TRANSDERMAL DELIVERY OF *CLOSTRIDIUM BOTULINUM* TOXIN TYPE A BY PULSED CURRENT IONTOPHORESIS

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INTRODUCTION

Botulinum toxins are neurotoxins immunologically classified into types A to G. The therapeutic possibilities of botulinum toxin type A are manifold and certainly not yet fully exhausted. Botulinum toxin type A (Fig. 1) has a molecular weight of 150,000 Da; it acts blocking the cholinergic release from the peripheral nerve terminus by the cleavage of proteins involved in the process of exocytosis of the neurotransmitter (Fig. 2: A, and B).

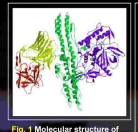

Fig. 1 Molecular structure of botulinum toxin type A

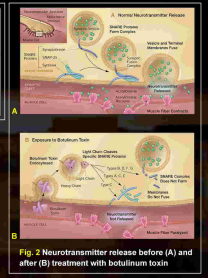

Fig. 2 Neurotransmitter release before (A) and after (B) treatment with botulinum toxin

AIMS

In this study we evaluated: - the possibility of administering botulinum toxin type A by pulsed current iontophoresis in order to avoid the hindrances related to the technique of injection; - the possibility of using current pulsed iontophoresis to deliver botulinum toxin type A through the skin in patients affected by focal hyperhydrosis.

MATERIALS AND METHODS

To investigate the efficacy of pulsed current iontophoresis in delivering botulinum toxin type A through the skin, experiments were performed on Wistar rats (Fig. 3), age 6-8 months, weight 350+50g. Each experimental point was repeated three times (*i.e.* in three different animals). Rats were anaesthetized and depilated. After mild abrasion of the selected skin areas and of control areas, the botulinum toxin type A was applied (4 U/0.1 ml) onto the skin and the pulsed current iontophoresis performed (current intensity waveform showed bursts of alternate symmetric 5 mA square pulses; electric treatment was performed for 10 min). The device used in this study (Fig. 4) (Mattioli Engineering, Firenze, Italy) is a powered iontophoresis drug delivery system that is indicated for the transdermal administration of ionic solutions for medical purposes and can be used as an alternative to injections (FDA approval n. K042590, 10.14.2004). Biopsies were taken both from control and from treated areas; to evaluate the transdermal delivery of botulinum toxin type A, specimens were prepared for immunohistochemistry reaction (monoclonal antibody against botulinum toxin type A: US Biological, Swampscott, Massachusetts, USA. Detection kit: Vector Laboratories, Burlingame, California, USA). Specimens were also prepared for Haematoxylin-Eosin staining (Sigma Aldrich, Milano, Italy) to verify the integrity of the tissue.

Fig. 3 Wistar rat

Fig. 4 Head of the electric device used to perform pulsed current ionthophoresis.
Actual size: 30 mm dia

RESULTS

1. Pulsed current iontophoresis elicits the transdermal delivery of botulinum toxin type A. The neurotoxin was detected in association to striated skeletal muscles localized below the deep dermis (Fig. 5); features of actin-myosin complexes of skeletal muscle fibers are shown in Fig. 6: A, B, and C.
Only a weak presence of neurotoxin was detected in association with the epidermis (the site of application) and with the adnexa (Fig. 7: A, and B). No botulinum toxin type A was detected after application of neurotoxin on the skin in absence of iontophoresis (Fig. 8). The electric treatment alone resulted in a negative immunohistochemistry reaction (Fig. 9). Haematoxylin-Eosin staining revealed no significant histological alterations in the skin areas where iontophoresis was performed (Fig. 10: A, B, and C).

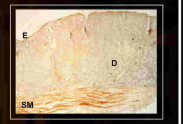

Fig. 5 Rat skin: epidermis (E), dermis (D) and skeletal muscles (SM). Immunohistochemistry reaction; 40X

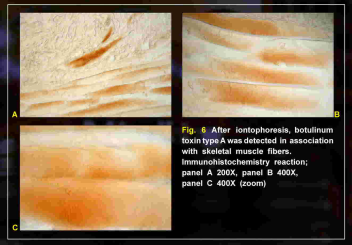

Fig. 6 After iontophoresis, botulinum toxin type A was detected in association with skeletal muscle fibers. Immunohistochemistry reaction; panel A 200X, panel B 400X, panel C 400X (zoom)

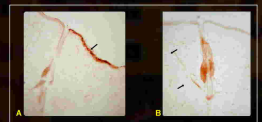

Fig. 7 Botulinum toxin type A, after the electric treatment, was evident on the site of application (panel A, arrow). Toxin was also associated with appendages (panel B). The arrow indicates the *arrector pili* muscle. Immunohistochemistry reaction; panel A 100X, panel B 100X

Fig. 8 Negative control: application of botulinum toxin type A on the skin without iontophoresis. Immunohistochemistry reaction; 100X

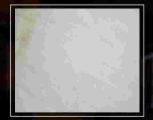

Fig. 9 Negative control: skeletal muscle fibers after electric treatment without application of botulinum toxin type A. Immunohistochemistry reaction; 200X

2. A preliminary clinical trial on 6 patients affected by focal hyperhydrosis was performed; palm and axillary regions were affected in 2 and 4 patients respectively. After a mild abrasion of the affected skin areas, 100 U of botulinum toxin type A was applied onto the skin, then the electric treatment was performed for 10 min on each skin area. All the patients undergoing the treatment showed no significant side effects during and after the electric treatment; patients' satisfaction was high.

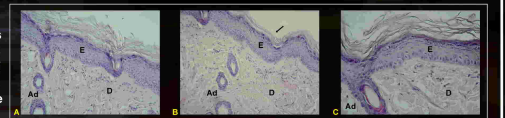

Fig. 10 Control and treated rat skin. Untreated skin (panel A) showed a thick *stratum corneum*; epidermis (E), dermis (D) and adnexa (Ad) are evident. Treated skin (panel B) showed a *stratum corneum* reduced in thickness. Epidermis, dermis and adnexa appear unaffected (panel C). Heamatoxilin-Eosin staining; panel A and B 100X , panel C 200X

CONCLUSIONS

This study demonstrates that pulsed current iontophoresis allows the transdermal delivery of botulinum toxin type A; this therapeutic approach is effective and stable for patients with focal hyperhydrosis. Thus, we hypotesize that pulsed current iontophoresis could satisfy the request for a wide use of neurotoxins in medicine and surgery.

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