UV-B Irradiation Attenuates Dermal Effects of Botulinum Toxin A: A Randomized, Double-Blind, Placebo-Controlled Study

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BACKGROUND Botulinum toxin type A (BoNT/A) is frequently used for cosmetic indications and hyperhidrosis.

OBJECTIVES We investigated whether UV-B irradiation alleviates the BoNT/A effect on local sudomotor activity.

MATERIALS AND METHODS In a randomized, double-blinded trial, the anhidrotic areas after BoNT/A (100 mU) injection 48 hours before and 14 days after UV-B irradiation were compared in six healthy volunteers.

RESULTS UV-B irradiation alleviated BoNT/A effect by approximately 30% ($p = .0017$). The UV-B–evoked reduction of anhidrotic areas was constant over the observation period of 14 weeks.

CONCLUSIONS When BoNT/A is applied intradermally, excessive exposure to UV-B and sunburn should be reconsidered.

The authors have indicated no significant interest with commercial supporters.

Botulinum toxin type A (BoNT/A) is a clostridial neurotoxin that inhibits acetylcholine release from peripheral cholinergic terminals and thereby blocks the neuromuscular and neuroglandular junction. The approved dermatologic indications for BoNT/A are glabellar rhytides[1,2] and focal hyperhidrosis.[3–5]

The optimal treatment requires proper patient selection,[6,7] precise dosing,[1,2,8] and accurate injection techniques.[9,10] Furthermore, the protein structure of BoNT/A implies its instability, although this appears not to be as problematic as previously reported. Recent studies show that agitation while reconstitution and the storage of solved toxin for extended periods do not affect BoNT/A's potency.[10] Nevertheless, exposure to ultraviolet radiation may be expected to alleviate BoNT/A effects after intradermal injection.

In this study we explored whether UV-B exposure can attenuate the effect of intradermal BoNT/A. We thereby sought to clarify the importance of avoiding intensive sun exposure after intradermal BoNT/A treatment.

Methods

Subjects, Study Design, and Drug Administration

The study was approved by the local ethics committee and the study protocol conformed to the guidelines of the 1975 Declaration of Helsinki. Six healthy volunteers (five male, one female, age 30–40 years) participated in the study after written informed consent and medical history were obtained. Subjects agreed to avoid additional UV exposure during the study period.

The first part of the study was performed using a randomized, double-blind design. For each subject, two syringes were prepared, one containing 0.5 mL [= 100 mouse units (MU)] of BoNT/A (Dysport, Ipsen, Wrexham, UK; reconstituted from 500 MU with

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 • Published by Blackwell Publishing •
ISSN: 1076-0512 • Dermatol Surg 2007;33:S92–S96 • DOI: 10.1111/j.1524-4725.2006.32338.x

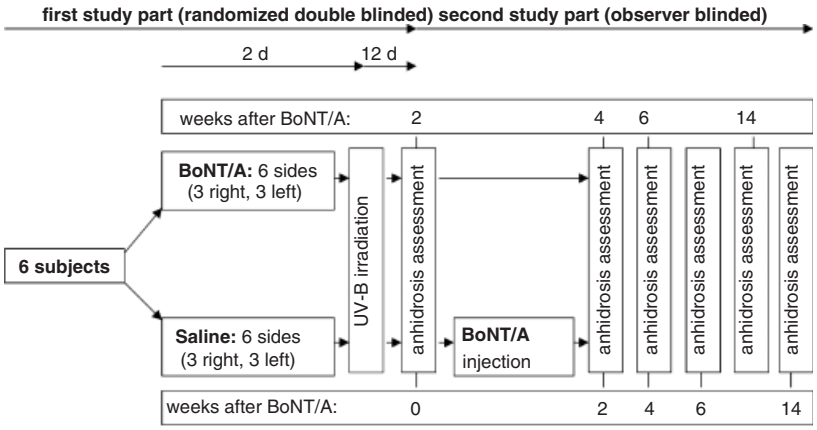

Figure 1. Overall subject disposition.

2.5 mL of unpreserved saline), the other with 0.5 mL saline only. Drugs were prepared by staff not otherwise involved in the study. The injection side for BoNT/A and placebo was randomized. Intracutaneous injections were performed on both thighs in the middle of the ventral aspect (the midpoint and vertices of a square with 3 cm sides). Forty-eight hours after injection, both sites were exposed to UV-B (see below).

Assessment of anhidrotic areas 14 days later led to unblinding of the subject and observer. After this assessment, in the second part of the study, BoNT/A was injected in the saline-treated area the same way as in the first part. All further assessments and analyses were done by one single investigator who was blinded to the sequence of BoNT/A injections. The overall subject allocation and study design are depicted in Figure 1.

UV-B Exposure

As previously described[11] a calibrated UV-B-source (Sellasol, Sellas Medizinische Geräte GMBH, Gevelsberg-Vogelsang, Germany; wavelength 290–320 nm) was used to induce erythema (circular spot, 5 cm diameter, three times the individual minimal erythema dose).

Assessment of Focal Anhidrosis

Focal anhidrosis was assessed 2, 4, 6, and 14 weeks after injection using the iodine starch reaction[12] by one single blinded investigator. The subjects were acclimatized in a temperature- and humidity-controlled room. Sweating was induced by drinking 500 mL of hot tea and bicycle exercise (100–150 W, 10 min). A transparent acetate sheet was placed over the relevant area to facilitate sweating; it was also used for drawing the borders of the anhidrotic area. The areas were digitized and automatically quantified using home-written software in IDL (RSI, now ITT Visual Information Systems, Boulder, CO) based on a two-dimensional region-growing algorithm.

Statistical Analysis

The data were analyzed using a repeated-measures mixed-effect ANOVA model with fixed effects for treatment and time and subject as a random effect. All computations were done using SAS software system V8.2 (SAS Institute, Inc., Cary, NC; 2002).

Results

Tolerability of the UV-B Model and the BoNT/A

All subjects completed the study. No subject reported pain during the UV-B irradiation or due to the UV-B erythema. No blisters or local weakness occurred.

Effects of BoNT/A and UV-B on Sudomotor Activity

BoNT/A, but not saline, injections produced substantial areas of anhidrosis. In all cases, however,

these areas were smaller on the side where UV-B irradiation was applied after BoNT/A versus the UV-B–pretreated side (p = .0017). The estimated mean difference (95% CI) between the anhidrotic area on the UV-B–pretreated versus the posttreated side was 8.3 cm^2 (4.2–12.4 cm^2). The UV-B–evoked reduction of anhidrosis was constant over 14 weeks at a level of approximately 30% (Figure 2). For both groups, we found a slight but significant decline of the size of anhidrosis over the time course ($p = .044$); this did not differ between the groups ($p = .87$).

Discussion

In this study, we have found that UV-B exposure substantially, significantly, and stably limits the effect of intradermal BoNT/A on local sudomotor activity. The mechanism of BoNT toxicity is thought to involve three steps.[13] After binding to the presynaptic membrane and internalization by receptor-mediated endocytosis, BoNT/A cleaves synaptosomal-associated membrane protein (SNAP-25), disabling acetylcholine release. It has been shown that the size of anhidrotic skin areas after intradermal BoNT/A injections is dose-dependent[14,15] and that the area of anhidrosis steadily increases over 2 weeks.[16–18] This has been explained by a quick local specific binding of BoNT/A and slow diffusion of excess toxin molecules to the surrounding area.

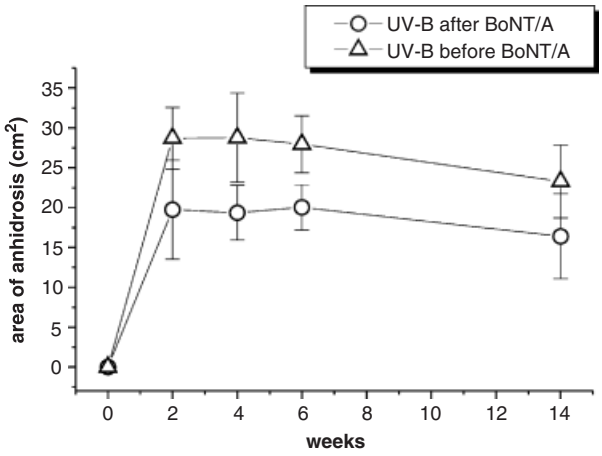

Figure 2. Time course (0 = baseline, 2, 4, 6, and 14 weeks after intradermal BoNT/A administration) of anhidrotic skin areas. The size of anhidrotic skin areas of both groups (O, UV-B posttreatment; △, UV-B pretreatment) is depicted and expressed as mean ± SD. UV-B irradiation after BoNT/A application significantly reduced the size of focal anhidrosis ($p = .0017$).

The results of this study, together with the time course of anhidrosis after intradermal BoNT/A,[16] suggest denaturation or degradation of the intercellular excess of BoNT/A by UV-B irradiation or the ensuing inflammation. The effect of BoNT/A on sweating was not fully blocked by UV-B, but the area of anhidrosis was reduced by approximately 30%. This reduction was stable over 14 weeks, indicating that UV-B irradiation did not shorten BoNT/A's sudomotor effects. Hence, the intracellular (internalized) proportion of the applied toxin appears unaffected.

Various mechanisms can account for this effect: (1) There might be a direct effect of UV-B on BoNT/A resulting in photodegradation and inactivation of the molecule. Although UV-B is mainly absorbed in the epidermis, significant amounts penetrate into the dermis, and thus interaction with intradermally injected molecules might occur.[19] According to the literature and information from the manufacturer, however, the photochemistry of BoNT/A and particularly its absorption spectrum in the UV-B range are currently unknown. (2) There might be an indirect proteotoxic effect of UV-B through reactive oxygen species (ROS). Although UV-induced oxidative damage is mainly caused by UV-A (320–400 nm), UV-B has also been described to induce the formation of ROS.[20] Accordingly, protein oxidation is a possible consequence of UV-B radiation. Modifications of single amino acids, cleavage of the polypeptide chain, and formation of cross-linked protein aggregates might be the result of protein oxidation. Furthermore, functional groups of proteins can react with oxidation products of polyunsaturated fatty acids and with carbohydrate derivatives. All these mechanisms can lead to inactive derivatives.[21] Thus, UV-B might have inhibited the further increase in the anhidrotic area in our study by inactivation of interstitial BoNT/A through

oxidative damage. (3) Inhibition of BoNT/A activity after UV-B might not be related to the radiation itself or photochemical metabolites but rather to the skin changes associated with inflammation. We applied UV-B at a sufficiently high dose (3 × MED) to induce a strong inflammatory response at the exposed skin sites. Owing to the production of inflammatory mediators, mainly nitric oxide and prostaglandins, UV-B leads to vasodilation and erythema. Other inflammatory substances, including cytokines, growth factors, and adhesion molecules, have additional important roles in the complicated immediate response of human skin to UV light resulting in the generation of a UV-B specific "inflammatory"-linked tissue reaction.[22] It is therefore possible that UV-B–associated changes in dermal connective tissue, e.g., edema, increased blood flow, and vascular permeability, alter the diffusion kinetics of BoNT/A and/or that BoNT/A interacts with molecules that are released into the inflamed tissue. Inhibition of BoNT/A activity by warming of the skin during UV-B irradiation seems unlikely because no heat sensation was reported by the subjects.

Owing to the small number of subjects and the single BoNT/A preparation (Dysport) used, generalizations from our study to a large population may be limited. Furthermore, our conclusions are restricted to dermal applications of BoNT/A for focal hyperhidrosis treatment. An effect of UV-B irradiation on superficial BoNT/A injections for cosmetic indications appears unlikely because UV-B has a very short wavelength and penetration.

In conclusion, we demonstrated for the first time that UV-B irradiation can substantially reduce the dermal effects of intradermal BoNT/A. The results of our small pilot study need to be confirmed, however, by larger studies before drawing any major conclusions.

Acknowledgments We thank Dr. Stephan Lehr (Institute for Medical Computer Sciences, Medical University of Vienna, Vienna, Austria) for statistical analysis of the study data and Dr. Boris Chizh (Addenbrooke's Center for Clinical Investigation, GlaxoSmithKline, Cambridge, UK) for his critical review of the manuscript.

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