the cytoplasm, the transcription factor of SMAD1/5/8 phosphorylated by the BMP receptor. Phosphorylated SMAD will increase the expression of target genes by binding to the promoter together with co-SMADS (SMAD4) and other transcription factors (DNA binding protein).[30] R-SMADS is a SMAD receptor protein found in the cytoplasm that capable of bonding with several membrane proteins such as CD44 and endorphins. Through that phosphorylation process stimulated by the type I responders, R-SMADS will form molecular complexes with co-SMADS (SMAD4), which enter the nucleus and regulate the transcription of target genes through interactions with various other transcription factors.[31]
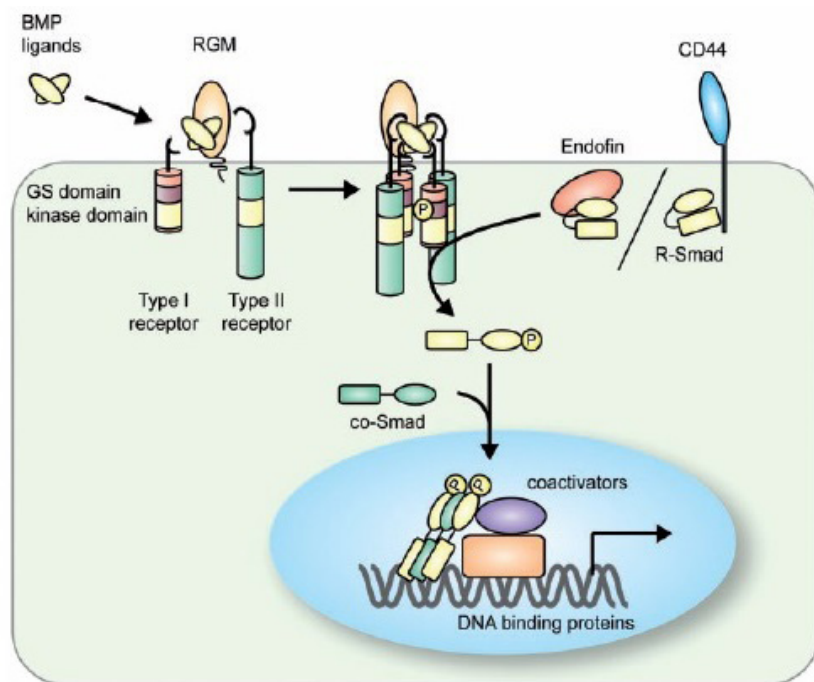
FIGURE 6. Signal transduction of BMP and SMADS.[31]

CONCLUSION

Tooth extraction is a treatment commonly performed in a dental practice that can initiate a subsequent healing process in the alveolar bone. This process consists of the inflammatory phase, the proliferation phase, and the remodeling phase. It involves several types of growth factors and takes a long time to complete. The administration of HA can accelerate bone formation due to it can enhance BMP which belongs to the TGF-β superfamily that has high osteogenic capacity. Hyaluronic acid works through a passive mechanism that depends on its molecular weight and an active mechanism by increasing BMP activity.

ACKNOWLEDGEMENT

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