Dental Clinics
JOURNAL OF GENERAL DENTISTRY
ADVANCE EXTRACT

Hyaluronic acid: biological effects and clinical applications

Demarosi F, Sardella A, Lodi G, Carrassi A
Oral Medicine and Pathology Unit
Department of Medicine, Surgery and Dentistry
Milan State University

■ STATE OF THE ART: *Hyaluronic acid (HA) is one of the main constituents of the extracellular connective tissue matrix of the skin, joints, eyeballs and other tissues, including the periodontal tissues. It is a particularly active molecule in biological terms, and has no significant side effects. It has been used in various fields of medicine for some time. Its use was initially associated with its viscoelastic characteristics, but is now mainly connected with its analgesic, anti-inflammatory and mucoadhesive effects. The function of HA mainly researched seems to be connected with the kinetics of healing processes, by means of complex action mechanisms which include activation and modulation of the inflammatory response, promotion of cell proliferation and re-epithelialisation processes, and inhibition of the CD44 receptors, cell adhesion molecules which are mainly expressed in the leucocytes.*

■ THE PROBLEM: *Although there is a great deal of literature about HA, its applications in dentistry have not yet been given sufficient attention. The specialist literature indicates that the viscoelastic properties of HA are exploited in this sphere, together with its analgesic and anti-inflammatory effect and promotion of wound healing. Its applications in support of the usual mechanical and motivational treatment of patients with gingivitis, and assistance with the treatment of oral surgical wounds, are particularly interesting.*

■ CONCLUSIONS: *Expectations regarding the use of this product could extend to implant surgery and surgical procedures, which can benefit from the osteogenic induction capacity demonstrated by HA in various research projects.*

A significant amount of the progress made in the field of treatments for oro-dento-periodontal disease is associated with the use of substances which are active against oral biofilms, and molecules or materials able to modify the trajectories of the oral tissue healing processes. Understanding of the mechanisms of formation and growth of oral biofilms has led to the development or perfection of molecules that slow the adherence of bacteria to the hard and soft tissues of the oral cavity, or inhibit their growth. These molecules, used locally, are released by a local delivery system or administered systemically, and now represent well-established aids which can improve our clinical results. Dentists currently have some useful tools for perfecting their treatments and pursuing the protection of the patient's health more efficiently (1). The study of oral wound-healing procedures has also led to the development of innovative treatment methods which enable the results of oral surgical procedures to be predicted with a reliable level of accuracy. The use of barrier membranes and morphogenetic protein, for example, has objectively modified the treatment of infraosseous periodontal pockets (2,3,4,5).

In addition to the better-known antimicrobial and anti-inflammatory molecules and biomaterials there are a number of substances, which are less well-known and less used at present, that could lead to further progress in the management of dental treatment. Some of these products are not yet available in Italy. One example is matrix metalloproteinase inhibitors (MMPs) such as low-dosage semisynthetic tetracyclines, which have no antibacterial effects, but counteract the action of the collagenases in the soft periodontal tissues (6).

Other substances are available, but not yet very well known in the dental sector: one such is hyaluronic acid (HA). This article analyses the available literature to provide an overview of this molecule, its use in wound-healing processes, and its possible applications in clinical dentistry.

KEY POINT

Hyaluronic acid, identified for the first time in 1934, is a component of extracellular matrix.

Biological characteristics

HA is a polysaccharide, present in the connective tissue of vertebrates, a polymer of glucuronic acid and N-acetylglucosylamine, and a member of the glycosamine family with high molecular weight. HA was discovered by Meyer and Palmer in 1934 (7) in the vitreous humour of the eye, and is one of the main constituents of the extracellular connective tissue matrix of the skin, joints, eyeballs and other tissues, including periodontal tissue. Unlike other glycosamines, which are synthesised at intracellular level in the endoplasmatic reticulum, HA is biosynthesised in the inner portion of the cell plasma membranes by three different synthetases (HAS1, HAS2 and HAS3) (8); HAS1 and HAS2 synthesise HA with high molecular weight, while HAS3 generates HA with low molecular weight. This information is very important, because the forms with high and low molecular weight seem to be involved in different functions. In humans, the half-life of HA varies from 1 to 70 days, depending on the tissue concerned, and its degradation mainly takes place in the liver and kidneys, where it arrives, under physiological conditions, through the lymphatic system (9). In healthy tissue, HA is present in the form of a polymer with high molecular weight. Fragments with a lower molecular weight can accumulate following a trauma or wound. Each of these polymers possesses specific properties, ranging from the metabolic inertia of the stabilised HA used in cosmetic surgery to the accentuated biological properties demonstrated by the polymers with a lower molecular weight (10). HA performs numerous functions, some of which are associated with the major disproportion between its osmotic power and

KEY POINT

It performs numerous physiological functions: it is a homeostasis regulator and a joint lubricant.

its molecular weight. Due to the hydroscopic effect, HA can absorb over 1000 times its volume of water, producing effects on the body distribution and movements of this fluid, and playing a leading role in homeostasis. HA bonds with different hydrophilic proteins and molecules via hydrogen bridges, forming a viscous microaggregate that participates in the fluid regulation of the tissues and the passage of molecules through the interstitial compartment, at the same time acting as a barrier to the intra-tissue diffusion of macromolecules and harmful external substances. The particular viscosity of HA also suggests that it may act during skeletal movements as the ideal lubricant, allowing the joint heads to slide and reducing the loads on them, and this viscosity leads to important analgesic effects (11). Moreover, much of the elasticity and stability of the extracellular collagen matrix is associated with the presence of HA. Numerous studies have been conducted on its physio-chemical properties and its role in tissue physiopathology. Some of its characteristics (biocompatibility, biodegradability, low immunogenicity and viscoelasticity), together with its versatile properties, suggest that HA can be considered an ideal substance in some pharmacological and cosmetic medicine applications (12). In particular, in dermatological cosmetic medicine, it is used for its filler effect and the absence of adverse immunopathological reactions to its insertion in the skin. In medical applications, its rheological, painkilling and anti-inflammatory properties are mainly exploited. The HA produced for commercial use is usually isolated from animal sources (synovial fluid, umbilical cord or skin) or from bacteria, by direct isolation or fermentation. The absolute value of HA in the human body, in terms of weight, is not particularly high. It has been estimated that the quantity of HA present in the cutaneous compartment is equivalent to approx. five grams (13), and represents some 30% of the total HA in the human body (14).

Repair processes

The complexity of this subject deserves fuller analysis. For further information about this important subject, see the recent reviews (15,16,17).

Wound healing involves a complex set of successive, correlated biological mechanisms which take place in a precise order in space and time. They include coagulation, deposit and differentiation of extracellular matrix, cell colonisation of the wound, and its re-epithelialisation. Processes that require more time, namely wound contraction and remodelling, take place at the end of the re-epithelialisation stage. These processes are of crucial importance in the homeostasis of the human body, and obviously also in clinical dentistry, in which they are routinely involved; some examples are extractions, scaling, oral and periodontal surgical procedures, and root canal treatments.

Many local and systemic factors can disturb the wound-healing processes (*Table 1*), and are reflected in a slowing of its trajectories, while others help to promote its development (*Table 2).* Traditionally, the repair processes which take place in an "*acute wound*", like those produced during intraoral surgical procedures, can be divided into three main stages: *initial*, *proliferative*, and *remodelling* (18;19) .

KEY POINT

In repair processes, HA promotes the migration and proliferation of the cells of the immune system

The initial biological response to bleeding resulting from a wound is based on stemming the bleeding. This purpose is firstly achieved by strong local vasoconstriction; this is a temporary, partial response, because the contraction of the smooth muscles of the vessels, which forms the basis of vasoconstriction, only lasts for a few minutes. Stable haemostasis is ensured by the formation of a fibrin cap following aggregation of the platelets and activation of the coagulation cascade. The fibrin "scaffold" also acts as a temporary matrix to house cells which are attracted by chemicals released by the platelets and thrombin, and colonise the wound area: polymorphonuclear leucocytes and fibroblasts. When the bleeding has been controlled, the increased permeability of the vessels near the site of the lesion facilitates further migration of inflammatory cells (PMNs), which are crucial to prevent bacterial contamination and infection of the area. During the initial stages of wound healing, the cell population is dominated by neutrophilic granulocytes, which represent the first line of defence against microbial infections, engulf the bacteria and keep the inflammatory response alive. A few days later, the epithelial cells at the edges of the wound begin to migrate and re-epithelialise the exposed connective tissue. The re-epithelialisation, when completed, represents the essential biological response for protection of the wound against infection and trauma during the healing process. Migration of the fibroblasts, which replace the temporary fibrin matrix with granulation tissue rich in collagen, begins on the second/third day.

MAIN SYSTEMIC AND LOCAL FACTORS THAT HINDER WOUND-HEALING PROCESSES

■ *Systemic*	■ *Systemic*
Malnutrition	Tissue hypovascularisation
Diabetes	Necrosis
Kidney failure	Repeated traumas
Immune deficiency	Radiotherapy
Steroid treatment	Tumours
Age	Smoking

Table 1

MAIN FACTORS THAT AID ORAL/PERIODONTAL WOUND-HEALING PROCESSES	
■ Wound closing	■ Perfrigeration of the area which is beginning to heal
■ Stitching technique	■ Diet and oral hygiene
■ Periodontal compress	■ Pharmacological support
■ General post-surgical treatment aspects	

Table 2

KEY POINT

HA is believed to play an important part in wound-healing in the foetus.

Extracellular HA promotes cell migration and proliferation at the initial stages of the repair response. The role of HA in wound healing can also be considered in the light of the differences between wound healing in the adult and the foetus. There are numerous differences involving various factors, including scar formation, which does not occur in the foetus, greater speed of the biological responses in the foetus, lower angiogenetic activity, etc. One of the main differences between healing in adult and foetal tissues is the absence in the latter of the inflammatory stage, and a much faster wound re-epithelialisation stage. Foetal tissues are particularly rich in HA. The foetal fibroblasts are believed to produce HA preferentially; this wealth of HA could be the reason for the faster and more efficient healing of foetal wounds. The cell proliferation stage is followed by “remodelling”, which lasts for several weeks, as a result of which the healing processes conclude with the formation of a clear line of demarcation from the peripheral tissue: the scar. A recent review of the literature on the role of HA in wounds and their healing (20) emphasises its crucial role in tissue integrity. Healing procedures are similar in the various parts of the body, but there are some unusual features in the oral cavity. The most important is the presence of the dental elements and the possibility that wounds will be created by tooth extraction which are unusual because the alveolar bone is exposed to direct contact with the oral environment. Numerous recent investigations have studied the post-extraction oral wound healing process and the healing of intraosseous wounds produced by the application of intraosseous implants. These studies have extended our knowledge of healing of the tooth socket after extraction of the corresponding tooth (21), and healing of the periodontal tissues after implants (22) or regenerative treatments (23), confirming that the healing trajectories and basic biological kinetics also maintain the same trends in these areas of surgery.

Therapeutic uses

HA was introduced into treatment long ago, but has only undergone rapid development in recent years. This is mainly due to the introduction of medical device legislation. HA is a substance with no real pharmacological effect, but is useful due to its biological characteristics, which explain its analgesic, anti-inflammatory and mucoadhesive properties (12, 24 and 25) and make it an invaluable therapeutic aid in various fields of medicine. For example, intra-articular infiltrations of HA in the treatment of inflammatory osteoarthritis reduce pain symptoms to a greater extent than injectable intra-articular treatments based on steroidal and non-steroidal anti-inflammatory drugs and a placebo (26). HA is used in ophthalmology to facilitate lens implants, due to its viscosity, and promotes faster healing of diabetic, corneal and varicose ulcers. HA was recently used, after endoscopic application, to treat vesicoureteral reflux, and cured that condition in some 80% of the patients treated (27). However, its most widespread use is in the field of cosmetic surgery: in the USA alone, some 900,000 procedures designed to increase the soft tissues with hyaluronic acid fillers are performed every year. This choice is due to the harmlessness of the molecule and the absence of adverse immune reactions (28). In dentistry, professional products based on HA were only introduced recently. A line of professional gels with 0.8% high molecular weight HA has been available in Italy for a few months. The international literature reports findings confirming that the properties of HA, which are appreciated in other medical fields, are also applicable to the treatment of oral disorders: primarily their ability to stem bleeding, reduce swelling and facilitate healing of damaged tissues. Some interesting experimental studies demonstrate its effect on tissue reconstruction. In a split-mouth study conducted on rabbits, from which the first upper and lower molars were extracted, the effect of an 0.8% HA gel on post-extraction

KEY POINT

HA is already used in a number of medical disciplines, including orthopaedics and ophthalmology.

socket healing was assessed in the test portion of the oral cavity, by comparison with treatment involving simple stitching at the control sites (29). The animals were sacrificed after 3, 7, 13, 20 and 30 days so that the healing process of the post-extraction test and control sites could be studied on histological sections. The total alveolar area, the area of new bone formation in the socket and the percentage of the socket recolonised by bone tissue were considered. The results confirmed that HA promotes healing in the case of a post-extraction wound, presumably due to its ability to promote and accelerate the replacement of clots with granulation tissue. Confirmation is also provided by a study conducted on rats to investigate the possibility that HA promotes the early differentiation of granulation tissue into osteogenic mesenchymal tissue. The authors used histological techniques to study the effects of implanting an HA ester compared with new bone formation without an implant in surgical wounds induced in the sinonasal cavities and calvaria of the rat (30). The results confirmed that osteogenic activity was boosted in the wounds treated with HA. The osteoinductive power of HA with high molecular weight had already been postulated in research conducted with transmission electron and scanning microscopes on the healing of wounds induced in the femur of the rat. The authors, after mechanically inducing standardised bone wounds in rats, filled some of the cavities with an HA-based preparation. From the fourth day of application evident signs of strong osteoinductive activity appeared in the test cavity, unlike the control sites, where no osteoinductive activity was observed (31). Research has been conducted to evaluate the possible use of HA as a support for periodontal treatment. It has been demonstrated that increased production of HA in the periodontal ligament, induced by *bFGF*, the basic growth factor of the fibroblasts, promotes the formation of new cement in infraosseous defects artificially created in the alveolar bone of the beagle and other primates, and in second-degree lesions of double roots. Eight weeks after the creation of the defects, there was an increase in cement apposition and osteogenesis in the sites treated with *bFGF*. The authors consider that the regenerative effect of *bFGF* may be correlated with increased HA synthesis (32). The ratio between *bFGF* and HA and its positive potential in periodontal wound healing was recently confirmed by the same group of authors (33).

KEY POINT

In periodontics, it can be a useful support to the formation of new cement in infradental defects

On the basis of knowledge of the regularisation role played by HA in tissues undergoing an inflammatory process, some clinical trials have been conducted on the treatment of gingivitis; however, in many cases, the forms used differed in terms of the quantity of HA and the method of administration.

In a randomised trial (34) conducted on 60 patients (40 treated and 20 placebo, observed for 1 week), who were non-smokers suffering from gingivitis but not periodontitis, the periodontal indexes recorded on the first day of the trial, after 3 days and at the end of the trial showed a significant reduction in bleeding on probing of the gingival furrow, the papillary bleeding index and the flow of crevicular fluid. The best clinical parameters, based on the consistency of the plaque index values in the two groups, may be attributable to the anti-inflammatory potential of the treatment tested. Other authors, who conducted a randomised double-blind trial on 50 patients suffering from plaque-generated gingivitis (35), reached similar conclusions. Another study on 60 patients treated for four weeks (36) showed an improvement in the gingival conditions, with a statistically significant reduction in the periodontal inflammation parameters used to evaluate the patients. Although the research published to date supports the efficacy of HA in acute clinical conditions, its effects in support of periodontal disease treatment are less clear. A randomised double-blind study conducted on 20 patients with a clinical history of periodontitis demonstrated that HA reduces the level of inflammation and extent of the inflammatory infiltrate on histological testing (37). The mechanisms behind this activity are probably associated with a barrier effect against bacteria, the bond of HA towards *Treponema denticola,* inhibition of the cellular transmembrane signals induced by the CD44 membrane receptors, and reduced expression of Ki-67 proliferation antigen. Conversely, two recent studies (38, 39) which investigated the clinical use of HA in periodontal disease confirmed its anti-inflammatory activity, but without detecting any significant modification in the clinical parameters taken as indicators of the disease. However, this finding may have been influenced by various situations which lead to uncertainty, such as the dosage regime used and the difficulty of effectively checking on the patient's compliance, which limit its predictability.

KEY POINT

Further studies are required to verify all the potential of HA in the various sectors of dentistry.

Conclusions

Analysis of the literature demonstrates that HA is a particularly active molecule devoid of side effects; it has therefore long been used in different fields of medicine, where its properties of non-immunogenic biocompatibility and its viscoelastic, analgesic, anti-inflammatory and wound-healing properties are exploited. In recent years a great deal of research has emphasised the importance of this glycosamine, and begun to uncover its many secrets. The main function of HA appears to be connected with the kinetics of the healing processes through different action mechanisms, which include activation and modulation of the inflammatory response, promotion of cell proliferation and re-epithelialisation processes, and inhibition of the CD44 receptors, a cell adhesion molecule particularly expressed in the leucocytes. The properties of HA have attracted the attention of dentists. Its applications in support of the usual mechanical and motivational treatment of patients with gingivitis, assistance with the treatment of oral surgical wounds, and use as a barrier in recurrent aphthous stomatitis, are particularly interesting. The expectations could extend to implant surgery and surgical procedures which can benefit from the osteogenic induction ability of HA. The possible clinical applications of HA in dentistry have not yet been fully researched; suitable clinical trials therefore need to be conducted on this molecule, which has significant application potential in dentistry. The only way of obtaining a definite response to these latest prospects is to obtain scientific confirmation from new randomised clinical trials.

References