J Fr. Ophtalmol., 2008; 31, 5, 465-471 **ORIGINAL ARTICLE**

Pilot study of a new matrix therapy agent (RGTA OTR4120®) in treatment-resistant corneal ulcers and corneal dystrophy

C. Khammari Chebbi (1), K. Kichenin (2), N. Amar (1), H. Nourry (4), J.M. Warnet (4), D. Barritault (2), C. Baudouin (1, 3)

(1) Department of Ophthalmology III, Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris
(2) OTR3 SAS, Paris
(3) INSERM UMRS 592, Institut de la Vision, Université Paris VI
(4) Pharmacy Department, Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris
Correspondence: C. Khammari, Service d'Ophtalmologie III, Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris
Email: cyrine.k@voila.fr
Received on 4 February 2008. Accepted on 26 March 2008.

Pilot study of a new matrix therapy agent (RGTA OTR4120®) in treatment-resistant corneal ulcers and corneal dystrophy

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Aims: This study's objective was to evaluate the tolerance and safety of a new ophthalmic solution based on ReGeneraTing Agent (RGTA) technology in a pilot noncontrolled exploration on compassion use for corneal ulcers and severe chronic dystrophies resistant to the usual treatments.
Rationale: RGTAs are large biopolymers engineered to replace heparan sulphates specifically bound to matrix proteins and growth factors destroyed after a lesion has occurred. The RGTA-bound proteins are protected from proteolysis and this allows the extracellular matrix micro-environment to restore its original proper organisation. The initial endogenous signals needed for tissues to regenerate are back on the restored matrix. They are expected to trigger the natural onset of events, signalling cells to migrate and multiply with the cascades and equilibrium found in tissue homeostasis. RGTA-induced matrix therapy is a possible alternative to cell or gene therapy in regenerative medicine. In a rabbit preclinical model of alkali-induced severe corneal ulcers, a single instillation of RGTA ophthalmic solution was found sufficient to enhance speed and quality of healing, restoring an almost normal corneal histology after only 1 week. These data prompted us to initiate this study.
Patients and methods: Eleven eyes from ten patients were included in this study. All patients had severe dystrophic cornea or painful corneal ulcers rated over 50 on the VAS pain scale ranging from 0 to 100 and had undergone unsuccessful treatments. The RGTA ophthalmic solution was administered by the investigator during each weekly consultation as a single drop over 1 month. Tolerance and efficacy were judged on subjective criteria based on pain evaluation and functional inconvenience as well as on objective clinical criteria through a complete ophthalmic examination at days 3, 7, 14, 21, 28 and after 2 and 3 months from the beginning of treatment.
Results: The study was conducted to completion for all patients included at the beginning. Tolerance was excellent both locally and generally: no uneasiness during instillation, no worsening of the initial pathology, no occurrence of ocular inflammation or increase in ocular pressure, and no general side effects were observed. In addition, we observed a noticeable analgesic effect, increasing with time and instillations, but pain reappeared in the majority of cases as treatment ended. The mean visual analogue scale pain score was 72.73 ± 7.86, it decreased significantly with the first drops of treatment.

After 1 month, the mean visual analogue scale pain score was 32 ± 15.49, then it increased after the end of the treatment, confirming the link between the effects observed and the treatment. Efficacy on keratitis was moderate but with an overall tendency towards improvement. The initial Oxford Score was 3.37 ± 1.06. After 1 month, it decreased significantly to 1.57 ± 0.97 and then it rose again after the end of the treatment. As for corneal ulcers, of the five cases included four healed during the protocol. Two reversed when the treatment stopped, two healed without reversion at the last follow-up visit. The last case was characterised by stem cell deficiency and no improvement was noted. It is important to keep in mind that these ulcers were all resistant to usual therapies.
Conclusion: This RGTA ophthalmic solution is the first matrix therapy product in ophthalmology. The RGTA OTR4120 was used in treating chronic and severe corneal dystrophies as well as corneal ulcers resistant to usual treatments. It was very well tolerated with no side effects. It significantly reduced pain and favoured corneal healing in almost all corneal ulcers. Weekly instillation of a single drop seems insufficient and these very promising data need to be confirmed on a larger population in a controlled trial with more adapted dosages.
Based on these preliminary data, a RGTA-based matrix therapy product may be a very innovative solution to unresolved pain and corneal surface healing problems.

Keywords: Corneal dystrophy, keratitis, matrix therapy, heparan mimetics, RGTA.

INTRODUCTION

Treatment resistant and painful corneal impairments are observed in many conditions of the ocular surface of different aetiologies, such as dry eye syndrome, auto-immune keratitis, toxicity of eye drops with preservatives over the long term or chemical or physical trauma. All these conditions result in an alteration to the ocular surface and the tear film, with loss of its protective properties and release of inflammatory mediators [1-3]. The condition variably affects the different layers of the cornea according to the etiology, the severity and the duration of the exposure, which can result in scar tissue reactions with permanent corneal opacity. Currently, the usual treatments for these impairments of the ocular surface are represented by vitamins, collagenase inhibitors, anti-inflammatories and tear substitutes. In severe cases, cyclosporine, autologous serum or grafts of the amniotic membrane may be used [4-7]. The tears contain several biologically active growth factors which play different roles in cell proliferation, migration, differentiation and survival and in the maintenance of corneal transparency [8-10]. A deficiency or imbalance of these factors is incriminated in several corneal pathologies, particularly those affecting corneal healing [8-9]. Some of these growth factors, such as *epidermal growth factor* (EGF), *nerve growth factor* (NGF) and *insulin-like growth factor* (IGF) [11-14], and also matrix proteins such as fibronectin [15], have demonstrated their efficacy on *in vivo* cell cultures and on animal models with corneal lesions. Recent studies have also shown encouraging results on the use of NGF in humans [16-17].

Regenerating agents (RGTAs for ReGeneraTing Agents) are biopolymers designed to mimic the protective properties of the heparan sulphates with regard to the matrix proteins and the growth factors [18-19]. The first members of the family of RGTAs are dextrans substituted with carboxymethyl, sulphate and hydrophobic groups [20-22], with the dextran being perfectly well tolerated and known in the pharmacopoeia. These RGTAs protect different growth factors and angiogenic factors which have heparin as ligand, such as *Fibroblast Growth Factors 1 and 2* (FGF-2 and FGF-2), *Transforming Growth Factor beta 1* (TGFbeta-1) or *Vascular Endothelial Growth Factor* (VEGF) [23-26], but in reality almost all growth factors, cytokines and chemokines bind to the heparin and heparan sulphates.

Thus the TGFbeta regulates the synthesis of collagen and glycosaminoglycans [23, 26] and contributes to the formation of the extracellular matrix. Its protection by the RGTA demonstrated *in vitro* can only favour this activity *in vivo*. These properties explain how upon tissue injuries, the RGTAs replace the heparan sulphates destroyed by binding to the matrix proteins and resist the remodelling enzymes because they are not destroyed by the heparanases. The binding of the RGTAs

to the matrix proteins also enables the growth factors and the cytokines to act on the injured site. The RGTA thus favours restoration of a matrix organisation similar to the physiological state. Through these dual bindings and protections, the RGTAs make it possible to reconstitute the micro-environment and the good positioning in space and time of the factors secreted by the cells involved in the regeneration process.

Like heparin, heparan sulphate and its analogues, RGTA inhibits *in vitro* the proteolytic enzymes such as elastases, plasmines and cathepsin G [27-29]. This property could reinforce this aspect of *in vivo* protection of the matrix. These hypotheses have been strengthened by many *in vivo* studies on animal models which demonstrated that local or systemic administration of RGTA improves the speed and quality of tissue healing. These studies related to animals having bone defects, injuries of the digestive tract, muscular and gingival lesions, or skin, oral or corneal ulcerations [30-39].

In order to carry out studies in humans, we identified RGTA OTR4120 both for its efficacy in preclinical models as tissue regeneration agent and as protector of the matrix proteins and the growth factors, and for its absence of predictable toxicity. Indeed, OTR4120 does not have potentially toxic substitutions such as the carcinogen benzylamine, nor residual traces of class 3 banned products such as the pyridine used traditionally in the stages of sulphation. Thus, the OTR4120 accelerates the healing of oral ulcers in a model of chemo-induced mucositis in hamsters and offers protection of the lamina basalis of the epithelia [40]. In rabbits, in a model of deep ulceration with sodium impairing the cornea up to two thirds of its thickness, a single drop of solution of OTR4120 sufficed to recover in one week an almost normal corneal histology. The cornea treated with a physiological solution was still very unconstructed and 3 times thicker and more inflamed than that treated with RGTA OTR4120. A marked difference in pain sensitivity was also observed, with the treated eye remaining open, while the untreated eye remained very sensitive and the eyelid closed (Brignole-Baudouin *et al*, ARVO abstract 2004). In these models, the RGTA OTR4120 acted as tissue protector stimulating the healing process by preserving the endogenous cytokines and factors.

These results motivated this initial compassionate pilot study in humans relating to volunteers affected with severe painful corneal dystrophy and corneal ulcers resistant to the usual treatments.

PATIENTS AND METHODS

Preparation of the RGTA

The RGTA OTR4120 is a carboxymethyl dextran sulphate polymer, the formula and synthesis process of which were described in 2005 by Papy Garcia *et al* [22]. The synthesis took place in accordance with the good manufacturing practice (GMP). The base motif was constituted of 15 glycoside units, repeated around 40 times and the mean molecular weight of which is 80,000 Da. The polymer was then, in a clean controlled-temperature room, dissolved in a solution of NaCl 0.9p100 (p/v) at a concentration of 100 µg/ml and in the presence of dextran at 40 mg/ml, then divided into type II glass bottles and labelled, still in accordance with GMP. The preparations were sterilised twice by filtration over 0.22 µm filters and the sterility tests proved negative for each of the preparation batches. The batches were stored at room temperature. The studies on the stability of OTR4120 alone or in ophthalmic solution were carried out in accordance with ICH standards in their final bottling. The product did not undergo any detectable alteration over durations of up to 18 months. The regulatory genotoxicity studies (Ames, microcore and Locus TK) showed an absence of toxicity. The skin and corneal tolerance tests by repeated applications on the eye of a rabbit for 28 days and on the skin showed complete tolerance topically. The measurement of the penetration of fluorescent OTR4120 applied locally to the corneas of healthy or ulcerated rabbits did not highlight any passage into the aqueous humour, which was expected for a molecule of this size.

Study protocol

This was a prospective explorative pilot study for compassion usage, non-controlled, evaluating the safety and efficacy of an ocular solution based on RGTA OTR4120 in chronic severe corneal dystrophy and corneal ulcers resistant to the usual treatments.

All patients included in the study were over the age of 18, capable of receiving full information and attending the monitoring visits. They presented with a severe chronic corneal ulcer or a corneal dystrophy with a pain rated at least 50 on a VAS scale (visual analogue scale) rated from 0 (no pain) to 100 (constant intolerable pain), and resistant to the usual treatments (healing eye drops, tear substitutes, anti-inflammatories, etc.). Patients presenting a perforated or pre-perforated ulcer, an infectious ulcer or a corneal abscess were excluded from the study. The same applied to patients under local anti-inflammatories if they had not stopped taking them at least eight days before the start of the study, and to those receiving systemic anti-inflammatory, anti-histamine, psychotropic or antibiotic treatment modified less than 8 days previously or that could be modified in the short term. Those with contact lenses had to stop wearing them at least eight days before the start of the protocol. A negative pregnancy test and effective contraceptive method were required for women of childbearing age. All patients included were informed by the investigator doctor of the purpose of this study, the monitoring protocol and the conduct to follow in the event of adverse effects. Their agreement was obtained by signing the voluntary informed consent form. The clinical trial application was granted by the CCPPRB of Ambroise Paré hospital on 22 June 2006.

At the inclusion all patients underwent questioning targeted at the functional symptomatology (pain, burning, stinging, etc.) with an evaluation of the pain on the visual analogue scale (VAS) and a full ophthalmological examination with measurement of the visual acuity of both eyes, examination of the eyelids, the conjunctiva, the cornea, the anterior chamber, the iris, the lens and the fundus oculi, and a measurement of the intraocular pressure. The presence of blepharitis was rated from 0 to 4. In the conjunctiva, the possible presence of a conjunctive hyperaemia was noted, and its intensity and the presence of papillae and/or conjunctival follicles. The examination of the cornea particularly looked for the presence of corneal opacity, corneal neovessels and endothelial dystrophy. It was completed in all cases by a test with fluorescein looking for diffuse, localised or ulcerative keratitis. Superficial punctate keratitis was graded on the Oxford scale from 0 to 5. Corneal ulcers were measured with the slit lamp with evaluation of the depth of the ulceration (epithelial, reaching the Bowman's membrane, the anterior or middle stroma). If they reached the deep stroma, the patient was not included. The anterior chamber was examined to check for a Tyndall effect.

Once the patient was included, the study protocol began by instilling one drop of the ophthalmological solution on D1, then an examination on D3. If an improvement was observed, the protocol was continued with an instillation on D7, D14 and D21 with clinical checks before each new administration of the eye drop. If on the 3rd day no improvement was noted, a second drop was instilled on D3 and a check conducted on D7. If no improvement was observed, the treatment was stopped. The patients included were then monitored once a month after D28 for 2 months.

At each check-up visit, the same examination protocol as previously described was followed.

RESULTS

Eleven eyes in ten patients were included in this pilot tolerance study. Six eyes presented painful corneal dystrophy with chronic superficial punctate keratitis, and five eyes presented painful corneal ulcers without tendency to heal. The inclusion of both eyes of a same patient was motivated by the improvement recorded in the treated eye while the condition of the untreated eye worsened and did not respond to any of the usual treatments. The second eye was therefore treated secondarily, on the request of the patient.

The initial pathologies were a limbic deficit in 4 cases, endothelial decompensation in 3 cases, ichthyosis in one case, chronic graft rejection in one case and immunological keratitis in one case. Some patients were awaiting a corneal graft.

All patients included followed the whole of the protocol. Their mean age was 67.8 years (extremes of 35 and 83 years). The group comprised 6 women and 4 men. The mean visual acuity upon inclusion was 0.12 (extremes: light perception at 0.5) and at the last check-up 0.12, so no significant difference in the sense of improvement or in the sense of worsening.

The ophthalmic solution based on RGTA was very well tolerated from the first instillation. On a functional level, no feeling of discomfort or inconvenience was reported by the patients during monitoring. No local or general allergic reaction and no general adverse effect were noted. With regard to the ophthalmological examination, no worsening of the initial pathology under treatment was noted, no increase in ocular pressure, no intraocular inflammation, and no modification to the fundus oculi when this was accessible for ophthalmoscopic examination.

The pain was evaluated on the VAS scale. The mean VAS on the inclusion visit was 72.73 ± 7.86. At the check-up visit at one week, the mean VAS was 49.09 ± 14.46. Therefore there was a significant improvement in the functional symptomatology from the first instillation with a significant difference between the mean VAS on inclusion and at one week ($p < 0.05$). The study of the trend line (*fig. 1*) showed an improvement from the start of treatment which continued over the instillations, but resumption of the pain after stopping treatment, with progressive worsening at the check-up visits after the end of the protocol. However, the pain had not returned to its initial level two months after the end of the treatment and the difference in the VAS remained statistically significant between the day of inclusion and the last examination two months after the end of treatment (mean VAS 55.71 ± 22.28; $p = 0.02$).

VAS score

Oxford score

Inclusion / end of treatment

Figure 1: Evolution of the VAS score during monitoring
Figure 2: Evolution of the Oxford score during monitoring

In the patients presenting superficial keratitis (N=6), the intensity of the keratitis was rated in accordance with the Oxford score. The mean Oxford score upon inclusion was 3.37 ± 1.06 (on a scale of 0 to 5). At one week, the mean Oxford score was 2.62 ± 1.30 ($p = 0.042$). After one month the improvement was more marked with a mean Oxford score of 1.57 ± 0.97 ($p = 0.01$). One month after stopping treatment, this result remained quite stable (mean Oxford score of 1.62 ± 1,18), then the keratitis generally worsened as shown in the trend line (*fig. 2*) two months after stopping treatment with a mean Oxford score of 2 ± 1.58 (the difference with the Oxford upon inclusion no longer being statistically significant, $p = 0.093$).

With regard to the five eyes included presenting corneal ulcers, the evolution was variable. Four patients experienced an improvement to the ulceration with progressive healing under treatment and reduction in pain. Out of these four patients, two relapsed upon withdrawal of the treatment: the first within a week following the end of the protocol, and the second two months later. The two other patients did not relapse after the monitoring for two months after the last instillation. Visual acuity did not for all that improve given the initial highly impaired condition of the cornea and the resulting opacity. In the last patient, no tendency for the ulcer to heal was observed (although there was a

marked reduction in the pain). In this case, the ulcer was characterised by its marginal juxtalimbic location.

DISCUSSION

Chronic corneal dystrophy, ulcers and erosions of the cornea can in some cases, such as in the patients included in this study, be resistant to all usual treatments, particularly when it is associated with a chronic inflammatory component. Several authors set out to find new molecules favouring and improving corneal healing. Thus, certain molecules such as collagenase inhibitors, fibronectin, heparin, EGF, IGF and NGF [11-17] have admittedly shown an effect on corneal healing in *in vitro* studies and on animals, but none is currently available in the ophthalmological pharmacopoeia.

In this study, the main aim of which was the evaluation of the tolerance and safety of an ophthalmological solution based on RGTA OTR4120, we found that at the dosage used this product was very well tolerated locally and generally. We did not note any functional complaint upon application or during monitoring or any adverse systemic effect. No worsening of the initial pathology or local or general allergic reaction occurred during the treatment or during monitoring. Furthermore, this ophthalmological solution did not cause any increase in the ocular pressure or intraocular inflammation and did not show, at least over this short period, any cataractogenic effect.

Furthermore, through our series of patients we noted that the OTR4120 solution had a significant, obvious and fast analgesic effect which began from the very first instillations, increased during treatment, and which seemed to stop after cessation of the protocol. The pain was rated on the VAS scale which is a composite discomfort score and which is widely used in clinical studies for analgesic products. This effect on the pain was also observed in a clinical trial studying the efficacy of a skin dressing also based on RGTA OTR4120 in patients with arterial ulcers linked to atherosclerosis not accessible to surgical treatment (study not published). The reduction in pain in the patients included in our study probably happened via an improvement in the quality of the extracellular matrix surrounding the sensitive nerve endings in the cornea. These results must however be moderated by stating the eminently subjective nature of pain and the impossibility of concluding on the absence of a placebo effect in this study without control group, the main aim of which was to evaluate the tolerance of the RGTA in topical ocular application.

In the group of patients presenting with keratitis, the improvement in the Oxford score was moderate but significant from the first applications. The trend lines clearly show the reaggravation of the keratitis after cessation of treatment and suggest that a longer duration would be desirable.

Moreover, the results obtained in the group of patients with chronic corneal ulcers are also promising, in spite of the small population of this group. Indeed, out of the 5 corneal ulcers included, 4 healed during treatment with the OTR4120 solution. It is important to state here that these ulcers were resistant to all usual treatments. The ulcer that did not respond to the treatment was characterised by its marginal limbic situation which suggested more aggressive immunological mechanisms making it even more difficult to treat. Furthermore, out of the 4 ulcers healed, 2 relapsed after cessation of treatment, which also suggests the interest of a longer duration and/or maintenance treatment.

In conclusion, and in spite of the limits of this initial clinical trial, absence of randomisation or control group, small population, low dosage and short monitoring period, it appears that this new matrix therapy agent based on RGTA is a very well tolerated product that offers through a new approach to corneal healing with promising prospects. Additional randomised studies with control group and more appropriate dosages in patients with less severe corneal conditions are therefore fully justified with the purpose of enhancing our treatment arsenal for patients we are often still unable to treat.

Acknowledgements: The authors would like to thank D. Lasnier (ARMM agency) for the submission of the files to the CCP-PRB of Ambroise Paré hospital. This article is dedicated to the memory of Professor Jean Pierre Caruelle, joint inventor of RGTA and who died in January 2006,

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