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Review

The topical application of nerve growth factor as a pharmacological tool for human corneal and skin ulcers

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Abstract

Aim: The nerve growth factor is a soluble protein produced by and acting upon a number of different cells located in the nervous, endocrine and immune systems. Recent studies have shown that nerve growth factor (NGF) exerts a critical role on epithelial cells and fibroblasts under normal and pathological conditions. In this review, we present data prospecting the clinical potentiality of NGF in cutaneous and ocular "non-healing" chronic ulcers.

Data synthesis: A consistent number of *in vitro* and *in vivo* studies carried out on animal models and in humans indicated that fibroblasts and epithelial cells are receptive to the action of NGF and that NGF promotes skin and cornea ulcer healing. These observations lead to the hypothesis that NGF can be a potential useful pharmacological agent for clinical investigations.

Conclusion: The available clinical evidences suggest that the topical application of NGF promotes healing action without side effects on corneal and cutaneous tissues damaged by chemical, physical and surgical insults and autoimmune disorders.

Keywords: Neurotrophins; Cutaneous ulcers; Epithelial cells; Eye; Topical application

Contents

1. Introduction

Nerve growth factor (NGF) is a soluble protein, belonging to a family of neurotrophic factors, which include brain-derived neurotrophic factor and neurotrophin-3, 4/5, collectively called neurotrophins. NGF is produced by and acting upon a number of different cell types and it is present in physiological relevant

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amounts in the bloodstream [1–3]. NGF transmits most of its known actions via two classes of transmembrane receptors that may but need not interact: the low-affinity pan-NT receptor $p75^{NTR}$ and the high affinity neurotrophin receptors of the tyrosinekinase (Trk) family TrkA [4,5]. Basically, the Trk receptors are responsible for most of survival and growth properties of the neurotrophin family. Tyrosine phosphorylation of these receptors stimulates the activity of cellular proteins responsible for regulating the cell shape and for activating the gene transcriptional machinery that controls the fate of neural precursors and axon and dendrite growth and patterning, as well as the growth, migration and morphology of keratinocytes and immune cells and other skin related cell types [6].

In vitro and *in vivo* investigations have revealed that NGF promotes the survival and function of neural crest-derived cells [3,4] and subsequently of forebrain cholinergic neurons, under normal and certain pathological conditions [2]. More recently, NGF has been used for investigating its protective action on its responsive cells. Thus, purified NGF has been administered in nasal cavity [7], intracerebroventricularly (i.c.v.) in subjects affected by Alzheimer Disease (AD) [8,9], intravenously in patients with peripheral neuropathies [10,11], and topically in subjects with corneal ulcer [12–14], pressure ulcer [15,16] and ulcers induced by autoimmune disorders [17–20].

The results of these studies indicate that the route of administration might represent a crucial point for evaluating the clinical benefit and safety of NGF application in human pathologies.

For example, the i.c.v. administration of NGF in patients with AD produces controversial results [8]. These side effects are in part due to the invasive methodology for intracerebral infusion, over-dosage, and to the fact that patients accepting this type of therapy were in advanced disease stage. Better results have been obtained by intraparenchymal transplants of primary autologous cells genetically modified to produce NGF in which no longer adverse effects were noted [9]. Recently, a non-invasive approach to deliver NGF into the brain has been suggested, raising a renewed therapeutic interest for the use of NGF in certain brain neurological disorders [21].

Intravenous injection of human recombinant NGF in patients with peripheral neuropathies can prevent the onset of neuropathy, but provides no convinced evidence that NGF can reverse the already present tissue and functional damage. Nonetheless, scientists continue to study the pharmacological potentiality of NGF for peripheral neuropathies, including studies with small molecules that either mimic the activity of NGF protein or stimulate their endogenous synthesis and action in a more natural way [22].

So far, the nasal spray [7] or ocular application [21] provides a more realistic approach for the pharmacological utilization of NGF in AD, while the skin topical application resulted highly effective in the treatment of cutaneous damage.

During the past 10 years we have been collaborating with clinicians to investigate the possible use of NGF in human pathologies, and to date more than 200 subjects have been treated with the murine NGF produced in our laboratories without reporting relapse or side effects. We here report published and unpublished results increasing the evidence that topical application of NGF exerts healing action on human corneal ulcer, ocular inflammation, diabetes, rheumatoid arthritis-associated ulcers, pressure ulcer, and cutaneous ulcer after autologous skin transplantation.

2. NGF and human corneal neurotrophic ulcers

Neurotrophic keratitis (NK) is a chronic progressive corneal disease due to the impairment of corneal sensitive nerves leading to epithelial defects and corneal ulcers [23]. Although NK is considered to be a rare disease, this condition may result from a variety of clinical disorders, either congenital or as a consequence of systemic disease, and a reduction in corneal sensitivity is very common in many corneal pathologies. NK is one of the most difficult and challenging ocular diseases, frequently leading to blindness and still lacking effective medical treatment.

Findings by us and later by others demonstrated that NGF administration accelerates wound healing of the corneal [12–14,24–26]. Our experience, based on more than 150 patients affected by neurotrophic corneal ulcer unresponsive to conventional treatment, such as artificial tears, eye patching, and soft contact lens bandaging, we have found that treatment with NGF eye drop induced a complete recovery of all corneal ulcers associated with a significant improvement of corneal sensitivity and visual acuity (both $p < 0.001$), within 3–4 weeks of NGF treatment, as illustrated in Fig. 1A and B. The rate of healing was not associated with the severity of the ulcer or to its depth in the stroma, nor with the age of the patients or the cause of the ulcer. In vitro and animal studies aimed at understanding the mechanism(s) through which NGF exerts its action, revealed that NGF induces proliferation and differentiation of epithelial cells, which stimulates fibroblasts migration, differentiation and collagen production, and restores corneal sensitivity, suggesting that the progressive corneal damage that occurs in patients with neurotrophic keratitis [27] was due to a deficit of endogenous NGF synthesis, release and/or utilization.

3. Skin and corneal ulcers induced by diabetes

It is known that skin ulcer associated with primary or secondary to diabetes mellitus can became chronic and difficult to heal [28,29]. A number of growth factors have shown to be implicated in the wound healing process [30], and among them, NGF has proved to play a prominent action in promoting healing processes [31]. In 1998, Matsuda et al. [32] showed that application of NGF into the wounds accelerated the rate of wound healing in normal mice and in healing-impaired diabetic mice. Based on this observation and our evidence with corneal ulcers, we have extended our investigation on patients affected by diabetes mellitus and progressive corneal or skin ulcerations unresponsive to conventional therapies.

Similar to what has been observed in previous studies, when applied as an eye drop, NGF was able to heal the corneal ulcers in diabetic patients in 3–6 weeks (see Fig. 1C and D).

As reported in the study by Generini et al. [19], 5–14 weeks of topical application were needed to produce the healing of