Original Article

Matrix therapy: a new branch of regenerative medicine and its application in the treatment of burns: from the fundamental to the clinical

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Abstract

RGTA®s, or ReGeneraTing Agents, form a new class of therapeutic products. These are polysaccharides, substituted with functionalised groups, chosen to mimic the heparan sulphates in their functions of organisation of the structure of the extracellular matrix and in their role of storage and protection against the proteolysis of natural protein signals called Growth Factors, Cytokines, Interleukins, Chemokines, etc.

Upon tissue injury or damage such as a burn, enzymes called heparanases degrade the heparan sulphates, thus releasing these cytokines which are then themselves degraded. RGTA® can protect these cytokines or growth factors. This protection will make it possible to prolong their action and therefore their efficacy. In the case of burns, this action notably has the consequence of a modification to the synthesis of collagen, which will result in better healing which is very quickly visible.

This article presents the experimental results obtained in the use of RGTA® to treat burns in animals and its actions at a molecular level and at a histological level. We will then illustrate the use in humans of a medical device based on RGTA®, CACIPLIQ20®, within the framework of the treatment for a severe burn exposing the tendons and joints of the knee and showing how bi-weekly topical application of CACIPLIQ20® was able to avoid a likely amputation.

Keywords: *RGTA®, burn, mimetic heparans, collagen, tissue regeneration.*

Introduction

RGTA®s or **ReGeneraTing Agents** form a new class of therapeutic products that increase the speed and quality of tissue repair and which, in some cases, lead to a real regeneration of the tissues.

RGTA®s are initially defined as polymers functionalised by carboxylic groups, sulphates and substitutions [1-2] favouring properties of penetration or anchoring in the tissues (alkyl chains, lipids, aromatics) or other therapeutic agents (corticosteroids, antibiotics, etc.). They have the property of mimicking the heparan sulphates that are naturally present particularly in the extracellular matrix, and which bind to natural protein signals such as growth factors, cytokines, interleukins, chemokines, etc. Their structure makes them resistant to proteolytic degradation [1]. They can thus, like the heparan sulphates, carry out the mission of protecting the growth factors such as FGF-1 and -2 and TGF-β even in environments rich in heparanases as injured areas are [3]. They have already demonstrated their regenerating ability in various animal models, but also in clinical trials on chronic peripheral ulcers [4-8].

The method of action of RGTA® is based on our molecular understanding of tissue homeostasis. During a tissue attack of any nature (physical, chemical, viral, bacterial, ischaemic, etc.), mass cell death occurs, and the whole of the matrix architecture is destroyed. The heparan sulphates are very

quickly degraded, entailing destruction of the growth factors which are then no longer protected. Circulating and inflammatory cells arrive very quickly to the site of the tissue injury and bring enzymes and growth factors which do not correspond to those originally present in the tissue. Their action is to repair the injury as quickly as possible without setting out to respect the local organisation of the cells. This action explains the appearance of a scar or fibrous regrowth tissues, also called fibrosis [9].

In the case of burns, the increase in the activity of fibrogenic cytokines such as TGF-β1 leads to changes to the network of collagen fibres, notably with a majority expression of type III collagen [10-11] and a persistence of this expression for at least 10 months. The use of heparin, which has a pro-healing action similar to that of heparan sulphates, in the treatment of burns is limited owing to the risk of adverse events: potentially serious haemorrhages linked to the anticoagulant activity, thrombocytopenia, or even allergies [12-13]. RGTA®s have barely any anticoagulant activity [14] and can therefore, through their action, re-establish the normal processes of collagen synthesis and avoid the onset of scarring while accelerating the process of tissue reconstruction.

Evolution of the collagens during treatment

The RGTA® OTR4120 was studied in an evaluation of the quality of remodelling of the matrix in an animal model with experimental skin burn in rats [15]. The burn was induced by a copper disc previously heated in boiling water and applied for 5 seconds to the skin of the back of hairless rats. The injury was immediately rinsed either with physiological serum or with a solution containing 0.1 mg/ml OTR4120 diluted in a saline buffer. The animals also received saline solution or OTR4120 (100mg/100g body weight) intramuscularly. Subsequently the skin applications of saline solution or OTR4120 were repeated as previously once every three days over the first month, then once a week over the following month. The intramuscular administrations were repeated once a week for 3 months, then once a month in subsequent months throughout the whole duration of the study, which was 10 months after the burn.

The animals were split into 4 groups: healthy (skin not burned), healthy treated (skin not burned and treated with OTR4120), control (skin burned and treated with saline solution), and treated (skin burned and treated with OTR4120). The fibrosis index, which is the ratio between the collagen III and the collagen I, is an indication for monitoring the quality of the scar tissue. In the healthy treated group no action of the RGTA® was visible owing to a lack of penetration of the product due to absence of injury. During healing of the burned skin treated with physiological serum, this fibrosis index increased considerably in comparison to the healthy skin in the first week after induction of the burn. It then remained high throughout the 10 months of the study (figure 1).

Another marker of interest in the evolution of the healing, matrix metalloproteases (MMP) constitute a family of proteases involved in the proteolytic degradation of many proteins of the extracellular matrix [16]. They can degrade all components of the extracellular matrix structure and therefore the dermis, but also the growth factors.

The treatment with OTR4120 made it possible to maintain in the burned skin a fibrosis index similar to that of the healthy skin (fig. 1). The abnormal increase in the synthesis of type III collagen observed in the control animals was absolutely not found when the animal was treated with OTR4120 (fig. 1). This specific effect of the RGTA® on the synthesis of type III collagen has already been described [17-18] and could involve an interaction with the FGF-2 (Fibroblast Growth Factors). This would be the reason for better tissue reconstruction since the increase in the synthesis of type III collagen is often associated with fibrosis and excessive scarring.

It has also been demonstrated in previous studies that treatment with RGTA® reduces the production of type III collagen in the intestinal tissues of patients suffering from Crohn's Disease [19].

% collagen
Control Control + RGTA® Burned Burned + RGTA®

Collagen I / Collagen III / Collagen V

Figure 1: Distribution of the synthesis of three types of collagen in a sample of skin taken from unburned (control) and burned (burned) mice, with or without treatment with RGTA®, 7 days after the burn (top) and 10 months after the burn (bottom).

In this study, the expression of metalloproteases MMP-2 and MMP-9 increased significantly in the burned skin, in accord with the data derived from studies in humans [10, 20]. These are key players in remodelling of the newly formed extracellular matrix, such as for example upon formation of the vascular neo-intima. MMP-2, which is expressed constitutively, participates in the constant regulation of the degradation of the collagen, while the expression of MMP-9 is induced during considerable degradation to the extracellular matrix. OTR4120 is capable of improving the activation of the pro MMP-2 and therefore the activity of the MMP-2. As the MMP-9 plays a crucial role in the remodelling of the scar tissue [16], by considerably increasing the activity of these two enzymes, OTR4120 can enable fast and appropriate tissue remodelling.

Another possible explanation for the effects of OTR4120 lies in its capacity to interact specifically with the TGF-β1 (Transforming Growth Factor) and to improve its bioavailability [17, 21]. The TGF-β1 is involved in the production and regeneration of the extracellular matrix in physiological conditions and in pathological conditions [22] and plays an essential role in the control of the fibrosis index by stimulating the production of type I collagen, and also type III collagen, MMP-2 and MMP-9.

Histological evolution of burns upon treatment

In the same model of heat burn induced in rats, a histological analysis of the burned skin, whether or not treated with OTR4120, was conducted [23]. It appears that OTR4120 stimulates the production of new vessels from the very first days. Furthermore, the epidermis is more mature in the group treated with OTR4120 than in the control group, where three layers of keratinocytes were visible, compared to four in the control group seven days after the burn. Generally, the epidermal repair is advanced by around one day. Indeed, the number of layers of keratinocytes is always higher in the group treated with OTR4120 in comparison with the control group (figure 2). Between 7 days and 30 days, the epidermis is constantly thicker in the OTR4120 group.

However, the quality of the newly formed epidermis seems similar in both groups. On day 14, the density of the fibroblasts is higher in the OTR4120 group. This effect could be attributed to the protective effect of the OTR4120 with regard to the FGF-2, which is chemotactic and mitogenic for fibroblasts *in vitro* and *in vivo*. The early development of a myofibroblastic appearance following treatment with OTR4120 can also be explained by the protection of the FGF-2.

Untreated OTR4120

Day 3
Day 4

Figure 2: Histological study on days 3 and 4 magnified x100. Three days after the burn, the control group does not present a layer of keratinocytes, while the OTR4120 group does. On day 4, two layers are visible in the control group, as compared with five for the group treated with OTR4120.

Keratin 14 was chosen for its properties of marker of the division of keratinocytes and the epithelium upon restoration of the skin [24]. Indeed, when the basal cells cease to divide in order to implement the terminal differentiation which results in the production of scales, the keratin 5 and 14 genes are no longer expressed and are replaced with the expression of the keratin 1 and 10 genes.

In the burn model, the presence of keratin 14 is more marked in the group treated with OTR4120 than in the control group 3 days after induction of the burn.

On day 4, the expression of the keratin 14 gene reached a peak in the treated group; this was much greater than in the control group.

On day 5, the keratin 14 is mainly found in the epithelium in the OTR4120 group, while it is confined to the granular layer and the stratum spinosum in the control group. Thus, treatment with OTR4120 induces faster evolution kinetics of the expression of the keratin 14 protein, in correlation with the histological elements showing an acceleration of the reepithelialisation.

Most heparin-binding growth factors play a key role in the healing of burns [25-27], accelerate the division of keratinocytes by stimulating the synthesis of the components of the matrix such as collagen, fibronectin and some proteoglycans [28], and improve the epithelialisation of wounds in a diabetic mouse model [29].

In humans, the topical application of FGF-2 on burns and chronic dermal ulcers accelerates healing [30-32]. In a wound model induced in rats, their administration clearly improves the reepithelialisation and the synthesis of collagen by the fibroblasts [33-35]. In humans, the TGF-β increases and regulates the angiogenesis by actions on the synthesis of the extracellular matrix, the activation of fibroblasts and the synthesis of collagen and fibronectin production [36]. OTR4120 seems therefore to exercise protective effects by maintaining the bioavailability of all these growth factors. These factors are normally stored on the heparan sulphates of the extracellular matrix, but upon injury or burn the heparanases, which are among the first enzymes activated, destroy them, thus releasing the growth factors which are degraded in turn.

As mimetic of the heparan sulphates, the OTR4120 can therefore constitute stable protection of these "heparin-binding" growth factors [1]. Being less sensitive to degradation, their effects could therefore last in spite of the presence of heparanases, consequently enabling better action of the growth factors in the reconstruction of the structure of the extracellular matrix and thus leading to better repair of the injured area.

From the laboratory to humans

RGTA® technology has resulted in several products which have been developed in human clinics. The first product, marketed as a class III medical device under the trade name CACIPLIQ20®, is indicated in the treatment of chronic skin wounds.

An initial clinical trial showed its efficacy on arterial ulcers resistant to the usual treatments on average for 7 months in patients suffering from critical ischaemia who could not or could no longer benefit from surgical revascularisation. Two months of treatment enabled the closure of half of the ulcers [37]. In another trial including patients with vein ulcers or sores over 2 years old, reactivation of the healing with CACIPLIQ20® has been observed from the first month of treatment.

With regard to burns, the action of the CACIPLIQ20® has not yet been published. The case presented here (figure 3) is ulceration by serious 3rd degree heat burning in a diabetic neuropathic patient, stagnating for over three months. Faced with the absence of healing and the real risk of

gangrene in this area, amputation was planned. Although CACIPLIQ20® is not indicated in the treatment of burns, this treatment made it possible to offer the patient an alternative. In compliance with its usual use, CACIPLIQ20® was applied twice a week using a compress imbibed with the product, covering the bare zones visible on the photo (figure 3). The compress was left on the wound for 5 minutes before being disposed of. The wound was then covered with a non-adherent dressing such as a petrolatum pack. Complete and good quality healing was obtained in 4 months with RGTA®.

D0
D+75
D+125

Figure 3: Evolution of the burn of a patient treated with CACIPLIQ20® at the rate of one application every 3 days. Complete and good quality healing was obtained in 4 months (125 days).

Several other patients suffering from deep burns on difficult areas were thus able to be treated and healed, which in some cases made it possible to avoid amputation. The same applied with chemical burns (acid) or boiling water burns that were not healing.

Thus the results on humans seem to confirm those obtained in the burn models in animals and make it possible to envisage clinical applications of CACIPLIQ20® in the treatment of burns, whether or not associated with difficult healing.

Conclusion

Treatment with RGTA®, called matrix therapy, has a real place in burns medicine through improving the speed and quality of the healing. Other investigations are necessary to evaluate the potential of matrix therapy used alone or combined with thin skin, whether or not expanded, or artificial dermis grafts, or the cultivation of keratinocytes.

Conflict of interest: Denis Barritault is the inventor, owner of patents over the RGTA® technology and President of the company OTR3 which manufactures CACIPLIQ20®.

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Summary

Matrixtherapya new branch of regenerative medicine and its developments in burned treatment : from fundamental to clinic

RGTA®, for RegeneraTing Agent, form a new class of therapeutics. These molecules are polysaccharides substituted by functionalized groups selected to protect signal proteins such as growth factors, cytokines, interleukins, chemokines, against proteolytic degradation. These proteins play a key role in cellular communication and are naturally stored in the extracellular matrix via interactions with heparansulfate. During tissue damage such as burning, enzymes called heparanases are released and degrade heparan sulfates, no more protecting signal proteins. RGTA® will replace natural degraded HS and again protect these cytokines or growth factors. This protection will extend their action and therefore their effectiveness. In the case of burns, this action would also result in restoring collagen synthesis to healthy tissue levels. This change allows a better quality of the repaired lesion readily visible on the appearance of tissues.
This review presents the experimental results of the use of RGTA® to treat burns in animal models analysed at a molecular and histological level. Then a case of a human treatment with a medical device CACIPLIQ20® based on RGTA® technology illustrated matrix therapy in the treatment of a burn.

Key words : RGTA®, burns, heparan-sulfates.