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Pocketed microneedles for rapid delivery of a liquid-state botulinum toxin A formulation into human skin

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ABSTRACT

Botulinum toxin A (BT) is used therapeutically for the treatment of primary focal hyperhidrosis, a chronic debilitating condition characterised by over-activity of the eccrine sweat glands. Systemic toxicity concerns require BT to be administered by local injection, which in the case of hyperhidrosis means multiple painful intradermal injections by a skilled clinician at 6-monthly intervals. This study investigates the potential of a liquid-loaded pocketed microneedle device to deliver botulinum toxin A into the human dermis with the aim of reducing patient pain, improving therapeutic targeting and simplifying the administration procedure. Initially, β-galactosidase was employed as a detectable model for BT to (i) visualise liquid loading of the microneedles, (ii) determine residence time of a liquid formulation on the device and (iii) quantify loaded doses. An array of five stainless steel pocketed microneedles was shown to possess sufficient capacity to deliver therapeutic doses of the potent BT protein. Microneedle-mediated intradermal delivery of β-galactosidase and formaldehyde-inactivated botulinum toxoid revealed effective deposition and subsequent diffusion within the dermis. This study is the first to characterise pocketed microneedle delivery of a liquid formulation into human skin and illustrates the potential of such systems for the cutaneous administration of potent proteins such as BT. A clinically appropriate microneedle delivery system for BT could have a significant impact in both the medical and cosmetic industries.

1. Introduction

Botulinum toxin A (BT) is a potent neurotoxin with an expanding list of therapeutic and cosmetic indications. Its primary therapeutic uses relate to the treatment of hypersecretory and muscular defects in peripheral tissues. These include primary focal hyperhidrosis (PFHH), a chronic, debilitating condition characterised by over-activity of the eccrine sweat gland, most commonly on the palms, soles and the axillae. PFHH impacts significantly on a patient's quality of life [1,2], with effects that are comparable to better-known clinical conditions such as acne vulgaris and psoriasis [3]. It affects up to 1% of the population, with over 1 million US citizens suffering from a severe form of the condition [4].

Traditional medical approaches to PFHH treatment include topical application of aluminium chloride salts, or glycopyrronium bromide iontophoresis. Clinical effects of these therapies are short-lived and ineffective for more severe forms of the condition. The traditional surgical approach involves sweat gland denervation or endoscopic transthoracic sympathectomy; both treatments are associated with potential for serious complications. In recent years localised administration of BT has proven to be a highly effective alternative therapy [5] and is increasingly used in the clinical setting due to its prolonged therapeutic effects, typically 6–12 months, and the absence of systemic side effects. However, effective delivery of BT to the eccrine sweat gland requires multiple intradermal injections into the hyperhidrotic site. The severity of the condition and the area of the anatomical site dictate the number of injections required; treatment typically involves up to 50 intradermal injections of 0.05–0.2 ml of a botulinum toxin solution, administered by a skilled clinician, at 1–2 cm intervals in a grid-like pattern. Administration of BT is often extremely painful, sometimes requiring patient anaesthesia in palmar and plantar regions. Furthermore, it is laborious and relies on high levels of administrator skill to achieve targeted deposition of BT in the dermis. A simple, less painful, yet effective alternative administration method for the targeted delivery of BT could therefore have a positive impact for both the patient and clinician.

In the previous decade there has been a considerable effort to develop minimally invasive delivery systems, such as the microneedle (MN) device [6], for intradermal administration of molecules and macromolecular biologics such as peptides [7], proteins [8] and nucleic acids [9,10]. MNs consist of one or more miniaturised needles, between 100 and 1000 μm in length, which are used to facilitate localised delivery

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of a therapeutic across the stratum corneum without significantly impinging on blood vessels and nerve fibres that occupy deeper regions of the stratified tissue. Numerous MN designs and compositions have been manufactured and developed to deliver pharmaceutical formulations using a diversity of approaches including (i) a dry coating on the MN shaft [11], (ii) topical application in combination with MN skin puncture [12,13], (iii) liquid delivery via hollow MNs [14] and (iv) biodegradation/dissolving of a polymer/sugar MN [15,16].

A further, less-characterised method is the use of pocketed MNs; devices containing cavities that function as integrated reservoirs in the MN shaft. Pocketed MNs have been used primarily for the intradermal delivery of dried formulations [17,18]. However commercial formulations of macromolecular therapeutics, such as BT, are most often delivered as a solution. In this study, we therefore investigate the pocketed MN as a means to facilitate intradermal delivery of a liquid formulation of BT. In addition to reduced pain and increased convenience, further proposed advantages of such a delivery system include the ability to deliver an existing commercial BT formulation without the need to reformulate, and to provide visual feedback to the user regarding the successful delivery of a formulation following application of the device.

2. Materials and methods

2.1. Materials

General laboratory reagents were used as received and were primarily purchased from Sigma-Aldrich Ltd (Poole UK) and Fisher Scientific (Loughborough, UK). Foetal Bovine Serum (FBS), penicillin–streptomycin solution, Dulbecco's Modified Eagle's Medium (DMEM) and 25 mM HEPES were purchased from Invitrogen Corporation, Paisley, UK. Optimal Cutting Temperature (OCT) embedding media was purchased from RA Lamb Limited, (Eastbourne UK). Potassium ferrocyanide trihydrate (A.C.S. reagent grade) was purchased from ICN Biomedicals Inc. (Aurora, Ohio). Harris' Haematoxylin and Gurrs' Eosin were purchased from Lab 3 (Bristol, UK). Micronised salbutamol sulphate (mean diameter of 50%; particle population ≤ 3.07 μm) was purchased from Micron Technologies Ltd (Dartford, UK). Botulinum neurotoxin type A toxoid and anti botulinum toxin type A chicken IgY were purchased from List Biological Laboratories (Campbell, California, USA). DAB$^+$ chromogen (3,3 diaminobenzidine chromogen solution) and dual endogenous peroxidase block was purchased from Dako UK Ltd. (Cambridgeshire, UK). EasyLink PE/Cy7® (3 × 10 μg PE/Cy7®) conjugation kit was purchased from AbCam (Cambridge, UK).

2.2. Methods

2.2.1. MN fabrication and characterisation

MNs, either with (pocketed microneedle devices; PMNs) or without (non-pocketed microneedle devices; NPMNs) cavities in the microneedle shafts, were cut from stainless steel sheets (Trinity Brand Industries, SS 304; McMaster-Carr, Atlanta, GA, USA) using an infrared laser and electropolished as described previously [19]. PMNs consisted of five individual needles, four of which contained central cavities and one of which was the central MN, without a cavity, which served as a non-pocketed control. For imaging, PMNs and NPMNs were mounted on aluminium stubs and characterised by scanning electron microscopy (SEM) (Veeco FEI (Philips) XL30, Eindhoven, The Netherlands). Images of MNs were also captured using a bright field light microscope (Zeiss Stemi 2000C Stereomicroscope, Welwyn Garden City, UK) associated with a digital image capture system (Olympus C3040-ADL digital camera, Watford, UK) and an external electronic light source (Schott KL150, Stafford, UK).

2.2.2. Liquid loading PMNs with a model formulation

Commercially available formulations of BT (Botox®, Vistabel® Dysport® and Xeomin®) contain BT protein complexes with different molecular weights, ranging from 150 kDa (Xeomin®) to 900 kDa (Botox®). A vial of Botox® contains 5 ng of BT (100 units), 0.5 mg of HSA (human serum albumin) and 0.9 mg of sodium chloride (NaCl) in a lyophilised form. At these low concentrations BT is notoriously difficult to detect and at higher concentrations there are significant safety concerns. Therefore, to characterise PMN loading BT was replaced by a well characterised and detectable model protein, β-galactosidase (β-gal) [20], and a blue dye (20%v/v) was included in the formulation. The Botox® formulation mimic therefore included 5 ng of β-gal, 0.5 mg of HSA, 0.9 mg of NaCl and 20% v/v blue dye in a final volume of 150 μl. PMNs (n = 3) and NPMNs (n = 3) were loaded by a single immersion in to a reservoir of the representative formulation and visualised by light microscopy. Residency of the BT mimic formulation within the PMN cavities was monitored using a digital camera (Olympus C3040-ADL).

2.2.3. Enhancing the retention of liquid-loaded drug formulations in MN pockets

A viscosity enhancer was investigated as a means to increase the residence time of the liquid film within MN pockets. Aqueous loading solutions containing brilliant blue as a visible indicator were prepared using increasing concentrations of glycerine BP; 0% w/v, 20% w/v, 50% w/v and 80% w/v glycerine BP. Each of these formulations was loaded into a PMN at room temperature and was continuously observed, using light microscopy, for 2 min. Subsequent observations were made at 30 min, 1 h, 24 h, 48 h, 120 h, 5 days, 12 days, 17 days, 26 days and 32 days.

2.2.4. Quantification of PMN loading capacity

The loading capacity of PMNs and NPMNs, after a single immersion into a loading formulation, was evaluated using established detectable model compounds. A low molecular weight drug, salbutamol sulphate (SS), and a high molecular weight protein, β-gal, were both evaluated.

2.2.4.1. Quantification of salbutamol sulphate (SS) loading. PMNs (n = 10) and NPMNs (n = 10) were loaded by immersion into a SS solution (0.1 g/ml in deionised water). Loaded microneedles were rinsed in 8 μg/ml of bamethane in methanol. Samples were analysed using high performance liquid chromatography (HPLC) using a Genesis C18 (Grace Vydac, Bannockburn, Illinois, USA) reverse phase column, a p200 pump, an AS 3000 autosampler, a UV 2000 detector and Chem Quest® 4.1 software (Thermo Electron Corporation, Altrincham, UK). The mobile phase was 40% methanol and 60% 1-heptane sulphonic acid in deionised water. The UV detector was set at a 278 nm wavelength and the retention time of the analyte was 6 min. Bamethane was included as an internal standard.

2.2.4.2. Quantification of β-gal loading. PMNs (n = 10) and NPMNs (n = 10) were loaded by a single immersion into the model formulation described in Section 2.2.2, containing a detectable concentration of the macromolecular analyte β-gal (1.3 μg/μl). Liquid-loaded MNs were then rinsed for 1 h in sodium phosphate buffer and analysed using an established spectrophotometric method [21]. Briefly, test solutions were added to a reagent containing $MgCl_2$ (30 mM), 2-mercaptoethanol (2-ME) (3.36 M) and o-nitrophenyl-β-D-galactoside (ONPG) (68 mM) and were incubated at pH 7.3, 37 °C. Addition of the protein, β-gal, to the colourless o-nitrophenyl-β-D-galactoside (ONPG) substrate results in a hydrolytic reaction and creation of a coloured product, O-nitrophenol (ONP) (λ_{max} = 420 nm) [22]. After 40 min the reaction was arrested using sodium carbonate (1 M) to increase the pH to 11. This assay was repeated for matched blank solutions (not containing the protein) and also a range of standards (0.06 ng/μl–6 ng/μl). Absorbance values were measured at λ = 410 nm using a Fluostar fluorometer (BMG Lab technologies, Ortenberg, Germany).