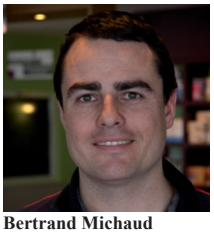
Medical Management of Corneal Ulcers

The Use of RGTAs

Corneal ulcers are commonly encountered in daily veterinary practice. Their treatment involves identifying the origin of the lesion. Therefore, this treatment must be customized to the root cause; there is no predetermined formula. Only the practitioner's clinical judgment and regular follow-ups will ensure that the ulcer is treated correctly.

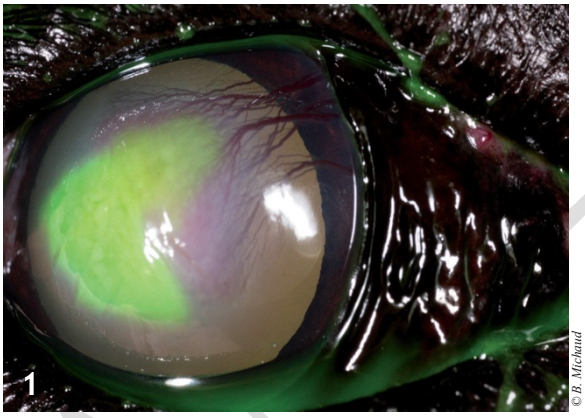


Bertrand Michaud
Veterinary physician
Colomby Veterinary Clinic
25, chemin des places
01170 CESSY
www.vetophtalmo.fr

A new class of drugs has recently emerged in the treatment of corneal ulcers and tissue healing in general, specifically RGTAs (ReGeneraTing Agents - Clerapliq®†). We will explain the action mechanism of OTR4120, as well as its benefits by presenting three clinical cases.

Obtaining a Diagnosis and History

A corneal ulcer is an often painful lesion on the ocular surface that can result from a variety of causes. The diagnosis is made by collecting specific information (lifestyle of the animal, breed, concomitant conditions, progress of the disease, etc.) before even examining the eyes; for instance, a very active dog that spends its days outside is a good candidate for traumatic ulcers.

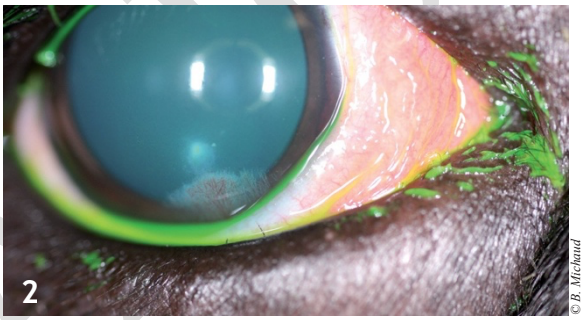


Epithelial ulcer with significant neovascularization.

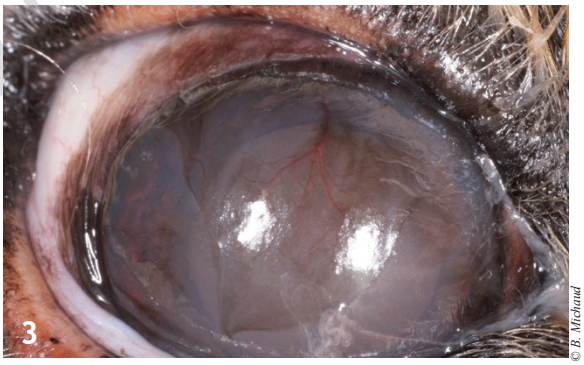
The main goal is to determine the origin of the ulcer. Before proceeding with an ophthalmic examination, a general checkup of the animal must be regularly conducted, along with an evaluation of the pain score. Moreover, the collection of data, e.g. the chronicity of the ulcer, the circumstances of its appearance, prior treatments administered, etc., is also of paramount importance.

Before conducting any other tests, such as fluorescein or eye cleansing, which may interfere with the quantification of basal lacrimal secretions, an evaluation of the lacrimal secretions (Schirmer's test or phenol red thread test) must first be performed. The types of secretions produced by the eye (mucous, seromucosal, mucopurulent, etc.) will then be classified.

A system of magnification, such as a direct ophthalmoscope, magnifying glasses, slit lamp or macro camera, must be used to thoroughly examine the eye and its attachments. Focusing only on the ulcer would be a mistake; consequently, it becomes necessary to look for any eyelid malposition, or ectopic cilia rubbing against the cornea (entropion, distichiasis, ectopic eyelash, trichiasis, and so on).



Corneal ulcer following a lower eyelid distichiasis.

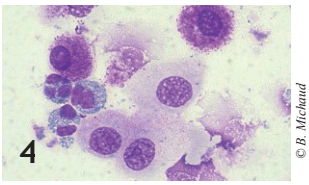


Untreated keratoconjunctivitis sicca, note the pigmentation and dryness of the conjunctiva visible in the glare of the flash which appears "blurred".

As a second step, the conjunctiva needs to be examined and the extent of the chemosis must be ascertained. Seeing as all possible evidence needs to be gathered, the third step consists of an examination of the actual cornea, which is of vital importance. Careful observation is then needed to determine the presence of corneal neovascularization, which would indicate the length of time the ulcer has been growing, as well as to evaluate the depth and surface area of the lesion (use of fluorescein, presence of corneal edema …). These factors are essential in the therapeutic management of this type of condition. The practitioner can use different stains (fluorescein, Rose Bengal, etc.) to classify the ulcer and evaluate the corneal damage.

†Translator's Note: OTR4120 is available in Europe as Clerapliq® and in North America as Optixcare EYE EMS.

Lastly, conjunctival and corneal cytology is a decisive weapon in establishing the ulcer's etiology. Its practical implementation is simple, even if reading the slides requires some training. Etiological investigations by PCR testing or bacteriology are rarely performed as a first step, but must be discussed at the very first consultation.



Example of ocular cytology.

It is equally important to display the ulcer (diagram, photograph, etc.), in order to optimize monitoring of the lesion during treatment.

Available Treatments

Treatment will depend on the stage of the ulcer and the extent of corneal lysis. Beyond treating the cause (blepharoplasty, removal of distichiasis, etc.), four key objectives must be considered:

Speed up the healing process of the ulcer

• This involves the topical use of trophic factors (vitamin A, vitamin B12, growth factors, autologous serum, etc.), which enhance the natural healing process of the cornea.

• Healing can also be stimulated by debridement of the ulcer in combination with a grid or radial keratotomy, the implant of a contact lens, or surgical sutures.

• Recently, techniques known as cross-linking have been adapted from human ophthalmology (using ultraviolet light and riboflavin), and have proven effective in animals. These bypass techniques create covalent bonds between distorted collagen fibers. The action mechanism here is similar to that of ReGeneraTing Agents such as OTR4120.

Limit pain

• Topical cycloplegic agents (atropine, tropicamide, etc.) are effective analgesics.

• Wetting agents (hyaluronic acid, carbopol, etc.) help reduce the eyelid rubbing against the ulcer, which leads to blepharospasm, a sign of ocular pain. Some of these are added to trophic substances, which improves treatment compliance.

• Contact lenses provide effective pain relief as well, despite their high cost, low durability and associated risk of trapping germs.

• Topical non-steroidal anti-inflammatory drugs, such as diclofenac, offer little pain relief and remain controversial.

• Non-steroidal anti-inflammatory drugs and/or systemic morphine should always be combined with topical treatments.

Avoid secondary infections

• The choice of antibiotic(s) as well as their administration route (topical and/or systemic) is determined based on:
- the severity of the ulcer,
- what treatments already been carried out,
- test results,
- and the progress of the lesions.

• The use of collagenolytic substances (NAC, EDTA, etc.) should not be systematic. They must only be used in specific contexts, for instance in cases of keratomalacia.

Optimize treatment compliance

• The galenic form of the products (eye drops or ointment, slow release or fast-acting, the type of the excipient which may or may not allow passage into aqueous forms) is quite essential when considering the willingness and the motivation of the owners.

• The greater the number of treatments required the less compliance follows, making it necessary to compete in terms of inventiveness to simplify their application, i.e. preparing adjuvant eye drops: artificial tears combined with antibiotics, cycloplegic drugs, anti-collagenases, and so on.

There is no actual cure for an ulcer; therefore, it is essential to remember that the cause of the condition must be established, so as to provide a more effective treatment.

Monitoring and Progress

Following the start of the treatment, the patient must be seen two days, five days, and ten days later. Any modifications to the treatment cannot be implemented before five days have lapsed, unless new test results indicate the need for a different treatment. If tissue loss persists beyond the fifth day, a surgical filling solution should be considered, i.e. conjunctival pedicle graft, synthetic biomaterial graft, application of cyanoacrylate …

Clinical Cases

We have selected three clinical cases to illustrate this approach and to present the benefits of RGTAs:

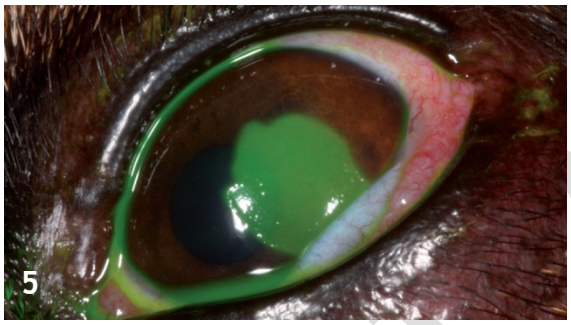
Clinical Case No. 1

A 5-year-old Jack Russell Terrier was brought in for consultation with pain in the left eye that had been progressing for five days.

Tear secretion was evaluated using phenol red: normal value (18 mm in 15") in the left eye, and an increase in value (>25 mm in 15") in the right eye. On direct examination, an epithelial ulcer involving 30% of the corneal surface, with a central and inferior location, was

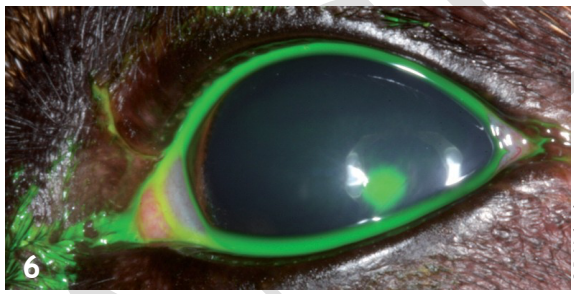
revealed with fluorescein: it appeared to be superficial. The eye was very painful, with blepharospasms and pronounced serous epiphora. Significant chemosis was also noted. An examination of the accessory structures did not reveal any traumas, i.e. ectopic cilia, distichiasis, entropion, foreign bodies, etc. Also, the cornea did not present any neovascularization, despite the age of the impairment. Therefore, this consisted of an atonic epithelial ulcer.

A treatment of acetylcysteine and a combination of neomycin + polymyxin B had been prescribed by the first veterinarian. Seeing as this ulcer appeared to be the result of a trauma, the use of an anti-collagenase agent was not justified in this case. Treating the pain was entirely overlooked, even though it plays a central role not only in the healing process, but also in the owner's opinion of the veterinarian.



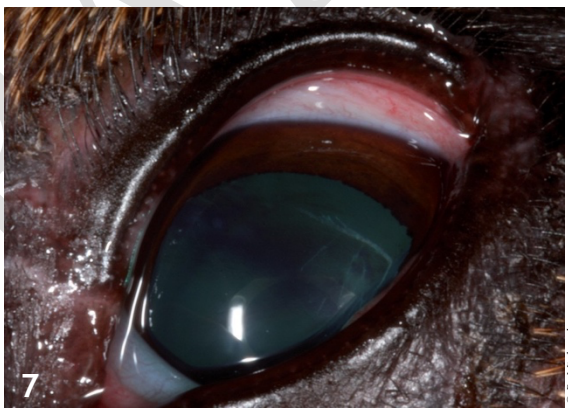
© B. Michaud

Appearance of the left eye on initial examination.



© B. Michaud

Left eye after 48 hours of treatment.



© B. Michaud

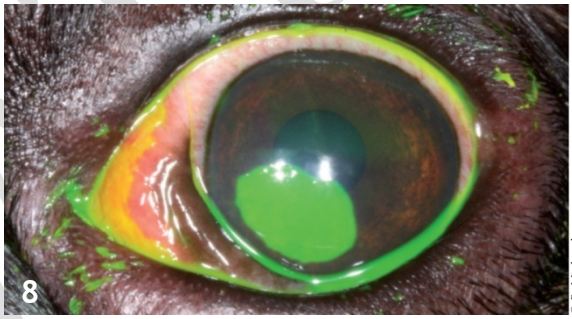
Left eye eight days after the start of treatment: total disappearance of the ulcer.

A treatment based on atropine (Atropine 0.5%® Alcon) administered twice daily for three days, with a combination of neomycin + polymyxin B (five times daily for seven days) and matrix regeneration agents (Clerapliq®† TVM) is recommended at a dose of one drop every two days for ten days.

The dog was re-examined on D2: the eye was wide open, the chemosis had faded and the surface of the ulcer had clearly decreased. On D8 the results were perfect: the dog was cured.

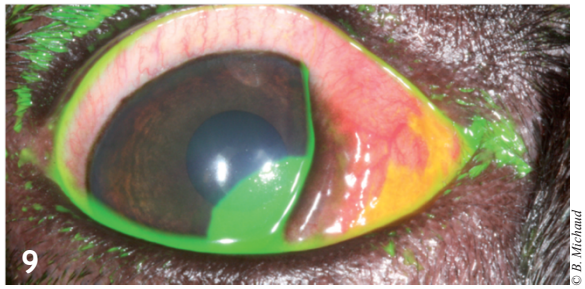
Clinical Case No. 2

A 17-month-old French Bulldog was brought in for consultation for a bilateral ulcer which had not progressed, despite having been seen by a colleague in the ER three days earlier. The dog had been started on a topical treatment with sodium hyaluronate, acetylcysteine and a combination of neomycin + polymyxin B.



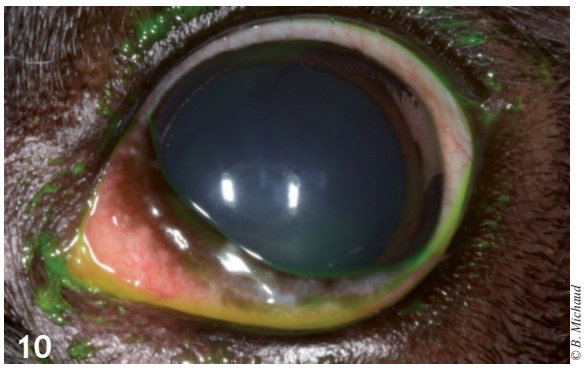
© B. Michaud

Left eye on initial examination after fluorescein staining.



© B. Michaud

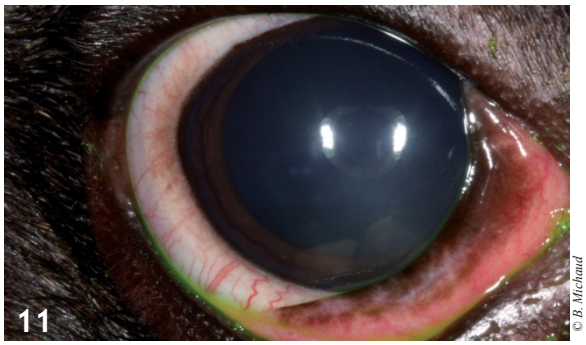
Right eye on initial examination after fluorescein staining.



© B. Michaud

Left eye after three days of treatment.

†Translator's Note: OTR4120 is available in Europe as Clerapliq® and in North America as Optixcare EYE EMS.



Right eye after three days of treatment.

During a far-off examination, pronounced pain was noted in both eyes (blepharospasms, epiphora, enophthalmos, etc.), associated with a bilateral protrusion of the nictitating membranes. The tear test was increased for both eyes because epiphora was present; this result was not taken into account.

An earlier examination revealed chemosis, hyperemia of the nictitating membrane in both eyes, as well as ulcerative keratitis on the projection area of flashing bodies. The examination of the accessory structures and anterior chamber was normal.

The ulcers were epithelial and very extensive.

After local anesthesia, the third eyelids were everted and showed hyperplasia of the lymphoid formations on the orbital side. These elements were then scraped off. A cytological screening revealed the presence of numerous lymphocytes and macrophages.

These lymphoid clusters were the cause of the corneal irritation, and were excised by scraping.

A treatment blending atropine, RGTA and diclofenac was recommended locally, in combination with a general treatment of meloxicam. Three days after the start of treatment, the ulcer had filled; ten days later, the congestion in the third eyelids had completely disappeared.

Clinical Case No. 3

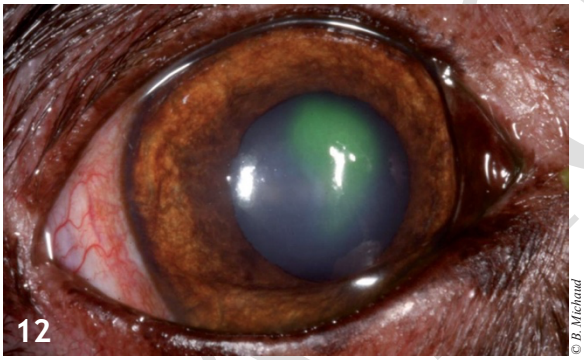
An 11-year-old Spitz dog was referred to the clinic for a traumatic ulcer in the right eye, which had appeared three weeks earlier. It was initially treated topically with gentamicin and sodium hyaluronate.

The far-off examination revealed moderate exophthalmos, as well as the presence of an epiphora and blepharospasms. The lacrimal secretions were within normal range.

An earlier examination revealed very significant chemosis, a very superficial ulcer and the total absence of any corneal neovascularization. Ocular pressure was normal.

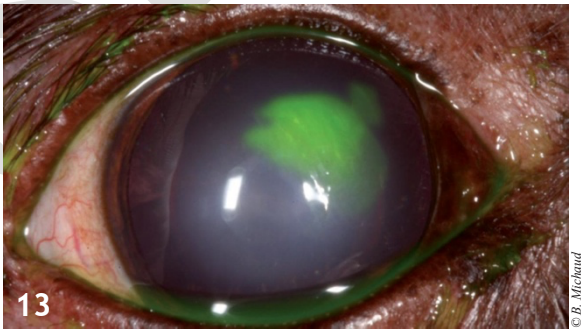
Therefore, this consists of an atonic ulcer, which in a dog over eight years of age generally suggests a systemic disease that can delay healing, or even fail to heal, namely Cushing's syndrome. A stimulation test can confirm the diagnosis of hypercorticism.

A trophic and analgesic treatment combining vitamin A, RGTA (Clerapliq®†) and Atropine 0.5%® is recommended. A trilostane-based treatment was initiated upon receipt of the ACTH stimulation test results.



Early examination of the right eye after staining on D0.

Eight days later, the dog was seen again. The ulcer had not progressed, even if the pain had significantly decreased. The treatment was completed with a radial keratotomy that aimed at stimulating vascular flow. The conjunctival hyperemia was nevertheless much less pronounced when compared to the first visit.

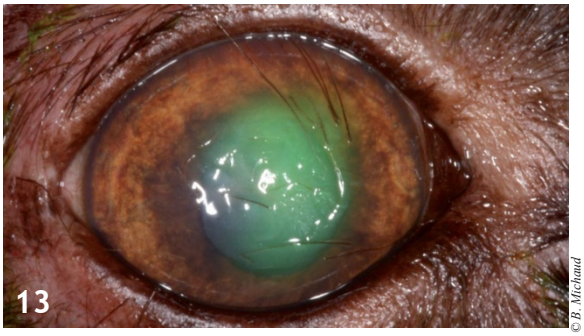


Right eye eight days later.

Three weeks after the start of treatment, or six weeks after the trauma, despite correcting the hypercorticism, the ulcer had still not filled up. It was therefore decided to proceed with conjunctival pedicle graft. Some substance shortages cannot be medically managed, despite the introduction of innovative therapies for the treatment of ulcers. A surgical filling solution is sometimes necessary, and the presence of

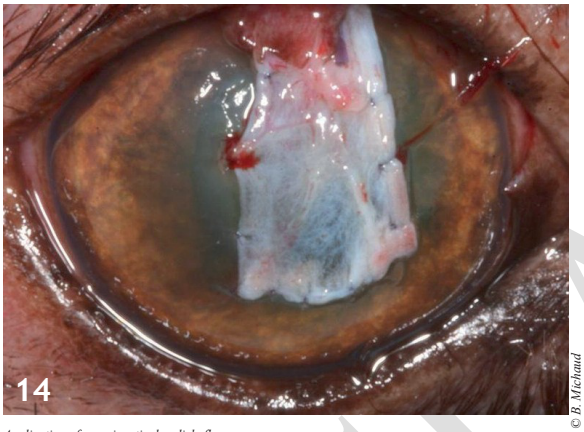
†Translator's Note: OTR4120 is available in Europe as Clerapliq® and in North America as Optixcare EYE EMS.

atonic ulcers very often indicates the need for surgery.



© B. Michaud

Right eye after 21 days of Clerapliq®† treatment.



© B. Michaud

Application of a conjunctival pedicle flap.

RGTAs, a Miracle Solution?

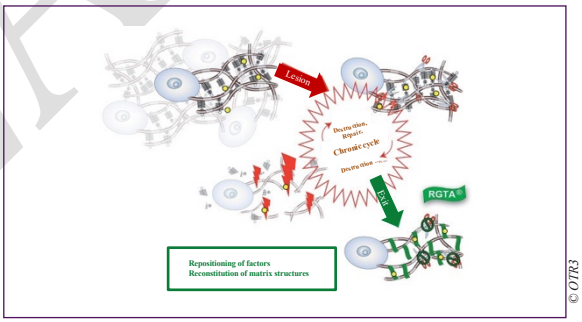
Corneal impairment that remains resistant to standard treatments can occur in a number of ocular surface disorders with different etiologies, such as physical or chemical traumas, dry eye syndrome, or autoimmune keratitis. All these disorders culminate in a change in the ocular surface and the lacrimal film, resulting in the loss of its protective properties and the release of inflammatory mediators. This damage affects the individual layers of the cornea differently, depending on the cause, severity and duration of the condition, which can lead to fibrotic scarring with permanent corneal opacities.

Currently, standard treatments for such ocular surface disorders include vitamins, collagenase inhibitors, anti-inflammatory drugs, and lacrimal substitutes. In the most severe cases, cyclosporin, autologous serum or conjunctival pedicle grafts, synthetic biomaterial, or even amniotic membrane grafts can be utilized.

ReGeneraTing Agents are a new family of innovative drugs in terms of their action mechanisms. They are "real" healers.

Functioning Principles

Tears contain several biologically active growth factors that play various roles in cell proliferation, migration, differentiation and survival, and are instrumental in maintaining corneal transparency[2]. A deficit or imbalance in these factors is implicated in several corneal conditions, especially those affecting corneal healing. Some of these growth factors, i.e. the epidermal growth factor (EGF), the energy growth factor (NGF) and the insulin-like growth factor (IGF), and some matrix proteins like fibronectin have shown their efficacy on *in vivo* cell cultures and in animal models of corneal lesions[5, 6, 9, 11, 13]. Recent studies also show encouraging results regarding the use of NGF in humans[11]. ReGeneraTing Agents (RGTAs) are biopolymers designed to mimic the protective properties of heparan sulphates with respect to matrix proteins and growth factors[1, 3]. The first members of the RGTAs family are dextrans, substituted by the carboxymethyl, sulfate and hydrophobic groups, with dextran being perfectly tolerated and well known in the pharmacopoeia[12]. These RGTAs protect different growth factors and angiogenic factors, which use heparin as ligand, for instance Fibroblast Growth Factors 1 and 2 (FGF-1 and FGF-2), Transforming Growth Factor beta1 (TGFbeta-1), or Vascular Endothelial Growth Factor (VEGF), but in reality almost all growth factors, cytokines or chemokines bind to heparin and heparan sulfates[6].



© OTR3

Diagram of the RGTAs means of action versus heparan sulphates (document OTR3).

Therefore, TGFbeta regulates the production of collagen and glycosaminoglycans, and contributes to the formation of extracellular matrices[1, 7]. The protection by RGTAs demonstrated *in vitro* can only favour this activity *in vivo*. These properties tend to indicate that in the presence of tissue lesions, RGTAs would replace the destroyed heparan sulphates by binding to the matrix proteins and resist the enzymes responsible for restructuring, seeing as they have not been destroyed by the heparanase enzymes[1, 3, 12]. RGTAs binding to the matrix proteins also allow growth factors and cytokines to act on the injured site. RGTAs would thus promote the return of a matrix structure that is closer to its natural physiological state.

†Translator's Note: OTR4120 is available in Europe as Clerapliq® and in North America as Optixcare EYE EMS.

By means of these double bindings and protections, RGTAs thus enable the restoration of the micro-environment and the proper positioning in space and time of the factors secreted by the cells involved in the regeneration process. Like heparin, heparan sulfate and its derivatives, RGTA inhibits *in vitro* proteolytic enzymes such as elastases, plasmins and cathepsin G[1,3].

This property may strengthen this aspect of the *in vivo* protection of the matrix. These hypotheses have been confirmed by numerous *in vivo* studies on animal models, showing that a topical or systemic administration of RGTAs improves the speed and quality of tissue healing. These studies focused on animals with bone defects, lesions in the gums, muscles or digestive tract and ulcers in the skin, mouth or cornea.

In order to leverage RGTAs in humans and animals, the RGTA OTR4120 was selected for both its efficacy in preclinical models as a tissue regeneration agent and as a protector of matrix proteins and growth factors, as well as for the absence of any foreseeable toxicity. Indeed, OTR4120 does not possess any potentially toxic substitutions, such as the carcinogen benzylamine, or any residual traces of prohibited class 3 products, such as pyridine, which are regularly used in the process of sulfation[8]. Consequently, OTR4120 speeds up the healing of oral ulcers in a model of chemically-induced mucositis in hamsters, and ensures the protection of the epithelia basal lamina[9, 13]. In a rabbit model of deep alkali-induced ulceration, which altered the cornea across approximately two-thirds of its thickness, a single drop of an OTR4120 solution was sufficient to restore the cornea to an almost normal histology within one week. The cornea treated with a physiological solution remained very distorted and three times thicker and more inflamed than the one treated with RGTA OTR4120 (Brignole-Baudouin et al., ARVO abstract 2004). A clear difference in pain sensitivity was also noted; the treated eye remaining open, while the untreated eye was very sensitive and the eyelid stayed closed. In these models, RGTA OTR4120 protected the tissue and promoted the healing process by preserving endogenous factors and cytokines.

Available since April 2012, Clerapliq®† is an OTR4120 identical to its counterpart for humans that has yet to be put on the market. It is indicated for its analgesic and structuring properties that often optimize the speed of corneal ulcer filling.

It is recommended as a first-line treatment, in conjunction with treatment of the etiology.

From a personal perspective (see box), RGTAs' properties make it feasible to expand their use as an adjuvant in the treatment of dry keratoconjunctivitis, pigmentary keratitis, as well as in the postoperative management of any keratoplasty, i.e. corneal transplants, keratectomy, endocular surgery, etc. A demonstration of their activity on all types of *in vitro* and *in vivo* tissue, such as bone, intestine, skin, and so on, suggests a wide field of application in both animal and human medicines.

Bibliography

1. Barritault D, Caruelle J.-P. Regeneration agents (or RGTAs): a new therapeutic approach. Ann Pharm Fr, 2006; 64: 135-44.

2. Baudouin C. A new approach for better comprehension of diseases of the ocular surface, J Fr Ophtalmol, 2007;30:239-46.

3. Fredj-Reygrobellet D, Hristova DL, Ettaiche M, Meddahi A, Jozefowicz J, Barritault D. CMDBS, Functional analogue of heparin sulphate as a new class of corneal ulcer healing agents. Ophthalmic Res, 1994;26:325-31.

4. Klenkler B, Sheardown H, Jones L. Growth factors in the tear film: role in tissue maintenance, wound healing, and ocular pathology. Ocul Surf, 2007;5:228-39.

5. Imanishi J, Kamiyana K, Iguchi I, Kita M, Sotozono C, Kinoshita S. Growth factors: importance in wound healing and maintenance of transparency of the cornea. Prog Retin Eye Res, 2000;19:113-9.

6. Lim M, Goldstein MH, Tuli S, Schultz GS. Growth factor, cytokine and protease interactions during corneal wound healing. Ocul Surf, 2003;1:53-65.

7. Matsumoto Y, Dogru M, Goto E, Ohashi Y, Kojima T, Ishida R, et al. Autologous serum application in the treatment of neurotrophic keratopathy. Ophthalmology, 2004;111:1115-20.

8. Mestries P, Borchiellini C, Barbaud C, Duchesnay A, Escartin Q, Barritault D, et al. Chemically modified dextrans modulate expression of collagen phenotype by cultured smooth muscle cells in relation to the degree of carboximethyl, benzamide, and sulfation substitutions. J Biomed Mater Res, 1998;42:286-94.

9. Mestries P, Alexakis C, Papy-Garcia D, Duchesnay A, Barritault D, Caruelle JP et al. Specific RGTA increases collagen V expression by cultured aortic smooth muscle cells via activation and protection of transforming growth factor-beta1. Matrix Biol, 2001;20:171-81.

10. Rouet V, Meddahi-Pellé A, Miao HQ, Vlodavsky I, Caruelle JP, Barritault D. Heparin-like synthetic polymers, named RGTAs, mimic biological effects of heparin in vitro. J Biomed Mater Res, 2006, 15;78:792-7.

11. Tan MH, Bryars J, Moore J. Use of nerve growth factor to treat congenital neurotrophic corneal ulceration. Cornea, 2006;25:352-5.

12. Tardieu M, Gamby C, Avramoglou T, Jozfonvicz J, Barritault D. Derivatized dextrans mimic heparin as stabilizers, potentiators, and protectors of acidic or basic FGF. J Cell Physiol, 1992;150:194-203.

13. Yamada N, Yanai R, Kawamoto K, Nagano T, Nakamura M, Inui M, et al. Promotion of corneal epithelial wound healing by a tetrapeptide (SSSR) derived from IGF-1. Invest Ophthalmol Vis Sci, 2006; 47:3286-92.

Personal feedback on 20 cases treated with Clerapliq®†

At my facility, I have conducted a retrospective study of 20 corneal ulcer cases of varying severity in both dogs and cats, which has been suspended for the time being. The Clerapliq®† treatment was recommended either in combination with polymyxin B + neomycin in the context of uncomplicated, simple, epithelial ulcers (12 cases), or in combination with tobramycin in the case of deep or superinfected ulcers (8 cases). Clerapliq®† was applied at a rate of one drop every other day for ten days, and tobramycin at a rate of six applications daily.

Although subjective because it was not conducted as a double blind study, this treatment seems to speed up corneal filling. This optimization is particularly seen with ulcers affecting the corneal stroma. According to the owners, the analgesic effect occurred as of the administration of the first drop. No side effects were noted in any of the 20 cases.

In 75% of the simple ulcer cases, healing was deemed complete (complete filling of the ulcer after five days of treatment) and in 62.5% of cases of complicated ulcers, ten days of treatment were required.

Of the 20 cases, four cases required a treatment adjustment: one consisted of switching antibiotics and three required surgical filling.

†Translator's Note: OTR4120 is available in Europe as Clerapliq® and in North America as Optixcare EYE EMS.