Joint Bone Spine 84 (2017) 283–292

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com/en

Review

RGTA®-based matrix therapy – A new branch of regenerative medicine in locomotion

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ARTICLE INFO

Article history:
Accepted 10 June 2016
Available online 20 September 2016

Keywords:
RGTA®
Heparan sulfate mimics
Extracellular scaffold
Regeneration
Ischemia
Tendon
Revascularization
Bone healing
Osteoblasts

ABSTRACT

Matrix therapy is an innovative, minimally invasive approach in the field of regenerative medicine, that aims to promote tissue regeneration by reconstructing the cellular microenvironment following tissue injury. This approach has significant therapeutic potential in the treatment of pathologies characterized by tissue inflammation and damage, or following injury, conditions which can be incapacitating and cost-consuming. Heparan sulfate mimics, termed ReGeneraTing Agents (RGTA®s) have emerged as a unifying approach to treat these diverse pathologies. Today, skin and corneal healing topical products have already been used in clinics, demonstrating a proof of concept in humans. In this review, we present key evidence that RGTA®s regenerate damaged tissue in bone, muscle, tendon and nerve, with astonishing results. In animal models of bone surgical defects and inflammatory bone loss, RGTA® induced healing of injured bones by controlling inflammation and bone resorption, and stimulated bone formation by coordinating vascularization, recruitment and differentiation of competent cells from specific niches, restoring tissue quality to that of uninjured tissue, evoking true regeneration. In models of muscle injury, RGTA® had marked effects on healing speed and quality, evidenced by increased muscle fiber density, maturation, vascularization and reduced fibrosis, more mature motor endplates and functional recovery. Applications merging RGTA®-based matrix therapy and cell therapy, combining Extra-Cellular Matrix reconstruction with cells required for optimal tissue repair show significant promise. Hence restoration of the proper microenvironment is a new paradigm in regenerative medicine. Harnessing the potential of RGTA® in this brave, new vision of regenerative therapy will therefore be the focus of future studies.

1. Introduction

In response to apoptotic cell death, local signals or communication peptides of the extracellular micro-environment trigger neighboring cells to migrate and multiply, replacing each dead cell by an identical new cell. This biological law, known as tissue homeostasis, applies to most tissues. The organization of the Extracellular Matrix (ECM) plays a central role in this local regulation, but remains poorly understood. The identification of various elements of the ECM, including structural proteins and glycosaminoglycanes (GAGs), in addition to the discovery of a multitude of signals involved in cell-to-cell communication in the past 50 years, has yielded further insight into an increasingly complex picture.

Following extensive cell death however, tissue homeostasis no longer occurs, and cell replacement is not identical, resulting in scars or fibrosis. This process is no longer regeneration but repair, and results in a loss of tissue quality and impaired functions. Furthermore, a chronic process is installed if the cause of cell destruction remains.

By identifying that a subclass of sulfated GAGs, the heparan sulfates (HSs), play a central role in the regulation of tissue homeostasis, we have developed a technology based on HS mimics, that restores the extra-cellular micro-environment and conditions, facilitating the regeneration of tissue following injury. These

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http://dx.doi.org/10.1016/j.jbspin.2016.06.012
1297-319X/

mimics, named RGTA®s (ReGeneraTing Agents), are polysaccharides engineered to replace degraded HS in injured tissues.

Numerous preclinical studies have documented the ability of RGTA®s to accelerate tissue regeneration in the context of various pathologies including corneal lesions [1], diabetic ulcers [2] and burns [3], giving a proof of concept that RGTA® induces tissue regeneration following both acute and chronic destruction in humans (see below). Within the scope of *Joint Bone Spine*, this review will summarize several studies demonstrating the regenerative action of RGTA® in models of bone, skin and muscle injury, and provide new perspectives for the use of matrix therapy alone or in combination therapies, an avenue which holds significant promise in the future of regenerative medicine.

1.1. How does HS regulate tissue homeostasis?

The ECM is a network of secreted molecules that forms a support framework for cells, fulfilling both a structural function by acting as a scaffold for cell components, and a biochemical function, initiating and transmitting signals that direct cell behavior [4]. The ECM is composed of fibrous proteins, including collagens and elastins and glycoproteins, which include fibronectins, laminins, and proteoglycans. Proteoglycans are comprised of a core protein to which one or several GAGs, long unbranched polysaccharides, are attached. HSs are a subclass of sulfated GAGs that play a decisive role in the regulation of tissue homeostasis by mediating and integrating communication events [5] (Fig. 1A, left panel). Briefly:

- HSs are expressed on the cell surface (syndecans and glypicans) and constitute a major component of the ECM (perlecans), together with structural proteins. The organization of the ECM is a scaffold consisting of structural proteins bridged by GAGs, among which HSs are a crucial element.
- The vast structural diversity of HS chains confers the ability to bind and interact with a wide variety of proteins, including most communication peptides such as growth factors, cytokines, chemokines.
- HS acts as a depot for communication peptides, *e.g.* growth factors.
- By positioning the communication peptides, HSs participate in the spatial organization of the cellular micro-environment.
- HS-bound proteins are protected from proteolytic degradation by steric hindrance as HSs are large molecules, thereby preventing the access of proteases.

When apoptotic cell death occurs, communication peptides trapped by HSs become available to trigger one to one cell replacement, a process underlying cellular regeneration. In contrast, in a context of extensive cellular destruction (induced by chemical, thermal, irradiation, external or internal mechanical aggressions, bacterial or viral infections, oxidative stress, etc.), inflammatory and neighboring resident cells release and activate proteases, glycanases and inflammatory communication peptides in the injured tissue. Glycanases degrade HSs, destroying the ECM scaffold, giving access to proteases, which destroy matrix proteins and stored growth factors (Fig. 1A, middle panel). At the same time, the communication peptides released by circulating cells provoke an emergency response facilitating the rapid repair of surrounding tissues. This repair process leaves scars or fibroses. However, if the cause of the lesion persists, a vicious cycle is maintained, as in certain chronic diseases.

1.2. RGTA®-based matrix therapy technology

RGTA®s are large polymers, comprising a saccharide backbone, to which functional groups have been added by chemical substitution (Fig. 1B) [6]. The choice, location and amount of the substituted chemical groups constitute the RGTA® technology and is based on the performance of these polymers *in vitro* (their ability to interact and protect matrix proteins and growth factors) and *in vivo* (their tissue regeneration properties). Since all RGTA®s bear carboxyl and sulfate groups, they can also be tailored with acetate, amino-acid and fatty acid groups to improve their *in vivo* properties.

In contrast to natural HS, RGTA®s are resistant to degradation by mammalian glycanases [7]. Hence, RGTA®s do not form metabolites and maintain their ability to protect the proteins they bind by blocking access to proteases (Fig. 1A, right panel). This is an essential property of RGTA®s: introduction in injured tissues restores the extracellular micro-environment by bridging and protecting scaffold matrix proteins [8,9]. Thus, newly synthesized communication peptides can resume a specific, spatial tissue organization, protected from proteases [10,11]. Like other ECM components, RGTA®s are biodegraded when internalized in cells and catabolized through lysosomal pathways [9]. By acting as a structural element of the ECM architecture and by blocking access to proteases, RGTA® acts mechanically and not as a pharmacological agent, and as such the first two marketed products developed as cicatrizing agents for skin and corneal lesions (see below) were classified as medical devices and not drugs. Interestingly, as predicted by its mode of action, RGTA®s only target available binding sites to replace destroyed HS. No specific receptors are known and RGTA®s do not form intermediates. Protection and repositioning of key factors of tissue homeostasis in the cellular microenvironment makes the use of RGTA® low risk, as the molecule itself is safely catabolized when the initial sugar backbone is polyglucose.

2. RGTA® - regeneration in locomotion

2.1. RGTA® triggers bone regeneration

Many reports have highlighted the importance of HSs in the bone environment. HSs stimulate human mesenchymal stem cell proliferation. These expanded cells possess a strong potential to commit to the osteoblastic and chondrogenic phenotypes and to form bone when implanted in rat segmental femoral defects [12]. HSs also participate in the maintenance of osteocyte pericellular space integrity, as mice deficient in perlecan have a reduced lacunocanalicular network [13] and disturbed mechanosensitivity [14]. HSs may also participate to the control of bone resorption by inhibiting RANKL-induced osteoclast differentiation and activity [15], adhesion of osteoclast precursors and spreading of differentiated osteoclasts [16].

Bone healing becomes a challenging issue in some pathological situations (*e.g.* delayed union, non-union, large loss of bone substance), for which the use of graft materials and/or osteoinductive molecules are prescribed. Similarly, bone destruction generated by inflammatory pathologies do not spontaneously heal. One goal of orthopedic research is to identify agents capable of enhancing bone formation in osseous defects and/or stimulating bone regeneration in compromised bone situations. The use of various growth factors has been proposed to favor bone healing, however the results are inconsistent and in some instances do not restore the anatomy of the treated bones. The ability of RGTA® to regenerate bone in defects where spontaneous healing does not occur, has been studied in surgical defects and in a model of inflammatory bone destruction.

2.1.1. RGTA® repairs bone defects

In a model of craniotomy in the rat (Fig. 2A), the defects were filled at the time of surgery with a piece of collagen soaked with RGTA®. After 1 month, a dose-dependent closure of the defect was observed (Fig. 2B–D). Notably, in the partially filled defects, bone