EFFICACY OF TOPICAL BOTULINUM TOXIN TYPE A CREAM FOR TREATMENT OF PRIMARY AXILLARY HYPERHIDROSIS: A RANDOMIZED, DOUBLE-BLINDED, SPLIT SITE, VEHICLE CONTROL STUDY

BY

MR. CHAIRAT SERMSILP

THE THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (DERMATOLOGY) CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE THAMMASAT UNIVERSITY ACADEMIC YEAR 2016

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was approved as partial fulfillment of the requirements for the degree of master of science (Dermatology)

on May 17,2017

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Thesis Title	EFFICACY OF TOPICAL BOTULINUM TOXIN TYPE A CREAM FOR TREATMENT OF PRIMARY AXILLARY HYPERHIDROSIS: A RANDOMIZED, DOUBLE-BLINDED, SPLIT SITE, VEHICLE CONTROL STUDY
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Academic Years	2016

ABSTRACT

Background: The treatment of Primary Axillary Hyperhidrosis (PAH) with traditional needle-based botulinum toxin delivery has been proven to be effective with Botulinum Toxin Type A (BTX-A). However, it is mainly associated with more pains and costs, as well as a number of adverse events following the injections, such as pains, redness at injection site, and possible muscle weakness. In a recent study, the effectiveness of topical BTX-A was significantly shown in PAH treatment with a decreased risk of side effects, resulting in no systemic and very few local adverse events. Therefore, this research is created for the purpose of testing the efficacy of low dose topical liposomal based BTX-A cream as a novel and cost-effective modality for treatment of PAH. Ultimately, this non-invasive topical method could yield treatment efficacy for PAH with minimally effective concentration of BTX-A.

Objective: This research has been created in order to test the efficacy and safety of BTX-A in multilamellar liposomal beaded capsule cream for treatment of PAH compared to vehicle cream.

Methods: A prospective, randomized, double blinded, split site study was conducted in participants, aged > 18 years, having symmetrical sweating with hyperhidrosis severity scale (HDSS) of 2-4. The amount of sweat reduction was assessed using a Tewameter. BTX-A (30U), combined with multilamellar liposomal based cream to bind the toxin, was applied to one axilla and the vehicle without BTX-A to the other axilla once daily before bedtime with a total duration of seven days. Clinical improvement was evaluated using Tewameter, Minor's Iodine Starch Test, HDSS, and Dermatology Life Quality Index (DLQI) every 2-week visit until 8 weeks. The data from questionnaires of patients' satisfaction and adverse reactions were recorded at every follow- up visit.

Results: Twenty participants, with mean (SD) age of 37.55 (9.41), were recruited into the study. Of these, 80% and 20% were female and male, respectively. At the 2nd, 4th, 6th and 8th week of follow-up, the topical BTX-A treated side demonstrated sweat reduction of 8.06, 6.47, 7.15 and 3.94, respectively with mean difference from Tewameter measurement relative to the same-patients in the vehicle-control treated axillae, with statistical significance ($p<0.001$). Also, clinical grading by panel assessment of IST photography showed statistically significant ($p<0.001$) improvement with mean difference of 1.50, 1.50, 1.10 and 0.75 at the 2nd, 4th, 6th, and 8th weeks of follow-up, respectively. According to the statistic result, HDSS score shows a great difference between the BTX-A treated and the vehicle treated groups ($p<0.001$). As well, DLQI score showed the improvement in 8 from 10 choices, with statistical significance ($p<0.05$). Nonetheless, no side effects were present in this study.

Conclusion: The results of the 30U of BTX-A inversion with multilamellar liposomal beaded capsule cream could provide effective treatment outcomes of PAH compared to the vehicle control treated side, evaluated by Tewameter. The clinical grading of improvement was noted by Minor's Iodine Starch Test (IST), HDSS, and DLQI. Tewameter demonstrated the statistically significant improvement of the BTX-A treated side compared with the vehicle control treated side. Meanwhile, HDSS score showed improvement in the BTX-A treated group compared to the control treated group, with statistical significance ($p<0.001$). Moreover, DLQI illustrated the significantly improved quality of life and greater patient's satisfaction outcomes with

no serious side effects. Hence, the topical BTX-A could be an innovative painless and cost effective treatment of PAH.

Keywords: Botulinum toxin; Topical botulinum toxin; Axillary hyperhidrosis; Liposome, Tewameter

ACKNOWLEDGEMENTS

First, I would like to express my sincere thanks to my thesis adviser and role model teacher, Dr. Suparuj Lueangarun who always motivates and supports me. He kindly gives me plenty helpful advices and ideas which are more than important to this study. Also, I would like to express my gratitude to Asso. Prof. Pichit Suvanprakorn for the greatest comments and meaningful guidance, Dr. Punyaphat Sirithanabadeekul who always gives me valuable suggestion, and all the teachers at Thammasat university those whose names are not mentioned here, this thesis would not be achieved without their help.

Moreover, I would like to extend my sincere appreciation to all the teachers from the cosmetic dermatology CICM and Pan-Rajdhevee clinic those who are not just my teachers but also my second family, including Dr. Anan Jiraviroon for his helpful support, Asst. Prof. Panida Laoratthaphong for the very big equipment support.

In addition, I am sincerely thankful to all my classmates who always help and support me when I was in trouble and many people those I could not completely mention here including the nurse, the staff, the Tewameter and all the subjects enrolled in this study for their time and their good compliance.

Finally, I would like to express my deeply grateful to my family who always support and encourage me. They are the most importance supporter in my life and the only one inspiration that keep me moving on against no matter what may come.

Mr. Chairat Sermsilp

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
BTX	Botulinum toxin
BTX-A	Botulinum toxin type A
PAH	Primary axillary hyperhidrosis
TEWL	Transepidermal water loss
IST	Iodine starch test
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment
H_N	N-terminal half of the heavy chain
H_C	C-terminal half of the heavy chain
LD50	Lethal dose
HDSS	Hyperhidrosis disease severity scale
DLQI	Dermatology life quality index

CHAPTER 1
INTRODUCTION

1.1 Introduction

Hyperhidrosis, also known as an excessive sweating is a chronic autonomic disorder that creates occupational, physical and psychological impairment, as well as emotional and public embarrassment (1). Nonetheless, the cause of hyperhidrosis is unknown in most cases (2). About 0.6%–1% of population are affected by primary hyperhidrosis which starts in childhood (3). The diagnostic criteria for hyperhidrosis includes unknown causes of excessive sweating for around 6 months or more with at least two of the symptoms as followings: decrease of daily activities, bilateral or symmetrically sweating at least once a week, people aged 25 or below, those who discontinuous sweating during sleep, or have hyperhidrosis patient in the family. In the meantime, drug-induced (sertraline) or toxin-induced (acrylamide) could also be possible causes of secondary hyperhidrosis (4).

Axillary hyperhidrosis is typically the symptom of an excessive sweating among the area of axillae, which usually occurs in bilateral pattern. It is usually encountered and likely to be urged by mentally stress and temperature (5), while being continuous and associated with dermatologic complications such as dyshidrosis and contact dermatitis (6). Despite the well-established PAH treatment with high doses of BTX-A, there has yet been no controlled study to confirm the more efficacy of high concentrated BTX-A compared with the low-concentrated in axillary hyperhidrosis treatment.

CHAPTER 2
HYPERHIDROSIS

2.1 Hyperhidrosis

2.1.1 Principle

Hyperhidrosis is also known as excessive sweating, which is a prolonged debilitating disease that leads to occupational, physical and psychological impairment, as well as public and emotional embarrassment (7). Unfortunately, according to many cases, the cause of this disease has not been discovered yet (2). Primary hyperhidrosis affects 0.6%–1% of the population, which mostly occurs during childhood (8). The diagnostic criteria includes excessive sweating of unknown causes for at least six months with two of the many symptoms as follows: decrease of daily activities, bilateral or symmetrically sweating at least once a week, people aged 25 or below, discontinuous sweating during sleep, or have hyperhidrosis patient in the family. Toxin-induced (acrylamide) and drug-induced (sertraline) are both the causes of secondary hyperhidrosis (4).

There are around four millions sweat glands in human body, which produce sweat nearly the whole entire surface, except inner preputial surface, nail beds, labia minora, nipples, lips, glans clitoris and glans penis. Over 75% of these glands are eccrine sweat glands and the rest are apocrine glands (9). Human's soles contains with the most numbers of sweat glands in the entire body, which is around $620 \pm 20/cm^2$, following with the forehead and axilla; $360 \pm 60/cm^2$, and the palms and cheek; $300 \pm 80/cm^2$. The least number of glands is on the trunk, which is around $65 \pm 20/cm^2$ and the extremities; $120 \pm 30/cm^2$ (10).

According to Biology of Sweat Slands and Their Disorders journal by Sato et al., it stated that "The eccrine sweat gland is a long-branched tubular structure with a highly coiled secretory and a straight ductular portions" (11). The base of epidermal papillae naturally fuses with ductular portions and create sweat pores onto the skin surface (10). The secretory coil normally appears deeply in hypodermis with the size of 0.5 mm, distinguished in clear cells and dark cells within its epithelium. The

function of clear cells is to secrete major components of water, sweat, and electrolytes. The function of dark cells works slightly different, creating the most important protein ingredients in sweat called glycoproteins.

There is a cholinergic fiber from sympathetic nervous system that innervates eccrine sweat glands. The main function of eccrine sweat gland is to produce an odorless sweat, which is clear fluid regulating body's temperature. Emotional and gustatory stimuli determine the rate of sweat (12). In the case of acetylcholine, released from cholinergic fiber activates the eccrine sweat glands, it causes an influx of calcium into the cell. A hypotonic fluid is formulated after the reabsorption of NaCl's secretion of from primary fluid. After the process of hypotonic fluid, it results some complex combination of various components such as sodium, potassium, calcium, as well as chloride ions (12).

The impulses pass through the medulla in order to the lateral horn of the spinal ganglia, the sympathetic ganglia, and then the eccrine sweat glands which creates in the pre-optic area of hypothalamus. Acetylcholine release at terminal neurotransmitter is the final result from the process (11). Most of human eccrine sweat glands activated from cholinergic pathway activation, more than 70% of all eccrine sweat glands. Although there are other activation of sweating from periglandular cholinergic, α-adrenergic or β-adrenergic nerves (13).

Moderate moisture to severe slushy from excess sweating causes the exacerbation of the associated anxiousness and tautness (14).

Different symptoms of hyperhidrosis can manifest onto various affected areas such as soiled or damaged clothing, paperwork, and shoes, particularly the apparent sweat marks on clothing or unpleasing cold wet handshakes. More importantly, excess sweating of the armpits, hands, feet, or face can result in substantial impairments among the patients, which include working limitations, social interactions, physical activities, and leisure, as well as emotional and psychological distress (15).

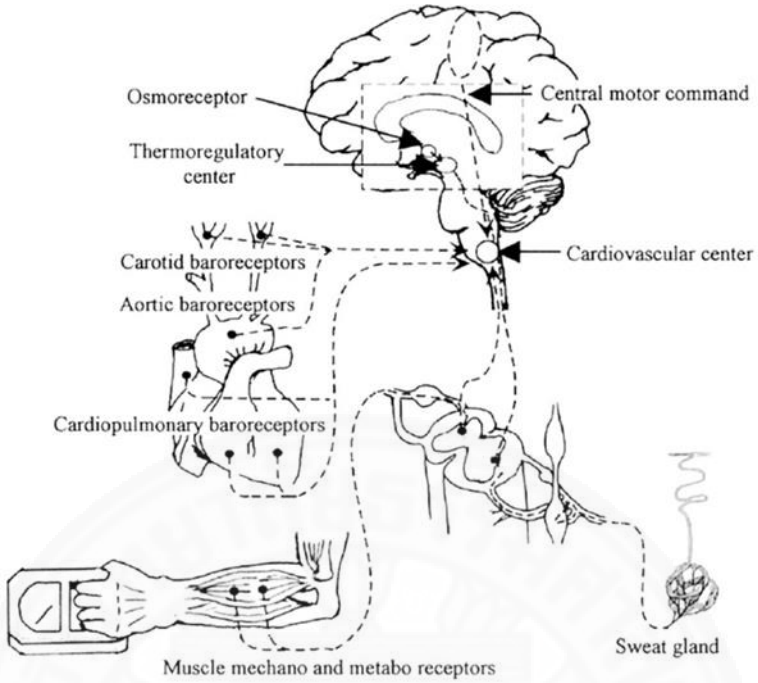


Figure 2.1 Schematic illustrating possible non-thermal modifiers of sweating (16).

2.1.2 Axillary Hyperhidrosis

Axillary hyperhidrosis or the sweat that specifically occurs in axillae area, bilateral in pattern. It is usually encountered and likely to be urged by mentally stress and temperature, while being continuous and associated with dermatologic complications such as dyshidrosis and contact dermatitis (5, 6).

Sweating level can be measured by Hyperhidrosis Disease Severity Scale (HDSS) to provide a qualitative criterion of the patient's severity grading of sweating status depend on how it affects or interferes with daily activities (Table 2.1).

Hyperhidrosis Disease Severity Scale	
"How would you rate the severity of your hyperhidrosis?"	
❑ 1.	My sweating is never noticeable and never interferes with my daily activities
❑ 2.	My sweating is tolerable but sometimes interferes with my daily activities
❑ 3.	My sweating is barely tolerable and frequently interferes with my daily activities
❑ 4.	My sweating is intolerable and always interferes with my daily activities

Table 2.1 Hyperhidrosis Disease Severity Scale (HDSS) (17).

There are many effective treatments of PAH including non-invasive treatments such as topical aluminium chloride hexahydrate, tapwater iontophoresis, and micro-focused ultrasound; and invasive treatments like fractional microneedle radiofrequency, subcutaneous injection botulinum toxin, microwave device, suction curettage, and surgical sympathectomy (18).

2.2 Primary Axillary Hyperhidrosis treatments

2.2.1 Topical Agents

Aluminum chloride is one of the alternatives of Hyperhidrosis treatment. This treatment process has taken place when there is an interaction between keratin and aluminum chloride which causes the sweat duct closure, causing atrophy of the secretory cells in the eccrine gland (19). However the usage of aluminum chloride can only treat in mild cases of hyperhidrosis, and could only be efficient within 48 hours (1). Skin irritation due to the high level of concentrated sodium is one of the most commonly seen affects from aluminum chloride usage (20).

Scholes et al (1978) reported the use of 20% aluminum chloride hexahydrate in absolute alcohol as the first treatment of choice for patients with axillary hyperhidrosis (21). To maintain efficacy, the application was required every week up to 3 weeks. However, the side effect was irritation of axillary skin.

Regarding to Ramon Grimalt et al (2006), it's stated the study of 1% of atropine sulfate with one milliliter of water applied on the affected area two times a day along with 30 seconds of massage, for the period of 15 days has proven to be an ineffective treatment for Hyperhidrosis (8). Therefore, there was no improvement of focal hyperhidrosis was identified after the application of topical anticholinergic drugs.

Another study conducted by Richard Glogau (2007) was to determine the efficacy of topical botulinum Toxin A in 12 patients with primary axillary hyperhidrosis of > 50 mg sweating every 5 minutes (22). BTX-A 200 U, toxin in a non-covalent manner, combined with a proprietary transport peptide molecule was applied on one of the axillae. The other side of axilla was applied with the vehicle without BTX-A. The measurement of sweat production rates were evaluated at the baseline and the 4^{th} week of the treatment. According to the result, 10 patients topically applied with

BTX-A demonstrated the mean reduction of 65.37 ± 21.5% in sweating. Nevertheless, the more favorable effects could not yet be confirmed when compared between the two concentrations.

Likewise, the study of Esther Cladellas (2008) studied the effectiveness of topical glycopyrrolate treatment in 10 compensatory sweating patients after sympathectomy (23). The research revealed that one milliliter of topical glycopyrrolate in 2% of water solution, applied once per day over the hyperhidrosis area along with 30 seconds massage, after the period of six weeks, 8 out of 10 patients were dramatically noted with improvement. Thus, it's proven that compensatory hyperhidrosis could be treated by a topical application of glycopyrrolate.

2.2.2 Oral Agents

Alpha-adrenergic agonists, such as clonidine and Anticholinergic agents, such as glycopyrrolate, menthatheline bromide, and oxybutynin; are the oral agents that create the inhibition of acetylcholine at muscarinic receptors (24). Nonetheless, the adverse events of these agents are unsatisfying. For sample, they create tachycardia, urinary incontinence, dry mouth, and etc. Moreover, the usage of these oral agents are contraindicated among those who have myasthenia gravis, pyloric stenosis, narrow angle glaucoma, and paralytic ileus. Furthermore, the patients with underlying of gastroesophogeal reflux disease, glaucoma, bladder outflow obstruction, and cardiac insufficiency, are absolute contraindicated to the usage of Alpha-adrenergic agonists and Anticholinergic agents (25).

2.2.3 Iontophoresis

Iontophoresis is an ionized substance enhancement through the skin which intact to the application a directly (26). Electricity is delivery of ions from the electrolyte liquid passed directly. Pads are applied to the axillae while using electrolyte solution onto the skin. The mechanism of action is to block the sweat glands reversibly with disrupted ion channel in the secretory glomeruli of the sweat glands and poral plugging (27). Initially, iontophoresis of tap water needs to be repeatedly done every few days in order to maintain the effective result. After the 2nd week of therapeutic

treatment and the affect reveals, the treatment may be performed once every two-three weeks (28).

2.2.4 Micro-focused Ultrasound

High intensity micro-focused ultrasound is objectively focus the target at dermis and produces thermal coagulation points and small thermal lesions. The focused ultrasound can deliver the energy depths of up to 4.5mm and must be passed into specific target at soft tissues within subcutaneous tissue and the layers beneath the superficial dermis. With thermal coagulation points at the depth of sweat glands, those specific soft tissues will be effectively damaged with no surface affected. Due to the non-limited capacity for regeneration of sweat glands, this efficacious effects will beneficially persist for at least 12 months (29).

2.2.5 Microwave-based device

Microwave-based devices delivery of heat to the selective areas between the skin and underlying fat in the axilla leading to generate the electrical heating and have been developed for treatment of axillary hyperhidrosis. In this process, the absorption of microwave heating in tissues can caused destruction effects of the eccrine sweat glands and apocrine sweat glands, beneficially last for at least 7 months (30).

2.2.6 Surgical treatment

Surgery method is offering to the definitive treatment of hyperhidrosis, but the risks of morbidity and mortality are higher than others modality of treatment. Sympathectomy is done to perform the ablation of sympathetic nerve which specific sweat glands supplying branch. Treatment of axillary hyperhidrosis is ablation at 2^{nd}, 3^{rd}, and 4^{th} thoracic ganglia (31). Electrocautery, laser, or clipped are the technique for locally excised and ablated the sympathetic chain which assist by endoscopic thoracic sympathectomy (ETS) and video-assisted thoracoscopy (32). The gold standard treatment of palmar hyperhidrosis is considering to be sympathectomy, yet likely to be more effective in palmar hyperhidrosis than axillary hyperhidrosis (33). Suction-assisted lipolysis or excision of the sweat glands are options of surgical

treatments (33, 34). Nonetheless, Horner's syndrome, pneumothorax and haemothorax are the complications implicated with these techniques (31).

Table 2.2 Review of axillary hyperhidrosis treatment

Author	Study name	Sample size	Intervention	Compare	Duration	Outcome Measurement	Result
MK Naumann 2001 (1)	Effect of botulinum toxin type A on quality of life measures in patients with excessive axillary sweating: a randomized controlled trial	N=307	Botox® (BTX-A) Subcutaneous injection 50 units per axillar	Placebo	16 wks	Gravimetric	- 95% Vs 32% at 1wk - 82% Vs 21% at 16wks (>50%improve from baseline)
Karolina ROSELL 2013 (35)	Botulinum toxin type A and B improve quality of life in patients with axillary and palmar hyperhidrosis	N=58	Xeomin® (BTX-A) Subcutaneous injection 107±22 units per both axillar	Before-After	3 wks	Vapometer, DLQI	Evaporation decreased >40% for all at 3 wks
Christian Tronstad 2014 (36)	Tumescent suction curettage vs. curettage only for treatment of axillary hyperhidrosis evaluated by subjective and new objective methods	N=17	Superficial tumescent suction with curettage	Curettage Only	3,6,12 mo	Gravimetric	Both reduction by gravimetric, greater in tumescent suction
MARK S. NESTOR 2014 (37)	Safety and Efficacy of Micro-focused Ultrasound Plus Visualization for the Treatment of Axillary Hyperhidrosis	N=14	Micro-focused Ultrasound 2 session / at day 0 and day 30	Sham-treated	1,2,4 and 12 mo	Gravimetric , HDSS , IST	At 2mo=67% 12mo = 83% (>50% sweat reduction from baseline)

SANG-JUN LEE 2013 (30)	The efficacy of a microwave device for treating axillary hyperhidrosis and osmidrosis in Asians: a preliminary study	N=12	Microwave-based device 10CC Local lidocaine injection + one microwave treatment	-	7 mo	HDSS , IST	83.3% (10/12) HDSS improve > 2 at 7-month
TALARICO-FILHO 2007 (38)	A Double-Blind, Randomized, Comparative Study of Two Type A Botulinum Toxins in the Treatment of Primary Axillary Hyperhidrosis	N=10	Subcutaneous injection Botox® 50u per axillar	Subcutaneous injection Dysport® 150u per axillar	Day 0,15,30 and 1 mo until 12mo	Gravimetric , Minor's iodine starch test	97.7% Vs 99.4% 77.8% Vs 88.9% at 1&4 mo (reduction from baseline of gravimetry)
Miri Kim 2013 (39)	Efficacy of Fractional Microneedle Radiofrequency Device in the Treatment of Primary Axillary Hyperhidrosis: A Pilot Study	N=20	Fractional Microneedle Radiofrequency 2 sessions / 4wks	Before-after	16 wks	Tewameter, IST, Biopsy	15 patients improve HDSS score, 14 patients improve in sweating, at 1 mo follow up and all increase after 2 mo
Ramon Grimalt 2006 (8)	Topical atropine sulfate for the treatment of axillary hyperhidrosis	N=10	Topical 1%atropine sulfate Applied twice a day + massaged for 30 s / 15days	Before-After	15 days	scale from 1 to 10 of satisfaction	Not effective for topical anticholinergic drug
RICHARD G. GLOGAU 2007 (22)	Topically Applied Botulinum Toxin Type A for the Treatment of Primary Axillary	N=12	Topical BTX-A 200 U apply for 60mins, one time	Vehicle (Placebo)	4 wks	Gravimetric	65.3±21.5% Vs 25.37±66.2% mean reduction in 4 wk by gravimetry

	Hyperhidrosis: Results of a Randomized, Blinded, Vehicle-Controlled Study						
K T SCHOLEs 1978 (21)	Axillary hyperhidrosis treated with alcoholic solution of aluminium chloride hexahydrate	N=64	20% Aluminium Chloride Hexahydrate Once a day for 6 month	Before-After	12 mo	Patient satification	100% improve Temporary effect
ESTHER CLADEL LAS 2008 (23)	A medical alternative to the treatment of compensatory sweating	N=8	2% aqueous solution of glycopyrrolate 1 ml bid and massage 30sec for 6wks	Before-After	6 wks	IST	Temporary effect, not improve in moderate-sever PAH

CHAPTER 3
BOTULINUM TOXIN

3.1 Botulinum toxin

Botulinum toxin (BTX) is the exotoxin-derived from clostridium botulinum, molecular weight of approximately 150 kDa comprising of disulfide bond with connected a heavy chain (100 kDa) and light chain (50 kDa). The BTX in commercially available contains complex proteins that stabilize the formulation (40). After BTX getting into target tissues, it endocytosis to the cholinergic nerve terminals and the heavy chain binding to glycoprotein receptors (41, 42). Then, the light chain binds with soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE), the high specificity to the protein complex. Following it cleaves the target proteins by proteolytic which normally acts as the docking of acetylcholine-containing vesicles to the inner surface of synaptic membrane (Fig. 3.1). This chemical denervation is completed after the restoration of SNARE protein complex turnover (41, 43).

Among the well-known of seven serotypes of BTX (A, B, C, D, E, F, and G), Humans are poisonous by serotypes A, B, E, and F. Types A and B are found in terrestrial environments; whereas, type E found in marine environments. Despite being antigenically distinct, all of these BTX serotypes possess similar molecular weights with a common subunit structure (44)

There is no limitation of BTX for inhibitory action to the neuromuscular junction. Autonomic cholinergic fibers also blocked from the BTX, including the sympathetic fibers to the sweat glands (45). Meanwhile, the released acetylcholine functions in eccrine glands through Chrm3 receptor, not via M1 or M2 types. The expression of Chrm3 in the secretory and myoepithelial cells is blocked with specific antagonists to inhibit sweat secretion (13).

BTX-A injections in local area have been shown to be effective treatment and safe for PAH and chronic hyperhidrosis (46, 47). Acetylcholine at neuromuscular junction and autonomic cholinergic nerve terminals is powerful inhibitor of the released by BTX-A. Small amounts of injection into or close to cholinergic target tissues (e.g. sweat glands, muscular,) can cause a localized, long-lasting, but ultimately reversible

decrease in cholinergic transmission. This property of BTX-A is beneficial for the widespread treatment of various disorders characterized by unpredictable local muscle hyperactivity. As well, it can be pondered as the choice of treatment for most focal dystonias (48, 49).

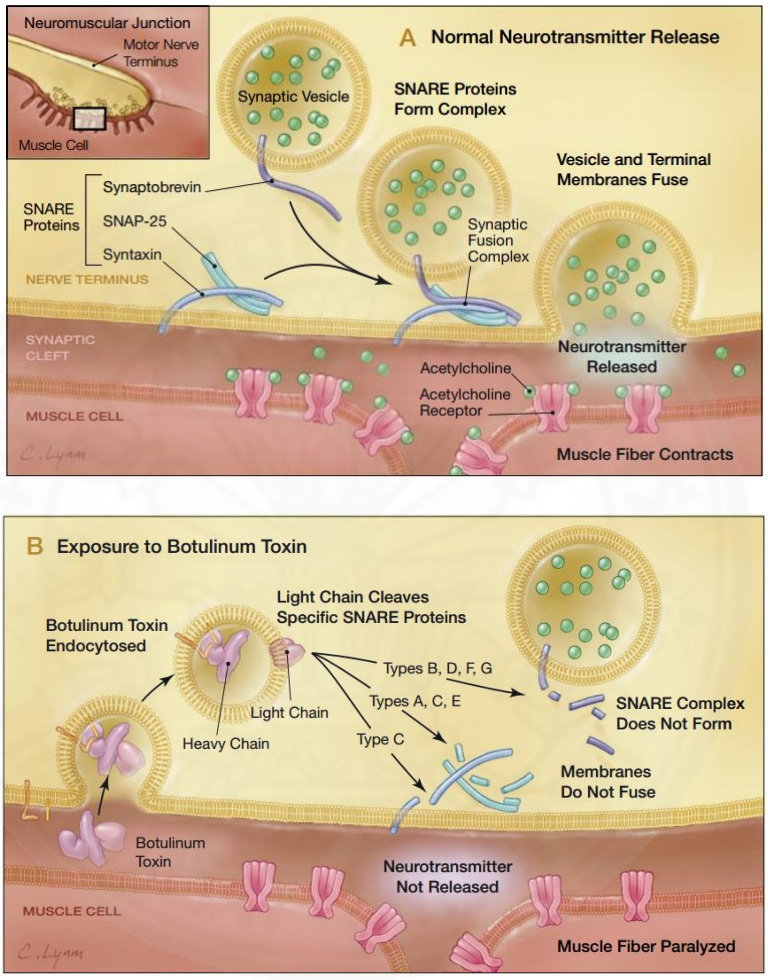


Figure 3.1 Mechanism of action of botulinum toxin (50).

A: Release of acetylcholine at the neuromuscular junction is mediated by the assembly of a synaptic fusion complex to allow the membrane of the synaptic vesicle containing acetylcholine to fuse with the neuronal cell membrane. The synaptic fusion complex is a set of SNARE proteins, including synaptobrevin, SNAP-25, and syntaxin. After the membrane fusion, acetylcholine is released into the synaptic cleft and then bound by receptors on the muscle cell.

B: Botulinum toxin binds to the neuronal cell membrane at the nerve terminus and enters the neuron by endocytosis. The light chain of botulinum toxin cleaves specific

sites on the SNARE proteins, preventing complete assembly of the synaptic fusion complex and thereby blocking acetylcholine release. Botulinum toxins types B, D, F, and G cleave synaptobrevin; types A, C, and E cleave SNAP-25; and type C cleaves syntaxin. Without acetylcholine release, the muscle is unable to contract (50).

BTX comprises three domains of light chains, N-terminal half of the heavy chain (H_N), and C-terminal half of the heavy chain (H_C), with each specific function in the mechanism of cell intoxication (Figure3.2). The C-terminal half of the heavy chain determines cholinergic specificity and is responsible for binding; whilst, the light chain provides intracellular toxic moiety. With the broken disulfide bond of the two chains before the cell can internalize the toxin, the light chain cannot then enter and loses its toxicity. After binding with the heavy chain to the presynaptic membrane at the non-identified receptors of peripheral nerve terminals, BTX is internalized inside endocytic vesicles. After internalization, the disulfide bond is cleaved by an unknown mechanism. The binding of light chain to the vesicle membrane is mediated by the carboxyl-terminal domain (H_C), while the N-terminal half of the heavy chain promotes penetration and translocation of the light chain across the endosomal membrane (51).

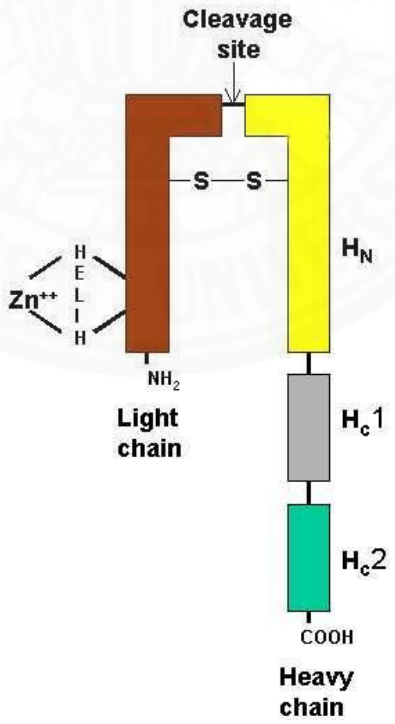


Figure 3.2 Structural architecture of Botulinum Toxin (52).

3.2 Botulinum toxin dose calculation

BTX products are determined for therapeutic preparations by a Swiss Webster mice LD50 analyze. The median lethal intraperitoneal dose are defined and labelled unit of activity which expressed in mouse LD50 units at a defined time-point. Potency and stability testing are required at several stages of the production process. Due to high precision, a large number of animals are used for testing at the final lot stage (53).

3.3 Botulinum toxin stability after reconstitution

Two randomized, double-blind studies by Yoon et al (2005) was created in order to test the safety, as well as the efficacy of Neuronox® compared with Botox®, at a 1:1 dose ratio for treatment of essential blepharospasm and spasticity in children with cerebral palsy. Neuronox® was proven to be non-inferior to Botox® in both studies, with no significant difference in safety profiles (54).

Regarding to the study of Lizarralde et al (2007), two clinical efficacy comparisons were performed. One side of the lateral canthal line were injected with the BTX-A, which was reconstituted and refrigerated for a week before the usage. Another lateral canthal line was injected with freshly reconstituted BTX-A. The result revealed similar clinical efficacies, both of the comparisons show no statistically significant differences (55).

Hui et al (2007) determined the efficacy of reconstituted and refrigerated BTX-A versus freshly reconstituted BTX-A (Botox Cosmetic, Allergan, Irvine, CA, U.S.A.) in treatment of lateral periorbital rhytids. BTX-A reconstituted and refrigerated 2 weeks before its application showed no significant effect on the time of onset and efficacy of BTX-A in treatment of lateral periorbital rhytids (56).

Yang et al (2008) studied the efficacy of two storage methods of BTX-A, compared with fresh reconstituted BTX-A onto the forehead dynamic lines. First storage method was reconstituted and refrigerate BTX-A in cold storage at 4 °C for two weeks before the injection. Second method was freezing the BTX-A at -20 °C for two weeks before the application. The result of these comparisons revealed no significant

difference between 2 weeks of refrigeration or freezing, and fresh reconstituted BTX-A. The storage method of reconstituted BTX-A in refrigerator does not affect the efficiency of the toxin (57).

3.4 Topical botulinum toxin

Topically administered drugs may be absorbed by penetration of the cutaneous layer or transport via the sweat pores (58). In similar way, topical BTX would reach target eccrine glands via the sweat pores. Intuitively, pore size and osmotic gradient would be directly proportional to rate of BTX absorption; whereas, the size of molecule would bear an inverse relationship (59). Factors likely to influence the success of this administration include concentration, temperature of the solution, and size of the sweat pores (43).

Due to its availability for absorption, painless and safety, we therefore would investigate the efficacy of topical botulinum toxin type A cream for treatment of axillar hyperhidrosis.

3.5 Botulinum toxin treatment of axillary hyperhidrosis

Naumann et al (2001) conducted a study to identify the effect of botulinum toxin type A in patients with excessive axillary sweating: a randomized controlled trial with subcutaneous injection of BTX-A 50 units per axillar. The result showed that those treated with BTX-A exhibited statistically significantly greater improvement in the physical component summary score at 16 weeks than the placebo-treated patients (1).

Talarico-Filho et al (2007) compared the efficacy, safety, and tolerability of two non-bioequivalents toxins, BOTOX® and Dysport®, in treatment of primary axillary hyperhidrosis, using a conversion factor of 1:3, respectively. To compare, 50U of BOTOX® was applied to one axilla and 150U of Dysport® to the other. Minor's test and gravimetry were performed at 0 day, 15 days, and 30 days for a period of 1 year. The result showed no significant difference on the mean of sweating reduction rate (97.7% for BOTOX® and 99.4% for Dysport®), without statistical difference. The mean

duration of beneficial effects was similar in both toxins (260 days for BOTOX and 290 days for Dysport), without statistical difference (38).

Rosell et al (2013) determined the effect of type A botulinum toxin (Xeomin®) in treatment of 58 axillary hyperhidrosis patients injected with 107±22U. The result at follow-up at 3 weeks showed the evaporation decreased >40%, and DLQI score improved from 12.0 to 1.7 ($p<0.05$) (35).

3.6 Topical botulinum toxin treatment of axillary hyperhidrosis

Topically administered drugs may be absorbed by penetration of the cutaneous layer or transport via the sweat pores (58). In similar way, topical BTX would reach target eccrine glands via the sweat pores. Intuitively, pore size and osmotic gradient would be directly proportional to rate of BTX absorption; whereas, the size of molecule would bear an inverse relationship (59). Factors likely to influence the success of this administration include concentration, temperature of the solution, and size of the sweat pores (43).

Glogau et al (2007) studied the effect of topical BTX-A 200U mix with transport peptide molecule to bind the toxin in a noncovalent manner technique for 60 minutes in 12 subjects with PAH. The result showed a decrease of sweat at 4 weeks follow-up by Minor's iodine starch test and photography. Few local adverse effects were reported with mild folliculitis or razor bumps, tenderness, erythema, and eczema, but no systemic adverse effects (22).

CHAPTER 4
LIPOSOME

4.1 Liposome

Liposomes are small artificial vesicles of spherical shape with particle sizes ranging from 30 nm to several micrometers which created from cholesterol and natural nontoxic phospholipids. The biocompatibility of liposome consists of hydrophobic and hydrophilic characters include their size. There are single or multiple lipid bilayer surrounding aqueous units. Liposomes are officiate as drug delivery. Hydration from the aqueous solutions will impulsive the closed structures of phospholipids. Lipids are amphipathic (both hydrophobic and hydrophilic) in aqueous media, the polar head groups are located in the pathway of the exterior and interior aqueous phases(60). Their thermodynamic phase properties and self-assembling characteristics influence entropic focused confiscation of hydrophobic sections into spherical bilayers. Those layers are referred to as lamellae (61).

Liposomes are encapsulated to delivery systems that can entrap unstable compounds and protect their functionality. Both hydrophobic and hydrophilic compounds can trap on the liposomes, release the entrapped at designated targets while still avoid decomposition of the entrapped combinations (62-64).

4.1.1 Classification of liposomes

Liposome sizes vary from 0.025 to 2.5 μm vesicles and have one or bilayer membranes. Liposomes can major divided into two categories: multilamellar vesicles and unilamellar vesicles (large unilamellar vesicles and small unilamellar vesicles) (65).

In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. In multilamellar liposomes, several unilamellar vesicles will form inside the others with smaller sizes, making a multilamellar structure of concentric phospholipid spheres separated by layers of water (66).

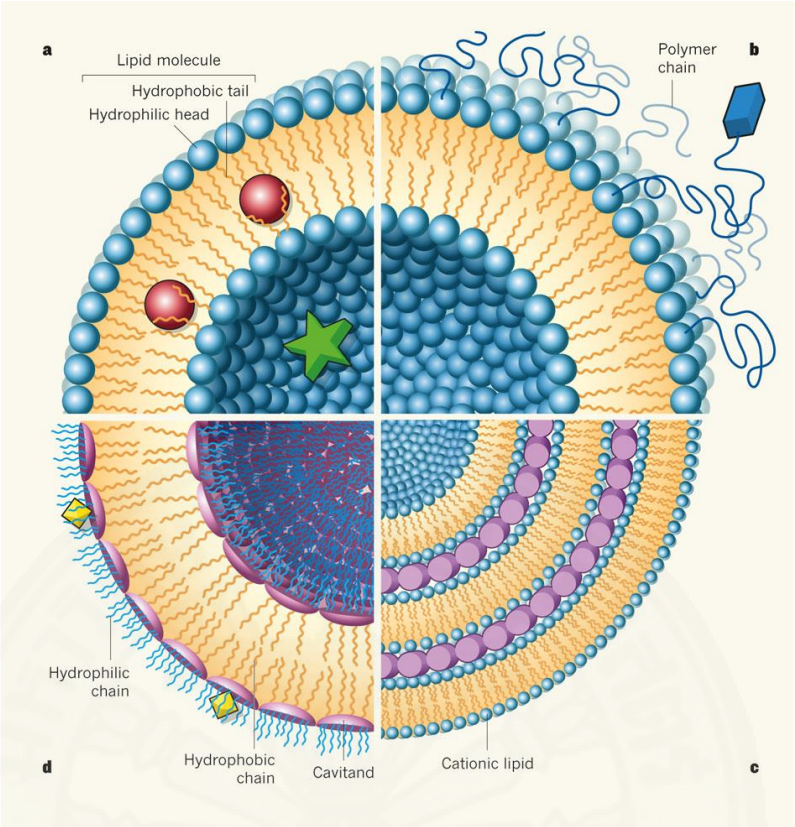


Figure 4.1 Liposome structure (67).

a, Simple liposomes are vesicles with a shell of lipid bilayer. A liposome can trap hydrophobic guest molecules of a few nanometres in diameter (red spheres) within the hydrophobic bilayer, and hydrophilic guests up to several hundred nanometres (green star) in its larger interior.

b, In 'stealth' liposomes developed for drug-delivery applications, the lipid bilayer contains a small percentage of polymer lipids. Peptides (blue rectangle) with specific biological targets may also be attached to polymers.

c, Most cationic liposome–DNA complexes have an onion-like structure, with DNA (purple rods) sandwiched between cationic membranes.

d, Kubitschke et al 1 report liposomes in which the bilayer assembles from cavitands — vase-shaped molecules — to which the authors attach hydrophobic and hydrophilic chains. The cavitands can trap ångström-sized guest compounds (yellow diamonds) in hydrophobic cavities. These vesicles can therefore encapsulate guest molecules of different sizes in cavitands, bilayer, and liposome's interior.

4.1.2 Mechanism of transportation through liposome

The limitations and benefits of liposome drug carriers lie critically on the interaction of liposomes with cells and their destiny in vivo after administration. In vivo and in vitro studies of the contacts with cells, the main interaction of liposomes with cells is either simple adsorption (by specific interactions with cell-surface components, electrostatic forces, or by nonspecific weak hydrophobic) or following endocytosis (by phagocytic cells of reticuloendothelial system, such as macrophages and neutrophils) (68). It is much rare for the fusion with plasma cell membrane by inserting the lipid bilayer of liposome into the plasma membrane, with simultaneous release of liposomal content into the cytoplasm (69).

The possible interaction is the exchange of bilayer components; for instance, cholesterol, lipids, and membrane-bound molecules with components of cell membranes. It is often difficult to determine what mechanism is functioning, and more than one may function at the same time (70).

4.1.3 Drug loading in liposomes

Drug loading can be divine into two methods, passively method (drug is loading and encapsulated during formation of liposome) or actively method (drug is loading and encapsulated after liposome formation). Hydrophobic drugs can be directly combined into liposomes during vesicle formation. Trapping effectiveness of 100% is often achievable, depending on drug solubility in the liposome membrane (71). Passive encapsulation of water-soluble drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation. Trapping effectiveness (generally <30%) is limited by the trapped volume delimited in the liposomes and drug solubility. The water-soluble drugs with protonizable amine functions can be actively entrapped by employing pH gradients, resulting in trapping effectiveness of almost 100% (72).

CHAPTER 5

TRANSEPIDERMAL WATER LOSS

5.1 Transepidermal water loss measurement

5.1.1 Principle of transepidermal water loss measurement

Skin acts as a protection barrier to prevent the organism from loss of essential components such as ions, water, and serum proteins. Beyond its structural components, stratum corneum encompasses the passage of water and electrolytes from the viable epidermis, as well as the active and passive transport of exogenous substances. Different noninvasive methods for the in vivo investigation of skin barrier properties have been developed during the past decades (73).

Transepidermal water loss (TEWL) is the parameter for the evaluation of epidermal permeability barrier function which is the most prominent in vivo. It is comprised by the insensible perspiration based on the diffusion of body water through the stratum corneum. Minimizing thermal sweating, hence, is crucial for quantifying TEWL under basal condition. To measuring of indirect prediction for the influence of topically applied substances and pharmaceutical compounds at the skin surface and assess the barrier can be done by using TEWL (74).

There are multiple methods for measuring TEWL, including the the open-chamber method, the ventilated-chamber method and unventilated-chamber (closed). The open-chamber method does not interfere with the microclimate and not occlude the skin. It is useful tool for both single and continuous measurements of the evaporative loss from the skin surface. The measuring principle behind the open-chamber devices is Fick's diffusion law which reveals the mass per cm2 being transported in a defined period of time and calculated by the formula (75):

$$\frac{dm}{dt} = -D \cdot A \cdot \frac{dp}{dx}$$

D = diffusion constant (=0.0877 g/m h mmHg)

A = surface in m^2

m = water transported (g)

t = time (h)

p = vapor pressure of the atmosphere (mmHg)

x = distance from skin surface to point of measurement (m)

Two pairs of sensors (temperature and relative humidity) is measured indirectly of density gradient and is analyzed by a microprocessor. After computer calculation, TEWL is displayed in $g/m^2/h$. The microclimatic changes near the skin surface could be influenced all methods. However, the measurements must be performed in climatized rooms with controlled air temperature and relative humidity without direct airflow into the measurement area. Practical aspects in performing the TEWL measurement include:

Climatized room with temperature 18–21 °C and relative humidity of 40–60 % shoud alwasys Perform formeasurements (when possible).

The study volunteers must allow adequate acclimatization time for 20–30 minutes before to the measurement.

Circadian rhythm: perform measurements at the same daytime and season; avoid measurements during summer.

Avoid direct airflow and direct light (resulting in temperature increase) at the test site.

Consider inter- and intra-individual variability in TEWL when calculating the size of study population.

Allow an equilibration time of about 20 s until a steady state is reached due to the heating of the sensors (shorter for devices with preheating function).

Allow for the vapor remnants in the probe to dry after each measurement (seconds) when performing repeated measurements.

Perform at least two consecutive measurements of neighboring areas of each test site, avoid the measurement from the exactly same site due to possible occlusion.

Place the probe perpendicular to the skin surface applying a minimal constant pressure. Do not moisten and damp the probe.

The interval from the last product (cosmetic, topical drug) application should be at least 12 h; otherwise, the occlusion effect of the product itself or its remnants is measured instead of its effects on the epidermal barrier properties.

The interval from the last skin cleansing should be at least 2–4 h.

5.1.2 Transepidermal water loss in sweating measurement

As sweat evaporates, producing vapour pressure, diffusion flow can be expressed by its pressure gradient. The measurement was created by measuring the passive diffusion of water evaporation through the skin. The vapour pressure gradient passing the stratum corneum shows the diffusion flow. In case of an absence in sweating, there is low amount of water vapour passing through stratum corneum by passive diffusion. Hence, this study indicates that sweat can be evaluated by measuring TEWL (76).

Table 5.1 Tewameter variable measurement influence factor

Variable		Influence on TEWL measurement
Individual related	Age	+
	Gender	-
	Race/ethnicity	+/-
	Anatomical site	+
	Skin temperature	+
	Sweating	+
	Intake of vasoactive substances (drugs, caffeine, nicotine)	+
Environment Related	Air convection	+
	Ambient temperature	+
	Humidity	+
	Direct light	+
	Season	+
	Circadian rhythms	+

Used symbols: “+ mean influencing”, “− mean no influence”, “+/− mean controversial” data (75).

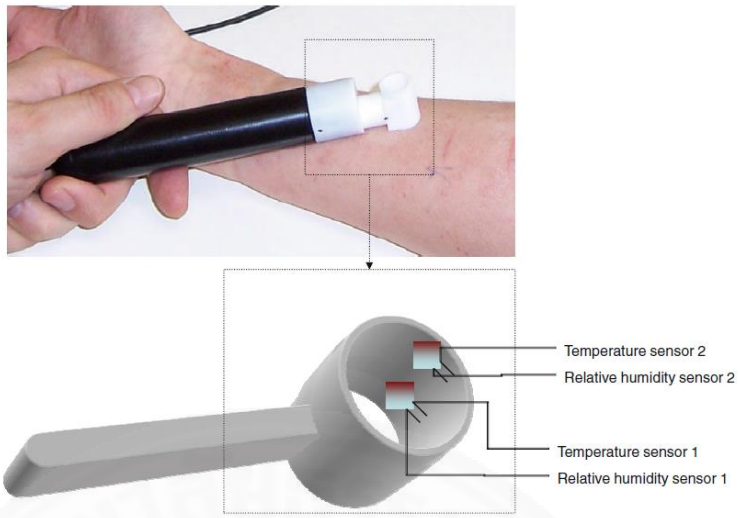


Figure 5.1 Transepidermal water loss measurement and a schematic overview of the open-chamber device measuring probe (75).

CHAPTER 6
RESEARCH METHODOLOGY

6.1 Objectives

6.1.1 Primary objective

To evaluate the efficacy of topical formulation of Botulinum toxin type A (BTX-A) with multilamellar liposomal beaded capsule cream for treatment of primary axillary hyperhidrosis (PAH) compared to vehicle cream from Tewameter measurement method.

6.1.2 Secondary objective

6.1.2.1 To assess the efficacy of topical formulation of BTX-A with multilamellar liposomal beaded capsule cream for treatment of PAH compared to vehicle cream from Minor's Iodine starch test method for improvement of hyperhidrosis severity scale (HDSS) and quality of life (DLQI).

6.1.2.2 To determine the safety of topical BTX-A for treatment of PAH.

6.2 Study design

Interventional: A randomized, prospective, double blinded, split site, vehicle-controlled therapeutic trial study

6.3 Target population

Patients aged ≥18 years with symmetrical sweating of Hyperhidrosis Severity Scale (HDSS) 2-4 who attended the OPD at TTMH Hospital

6.4 Selection criteria

6.4.1 Inclusion criteria

Male or female aged ≥18 years

Hyperhidrosis severity scale 2-4

6.4.2 Exclusion criteria

6.4.2.1 Pregnancy and those planning to get pregnant within 2 months

6.4.2.2 Lactating mother

6.4.2.3 History of botulinum toxin injection within the 6 months prior to the day of screening

6.4.2.4 History of using High Intensity Focus Ultrasound at axillar area within the 6 months prior to the day of screening

6.4.2.5 History of using hair removal laser within 6 months prior to the day of screening

6.4.2.6 Skin infections, inflammation or persistent scar at axillar area

6.4.2.7 No history of myasthenia gravis, hyperthyroidism, congestive heart failure, pheochromocytoma, diabetic mellitus

6.4.2.8 Concurrent medication within 30 days prior to enrollment (any anticholinergic, aminoglycoside, calcium channel blocker, antidepressant, anti-glaucoma, or opioid medications)

6.4.2.9 Presence of evidence indicating likely poor compliance with the protocol

6.4.3 Discontinuation criteria

6.4.3.1 Those getting pregnant during the protocol

6.4.3.2 Those unwilling to continue participating in the study

6.5 Sample size

Sample size = 20 (At least 15 from the statistical calculation), Reference value (μ0) = 1.3, Mean (μ) = 0.8, Standard deviation = 0.58 Glogau R (22) Alpha error = 0.05, Beta (β) = 0.1, Power of the test = 90%

Calculation of sample size using "Testing for on population mean formula" method based on reference value, mean, and standard deviation from the study of Glogau et al (22) as follows:

$$n = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \sigma^2}{(\mu - \mu_0)^2}$$

Figure 6.1 Sample size calculation formula

6.6 Recruitment process

To invite and persuade volunteers, we used posters with important details of the study in brief and placed them around TTMH Hospital.

6.7 Preparation of research

6.7.1 Preparation of subjects

6.7.1.1 Subjects were selected to enroll in the study according to the selection criteria

6.7.1.2 Details in the information sheet were informed to all subjects.

6.7.1.3 The subjects were required to sign the inform consent form for participation in the study. Those who asked for subject's consent were nurses or an investigator's staffs who had no benefits from the study. The process to inform the details was through the describing of all details to subjects and allowing some little time for them to read information sheet by themselves before signing in the study. The place for the informed consent was held at the TTMH Hospital. For the blind or incapable volunteers, a relative or someone who has no benefits from the study was responsible for communicating all the studies' details before the informed consent. Once deciding to join the study, those volunteers must pump their fingerprints onto the informed consent form with inerasable ink followed by the signing from their witness.

The investigator kept one of the two informed consent documents and gave the other to the subjects during the study period.

6.7.1.4 Important information such as subject's demographic data needed to be recorded after signing.

6.7.2 Preparation of measurement room

In this study, the investigator used the TEWL measurement equipment as an open-chamber device measuring probe, which could be invalid by many factors, especially air flow, humidity and environment temperature. Setting an ambient temperature (22–25°C) by using air condition (the evaluated thermometer measurement) and relative humidity (40–60%) (the relative humidity meter

measurement) before measuring (77). Avoid direct airflow by using curtains around the place for measurement.

6.7.3 Topical Botulinum toxin in liposomal cream based preparation

BTX-A (Neuronox®, Medytox, Inc. Republic of Korea) was dissolved in 1.0 mL of 0.9% sterile, preservative-free saline before using BTX-A solution 0.3 mL as equivalent to 30 unit of BTX-A mixed by inversion with 3 ml of multilamellar liposomal beaded capsule cream to be contained in a puff bottle. Whereas, the vehicle control was prepared in 0.3 mL of 0.9% sterile, preservative-free saline mixed by inversion with 3 ml of multilamellar liposomal beaded capsule cream to be contained in a puff bottle. Each bottle would be labeled as number 1 or 2.

6.8 Treatment

6.8.1 Preoperational subject

6.8.1.1 Subjects were asked to shave axillary hair and avoid using antiperspirant, deodorant and any topical products at axillar area for 3 days.

6.8.1.2 Subjects needed to be in the setting temperature room of 22-25^{0}C for 15 minutes to prepare their body conditions and lay down on the examination bed, with 90-degree hand abduction before starting sweating measurement by tewameter for 1 minutes. The measurement was done 3 times, onto nearby areas but not exactly the same sites, at mid-axillary fold and average as 1 result.

6.8.1.3 Minor's Iodine-starch test was used with the application of povidone iodine solution 1cc on each axillar. When the solution was dry, apply corn starch 4gm on both axillar with hand adduction for 10 minutes in the temperature control room, and then take a photograph. Subjects afterwards stood back to the wall at the mark area and rose both hands up with wall contracting of all hand parts. The distant between axillar was measured by a measuring tape, with a camera at 90 degrees (30 centimeters distant) for photographs.

6.8.2 Treatment

6.8.2.1 Subjects randomly received two bottles of cream: one with BTX-A cream and the other with vehicle cream. Both treatment regimens were double-blind. Each bottle was labeled as a number 1 or number 2. The subjects also received the instruction leaflets which precisely described how to use the products.

6.8.2.2 The cream was applied once daily for a total course of seven days. Each product must be used only 2 puffs (equal to approximately 4.4u of BTX-A) per side of the axilla (puffing with right hand for left axillar and left hand for right axillar), and gently applied all over without anything else afterwards. In order to check their compliance, all subjects were instructed to bring both products to the researcher at first follow-up visit.

6.8.2.3 Keep the products in refrigerator after use.

6.8.2.4 Measure the efficacy every 2 weeks for 8 weeks

6.9 Outcome measurement

All participants were physically examined to assess the treatment areas including general skin appearance, together with Tewameter measurement, Minor's Iodine starch test, and photography by a single physician. This assessment was done at baseline (before products' application), 0 day, 2 weeks, 4 weeks, 6 weeks, and 8 weeks.

Tewameter measurement was performed on the axillar for 1 minute, 3 times per one side in the room with setting control of temperature, humidity, and air flow during every visit (at baseline before applying the cream, 0 day, 2 weeks, 4 weeks, 6 weeks, and 8 weeks). The fixture ensured the mid of axillar skin fold.

A documentation of this study was compiled by collecting photographic records, using a digital camera with consistent setting, lighting, and positioning of the patients. All the patients were in the same position, raising arms with full abduction. Furthermore, the position between the patients and a camera were controlled under the fixed distance and angle. The photos were taken since the baseline before applying the cream, after the Minor's iodine starch test, 2^{nd} week, 4^{th} week, 6^{th} week, and 8^{th} week.

The HDSS and DLQI were recorded in the Case Record Form (at baseline before applying the cream, 0 day, 2 weeks, 4 weeks, 6 weeks, and 8 weeks).

Safety and Satisfaction were measured in every visit.

The data were collected in the Case Record Form, text file, and imaging file in the computer.

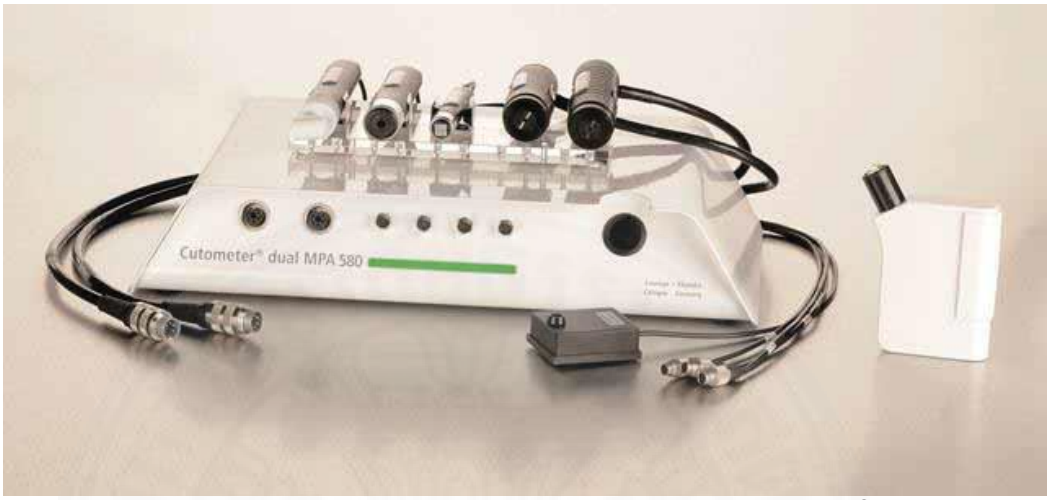


Figure 6.2 Cutometer® dual MPA 580 model with Cutometer®, Tewameter®, Corneometer®, Sebometer®, Mexameter®, Skin-pH-Meter and Probe Skin-Thermometer probe (78).

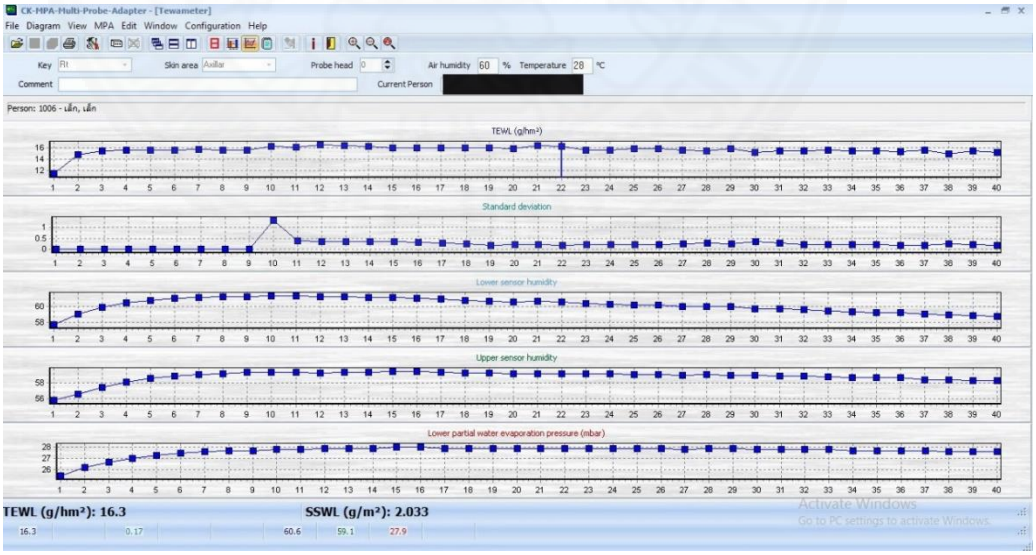
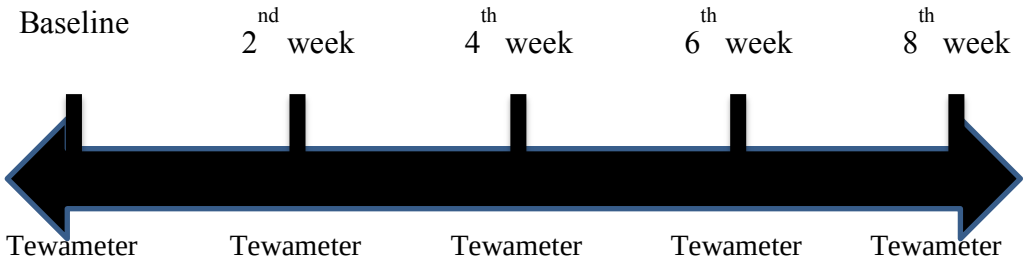


Figure 6.3 The parameter provided in the Tewameter program.

Table 6.1 The schedule for the result evaluation



Baseline	2nd week	4th week	6th week	8th week
Tewameter IST + Photography HDSS DLQI **Receive** **Drug**	Tewameter IST + Photography HDSS DLQI Safety Satisfaction	Tewameter IST + Photography HDSS DLQI Safety Satisfaction	Tewameter IST + Photography HDSS DLQI Safety Satisfaction	Tewameter IST + Photography HDSS DLQI Safety Satisfaction

6.10 Data collection

The data were collected in paper document, text file, and imaging file in the computer during every visit of follow up. The case record form used in this study contained all patients' demographic data as shown.

6.11 Data analysis

The patients' demographic data and other descriptive statistics were analyzed by using percentage, mean, and SD. Data comparison between topical BTX-A liposomal cream and vehicle control cream was analyzed by using dependent t-test and Mann Whitney U test. Whereas, The data on number of times and weeks were compared and analyzed by using Friedman test, repeated ANOVA, and Wilcoxon Signed Ranks test, with the adjusted P-value by using Bonferroni method.

6.12 Ethical consideration

This research was approved by the ethic committee of Thammasat University.

6.13 Significance of the research

This research could provide the efficacious and non-invasive topical method for primary axillary hyperhidrosis treatment using minimally effective concentration of botulinum toxin type A.

6.14 Conceptual Framework

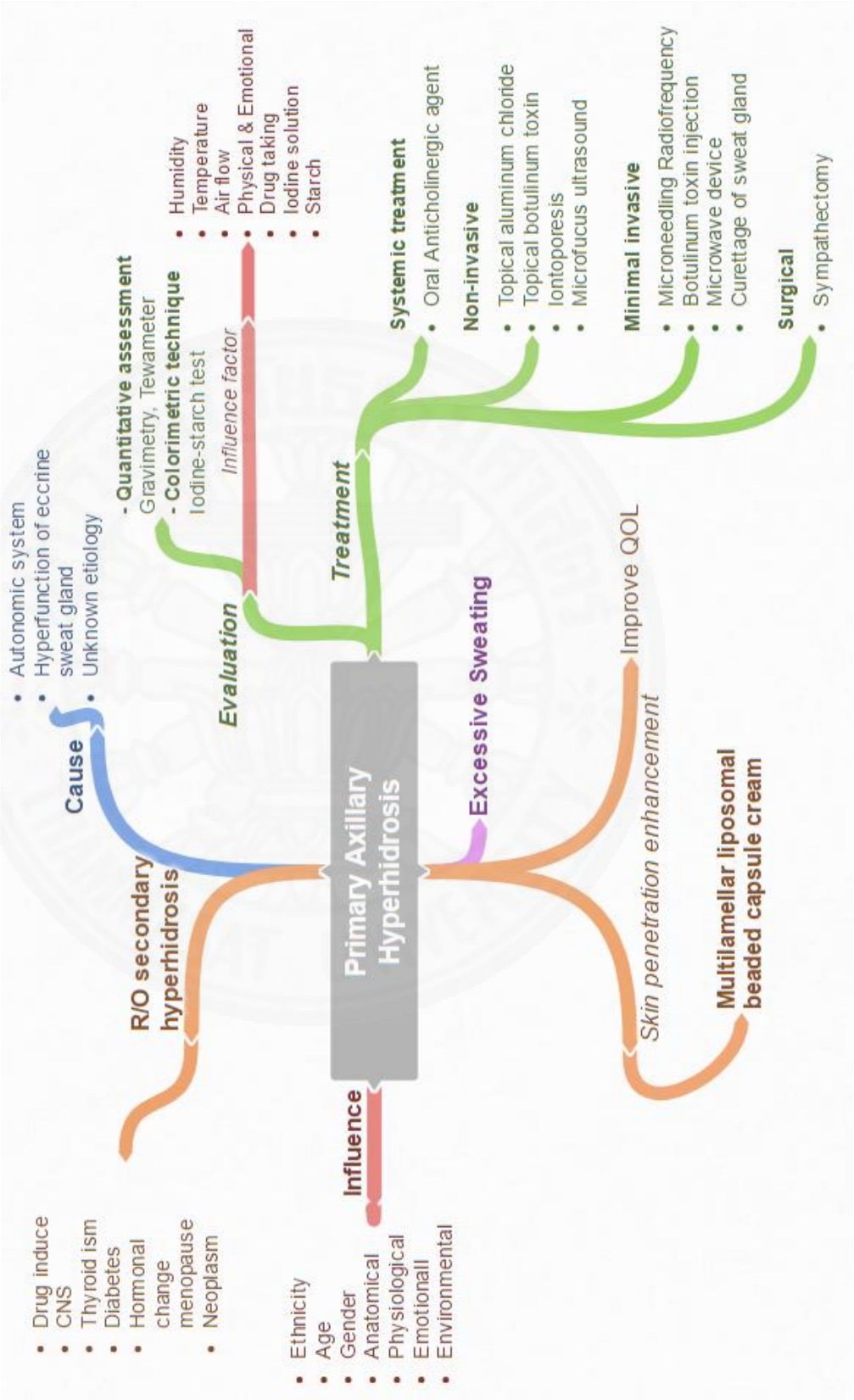


Figure 6.4 Conceptual framework

CHAPTER 7
RESULTS

7.1 Patient demographic data

Twenty subjects (4 males, 16 females) with the mean±SD age of 37.55±9.41 years were enrolled and completed the study. In details, most of them were HDSS 3 (12 subjects, 60%). In addition, 5 subjects (25%) were HDSS 4 and 3 subjects (15%) were HDSS 2. Moreover, all subjects had no underlying disease. For the history of axillary hyperhidrosis treatment, 18 subjects (90%) used to apply topical deodorant, with active ingredient of aluminum chloride hexahydrate, on both axillar every morning. Meanwhile, 1 subjects (5%) previously received hair removal laser with the last session longer than 6 months. Finally, 0 subject (0%) ever took botulinum toxin injection.

Table 7.1 Patient demographic data

Variable	N	%
Sex		
Male	4	20
Female	16	80
Age	37.55±9.41/ 37.50	
Hyperhidrosis Disease Severity Scale		
1	0	0.00
2	3	15.00
3	12	60.00
4	5	25.00
History of asymmetrical sweating		
Yes	0	0.00
No	20	100.00
History of current daily drug use		
Yes	0	0.00
No	20	100.00
Underlying disease		
Yes	0	0.00
No	20	100.00
Previous treatment in a		
Topical drug	18	90.00
Botulinum toxin injection	0	0.00
Laser	1	5.00

Variable	N	%
Iontoporesis	0	0.00
Microwave base device	0	0.00
Curette	0	0.00

7.2 Tewameter measurement

As sweat evaporates, producing vapour pressure, diffusion flow can be expressed by its pressure gradient. The measurement was created by measuring the passive diffusion of water evaporation through the skin. The vapour pressure gradient passing the stratum corneum shows the diffusion flow. In case of an absence in sweating, there is low amount of water vapour passing through stratum corneum by passive diffusion. Hence, this study indicates that sweat can be evaluated by measuring TEWL.

Following Tewameter measurement, the baseline value showed the difference of not more than 25% in the right and the left axillar according to the inclusion criteria of 31.13±6.63 g/m^2h and 32.27±5.71 g/m^2h, respectively.

The 2nd week of follow up demonstrated a statistically significant decrease value of Tewameter measurement between the BTX-A treated side compared with the vehicle control treated side of 25.43 ± 4.48 g/m^2h vs 33.49 ± 4.9 g/m^2h respectively, with mean difference of -8.06 (-10.57, -5.55) ($p<0.001$).

The 4th week of follow up noted a statistically significant decrease value of Tewameter measurement between the BTX-A treated side compared with vehicle the control treated side of 28.39 ± 4.46 g/m^2h vs 34.86 ± 5.01 g/m^2h respectively, with mean difference of -6.47 (-8.42, -4.52) ($p<0.001$).

The 6th week of follow up illustrated a statistically significant decrease value of Tewameter measurement between the BTX-A treated side compared with the vehicle control treated side of 28.94 ± 5.03 g/m^2h vs 36.09 ± 4.58 g/m^2h respectively, with mean difference of -7.15 (-9.99, -4.31) ($p<0.001$).

The 8th week of follow up revealed a statistically significant decrease value of Tewameter measurement between the BTX-A treated side compared with the vehicle control treated side of 34.41 ± 7.84 g/m^2h vs 38.35 ± 8.71 g/m^2h respectively, with mean difference of -3.94 (-6.58, -1.29) ($p=0.006$).

Additionally, there was a statistically significant difference of Tewameter measurement in the BTX-A treated side at baseline compared with the follow up measurement (p=0.007). In the other side, a statistically significant difference of TEWL in the vehicle control treated side was noted at baseline compared with the follow up measurement (p<0.001).

Table 7.2 Tewameter measurement result

Treatment	Baseline	Week 2	Week 4	Week 6	Week 8	p-value
BTX-A treated	31.19 ± 5.88	25.43 ± 4.48	28.39 ± 4.46	28.94 ± 5.03	34.41 ± 7.84	**0.007***
Vehicle control	32.21 ± 6.48	33.49 ± 4.9	34.86 ± 5.01	36.09 ± 4.58	38.35 ± 8.71	**<0.001***
Mean diff. (95%CI)	-1.01 (-2.77, 0.74)	-8.06 (-10.5, -5.5)	-6.47 (-8.4, -4.5)	-7.15 (-9.99, -4.3)	-3.94 (-6.58, -1.3)	
p-value	**0.242**	**<0.001***	**<0.001***	**<0.001***	**0.006***	

Values presented as mean ± SD. and mean difference (95% Confident interval). P-value corresponds to Paired t-test (between treatments) and repeated measurement ANOVA (within treatment)

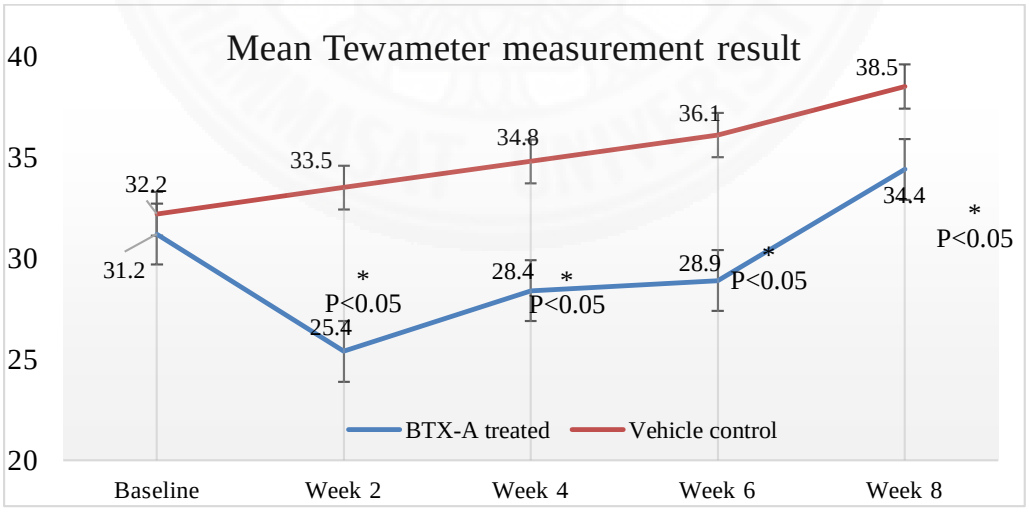


Figure 7.1 Mean Tewameter measurement result (*p<0.005)

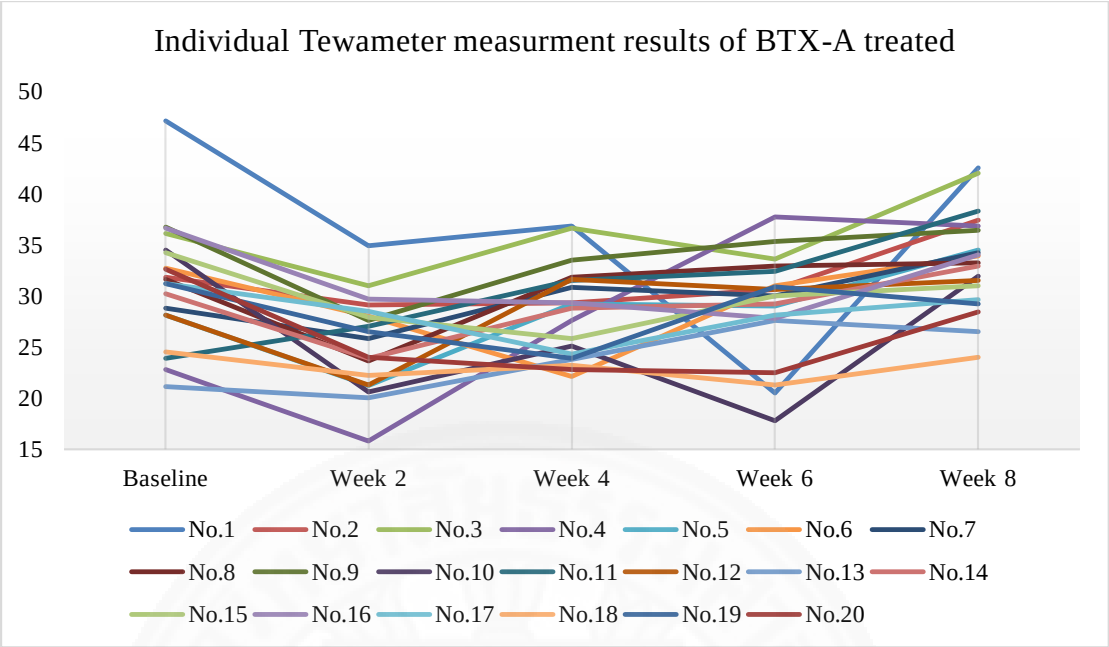


Figure 7.2 Individual Tewameter measurement results of BTX-A treated

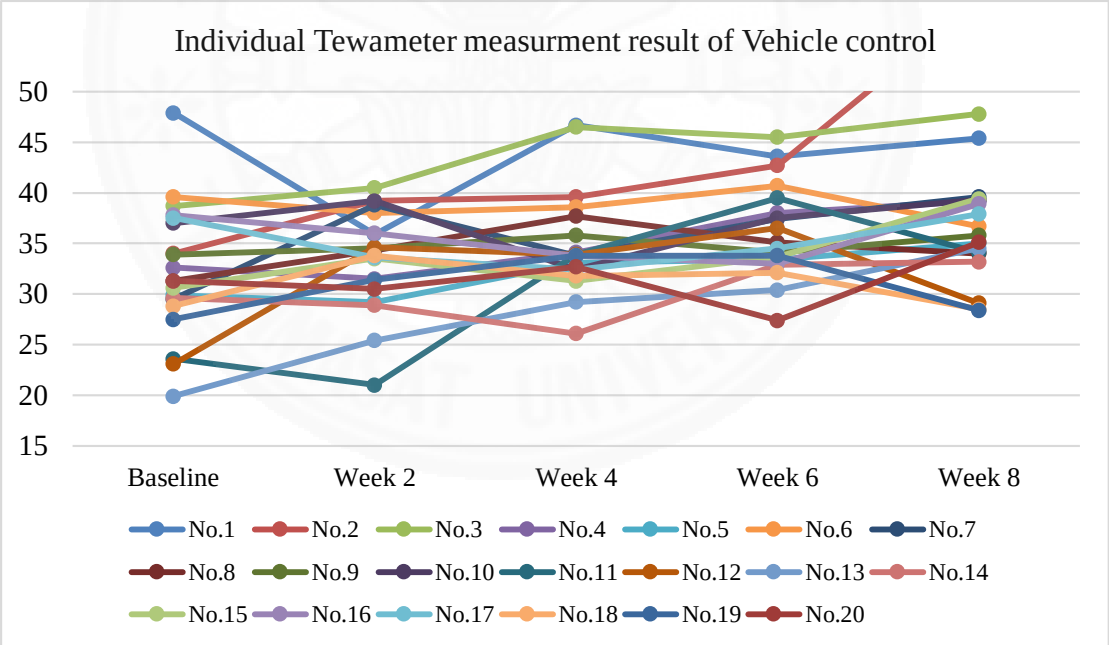


Figure 7.3 Individual Tewameter measurement result of vehicle control

7.3 Expert panel assessment and Investigator assessment

7.3.1 Expert panel assessment compared with baseline

The expert panel assessment evaluated the clinical data of subjects. All pictures of the subjects' axillar from baseline to every 2 weeks of follow up until 8th week using the Minor's iodine starch test and photography were compared in each follow up and assessed by the scores of 3 certificated dermatologists using double-blind evaluation, with the score -4 to +4 (-4: markedly worsen than baseline, -3: moderate worsen than baseline, -2: mild worsen than baseline, -1: minimal worsen than baseline, 0: no difference from baseline, +1: minimal improvement from baseline, +2: minimal improvement from baseline, +2: mild improvement from baseline, +3: moderate improvement from baseline, +4: markedly improvement from baseline).

The 2nd week of follow up showed a statistically significant improvement of score on the BTX-A treated side compared with the vehicle control side of 2.25±0.91 (mild-moderate) vs 0.75±0.85 (minimal-mild) respectively, with mean difference of 1.50 (0.94-2.06) ($p<0.001$).

The 4th week of follow up noted a statistically significant improvement of score on the BTX-A treated side compared with the vehicle control treated side of 1.90±1.02 (mild-moderate) vs 0.40±0.50 (minimal) respectively, with mean difference of 1.50 (0.98-2.02) ($p<0.001$).

The 6th week of follow up demonstrated a statistically significant improvement of score on the BTX-A treated side compared with the vehicle control treated side of 1.40±0.88 (mild-moderte) vs 0.30±0.92 (minimal) respectively, with mean difference of 1.10 (0.52-1.68) ($p<0.001$).

The 8th week of follow up illustrated a statistically significant improvement of score on the BTX-A treated side compared with the vehicle control treated side of 0.55±0.60 (minimal) vs -0.20±0.77 (minimal) respectively, with mean difference of 0.75 (0.31-1.19) ($p=0.001$).

The expert panel assessment revealed a statistically significant improvement on the BTX-treated side with the prolonged scores until the 8th week ($P < 0.001$). However, the vehicle control treated side was also shown with minimal worsening score at the 8th week of follow up ($p=0.001$).

Table 7.3 Expert panel assessment of photography of IST compared with baseline

Treatment	Week 2	Week 4	Week 6	Week 8	P-value
BTX-A treated	2.25±0.91[1,2]	1.90±1.02[3]	1.40±0.88[1,4]	0.55±0.60[2,3,4]	**<0.001[c]**
Vehicle control	0.75±0.85[1]	0.40±0.50[2]	0.30±0.92[3]	-0.20±0.77[1,2,3]	**0.001[c]**
Mean diff. 95% CI	1.50 (0.94-2.06)	1.50 (0.98-2.02)	1.10 (0.52-1.68)	0.75 (0.31-1.19)	
P-value	**<0.001[b]**	**<0.001[b]**	**<0.001[b]**	**0.001[b]**	

[b]Mann-Whitney U Test, [c] Friedman Test, Post Hoc: Wilcoxon Signed Ranks Test (p-value<0.008)

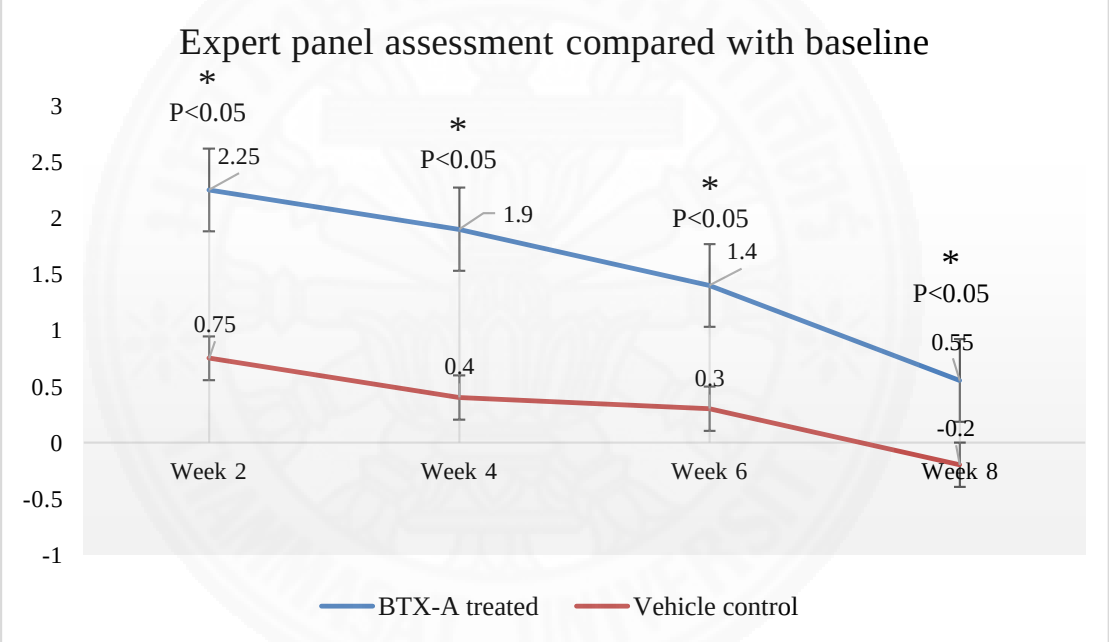


Figure 7.4 Expert panel assessment compared with baseline.

7.3.1 Investigator panel assessment compared with baseline

The investigator panel assessment evaluated the clinical data of subjects. All pictures of the subjects' axillar from baseline to every 2 weeks follow up until the 8th week using Minor's iodine starch test and photography in each follow up were compared and assessed by the scores of 3 certificated dermatologists using double-blind evaluation, with the score of -4 to +4 (-4: markedly worsen than baseline, -3: moderate worsen than baseline, -2: mild worsen than baseline, -1: minimal worsen than baseline, 0: no difference from baseline, +1: minimal improvement from baseline,

+2: minimal improvement from baseline, +2: mild improvement from baseline, +3: moderate improvement from baseline, +4: markedly improvement from baseline).

The 2nd week of follow up showed a statistically significant improvement of score on the BTX-A treated side compared with the vehicle control treated side of 2.05±1.00 (mild-moderate) vs 0.65±0.93 (minimal) respectively, with mean difference of 1.40 (0.78-2.02) (p<0.001).

The 4th week of follow up illustrated a statistically significant improvement of score on the BTX-A treated side compared with the vehicle control treated side of 1.95±0.83 (mild-moderate) vs 0.40±0.75 (minimal) respectively, with mean difference of 1.55 (1.04-2.06) (p<0.001).

The 6th week of follow up revealed a statistically significant improvement of score on the BTX-A treated side compared with the vehicle control treated side of 1.25±0.64 (minimal-mild) vs 0.30±0.92 (minimal) respectively, with mean difference of 0.95 (0.44-1.46) (p<0.001).

The 8th week of follow up demonstrated a statistically significant improvement of score on the BTX-A treated side compared with the vehicle control treated side of 0.80±0.89 (minimal) vs 0.20±0.70 (minimal) respectively, with mean difference of 0.60 (0.09-1.11) (p=0.024).

The investigator panel assessment identified a statistically significant improvement on the BTX-A treated side with the scores prolonged to 8 week (p< 0.001). However, the vehicle control treated side also showed minimal worsening in score at the 8th week of follow up without statistically significance.

Table 7.4 Investigator assessment compared with baseline

Treatment	Week 2	Week 4	Week 6	Week 8	P-value
BTX-A treated	2.05±1.00[1,2]	1.95±0.83[3,4]	1.25±0.64[1,3,5]	0.80±0.89[2,4,5]	**<0.001[b]**
Vehicle control	0.65±0.93	0.40±0.75	0.30±0.92	0.20±0.70	0.170[b]
Mean diff. 95% CI	1.40	1.55	0.95	0.60	
P-value	**<0.001[b]**	**<0.001[b]**	**<0.001[b]**	**0.024[b]**	

[b]Mann-Whitney U Test, [c] Friedman Test, Post Hoc: Wilcoxon Signed Ranks Test (p-value<0.008)

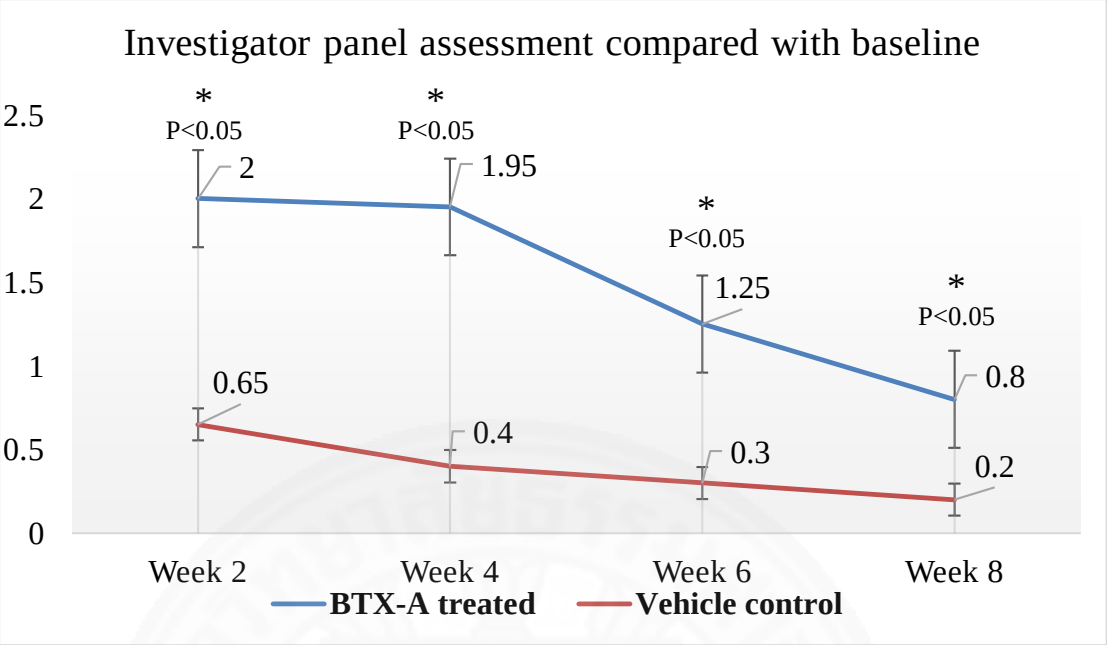
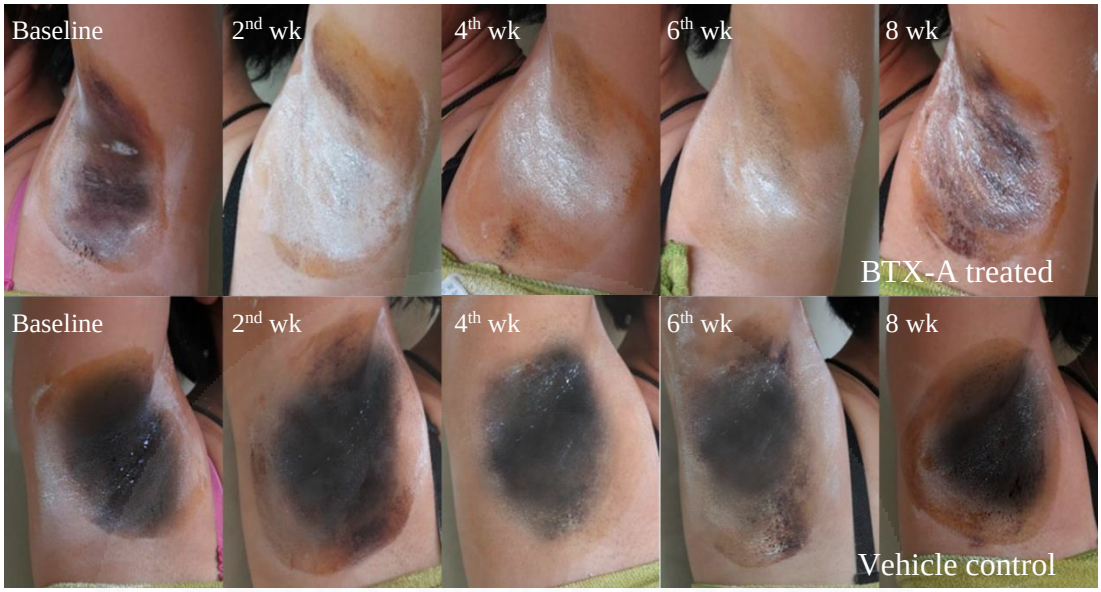


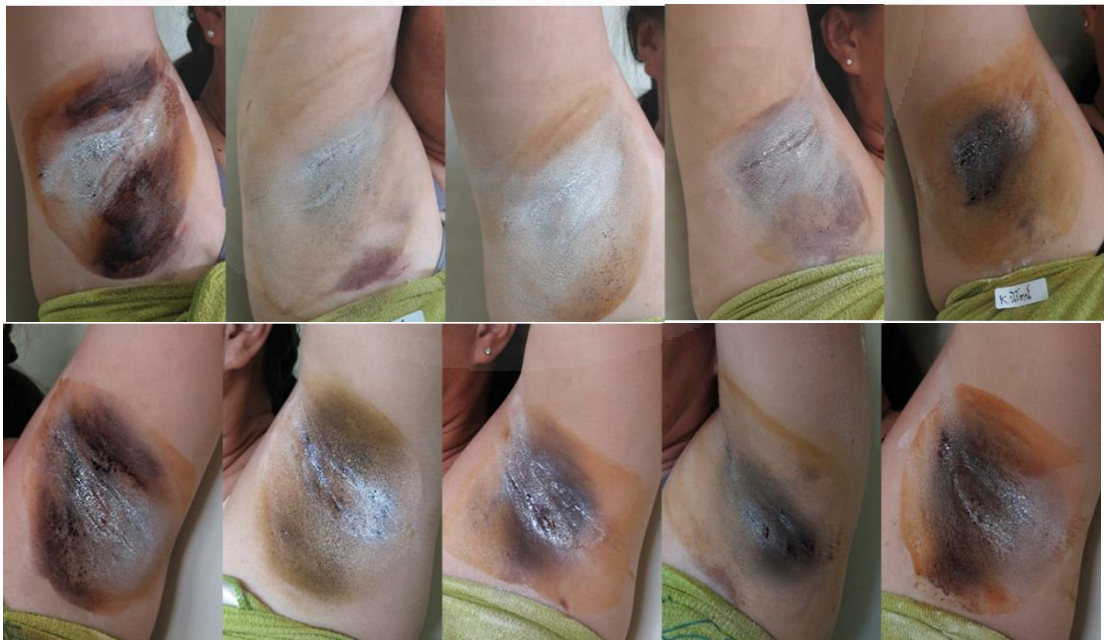
Figure 7.5 Investigator panel assessment compared with baseline

Figure 7.6 Patient axillar with iodine starch test in BTX-A treated (upper row) and vehicle control (lower row)

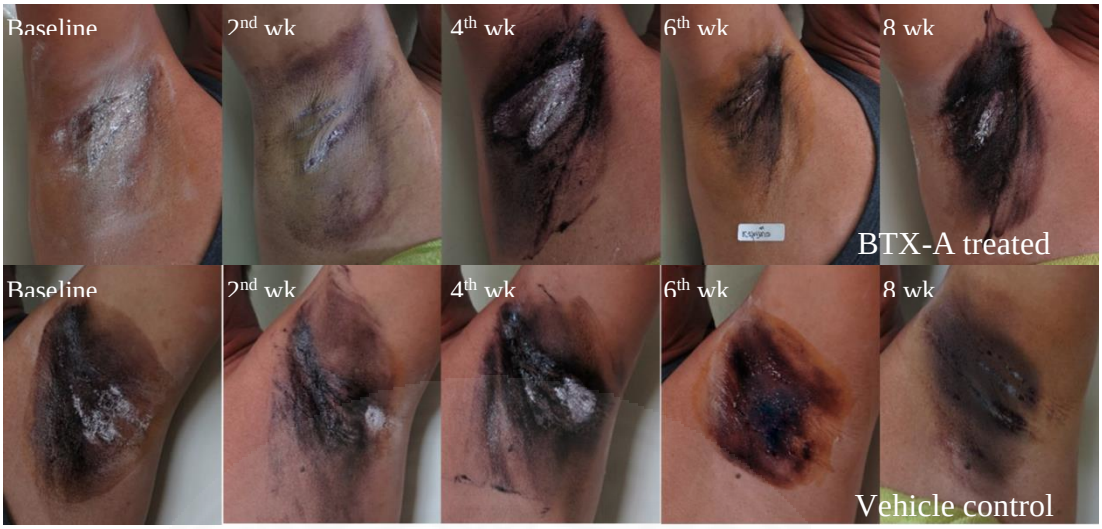
Case No. 1



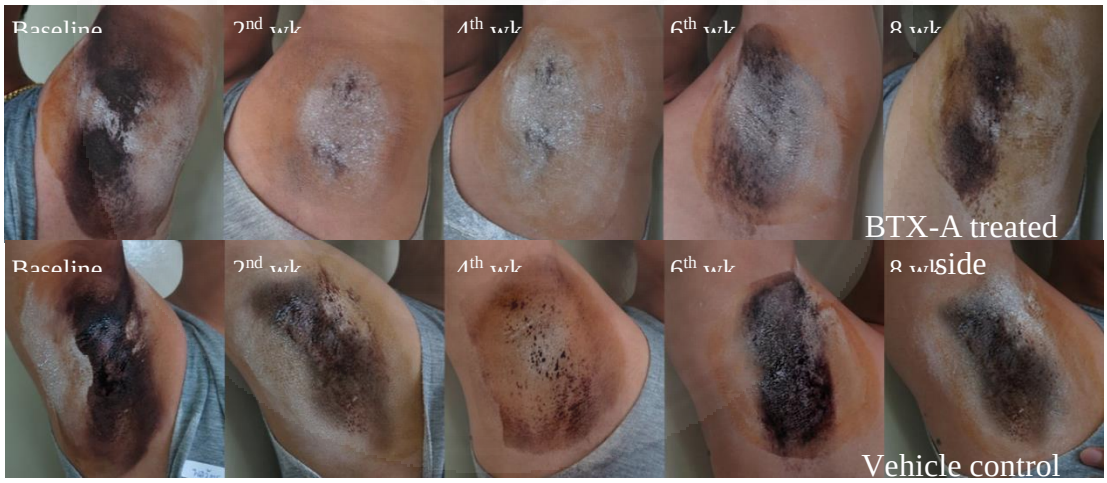
Case No. 6



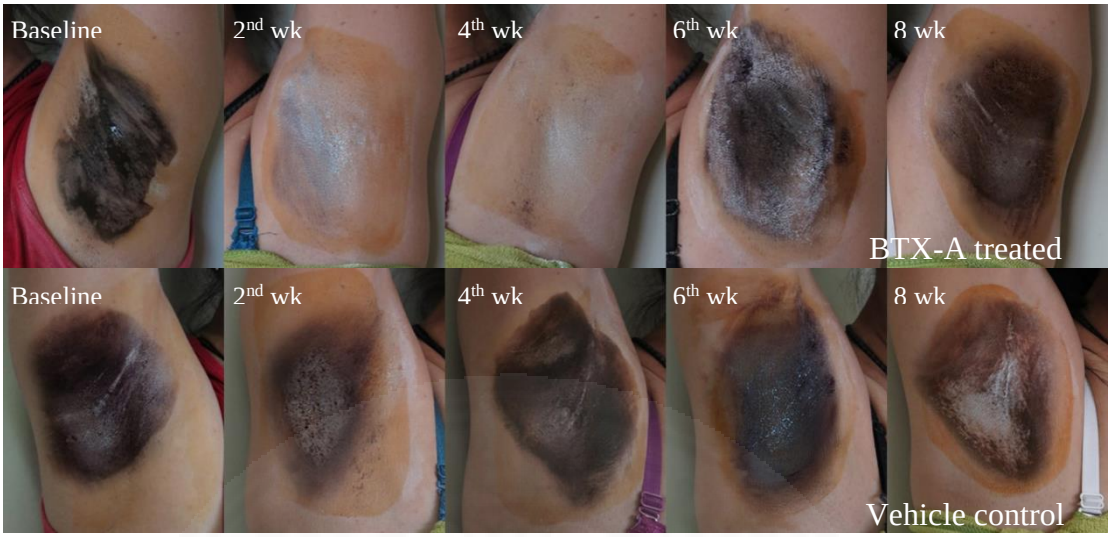
Case No. 8



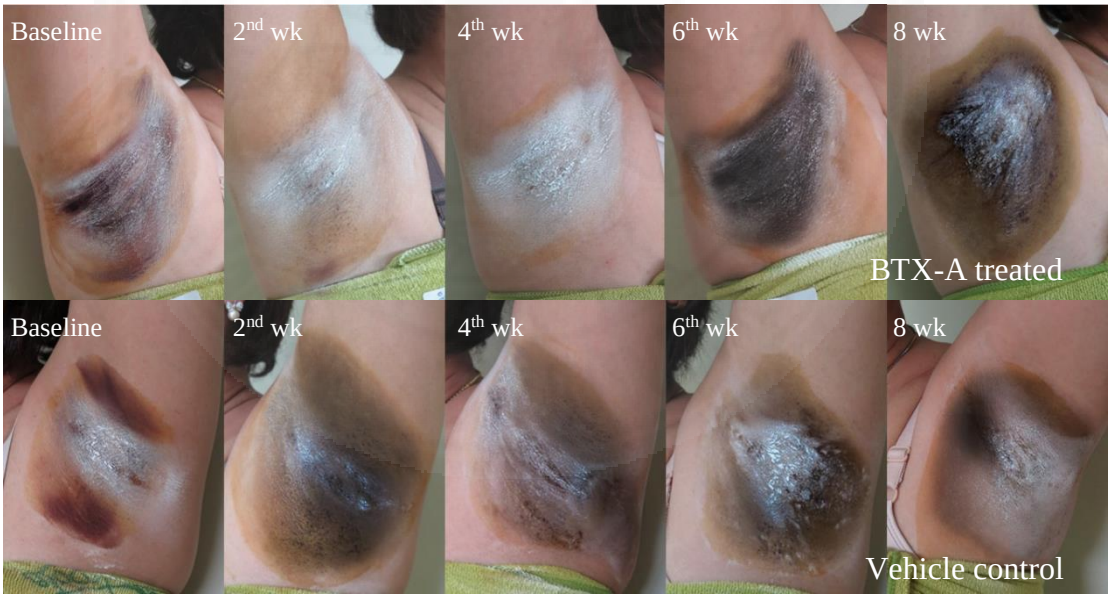
Case No. 10



Case No. 14



Case No. 18



7.4 Hyperhidrosis disease severity scale (HDSS)

Hyperhidrosis disease severity scale was evaluated by questionnaire and scoring from baseline to every 2 weeks follow up until the 8th week. "The score 1 mean sweating was never noticeable and not interfered with daily activities. The score 2 mean sweating was tolerable but sometimes interfered with daily activities. The score 3 mean sweating was barely tolerable and frequently interfered with daily activities and score 4 mean sweating was intolerable and always interfered with daily activities".

The subjects' scale showed HDSS improvement in every 2 weeks of follow up. The score became worsening during last follow up, but still better than baseline.

The 2^{nd} ,4^{th} ,6^{th} and 8^{th} week of follow up showed a statistically significant decrease value of Tewameter measurement on the BTX-A treated side compared with the vehicle control treated side of 1.55 ± 0.51 vs 2.3 ± 0.66, with mean difference of -0.75 (-1.01, -0.49); 1.75 ± 0.44 vs 2.65 ± 0.49, with mean difference of 0.9 (-1.04, -0.76); 2 ± 0.56 vs 2.95 ± 0.6, with mean difference of -0.95 (-1.23, -0.67); and 2.55 ± 0.6 vs 3.05 ± 0.6, with mean difference of -0.5 (-0.74, -0.26) ($p<0.001$), respectively.

HDSS revealed a statistically significant improvement on the BTX-A treated side with the scores prolonged to the 8^{th} week from baseline (P=0.044). However, the vehicle control treated side had statistically significant improvement in HDSS score prolonged to the 8^{th} week from baseline (p=0.004).

Table 7.5 Hyperhidrosis Disease Severity Scale

Treatment	**Baseline**	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**p-value**
BTX-A treated	3.1 ± 0.64	1.55 ± 0.51	1.75 ± 0.44	2 ± 0.56	2.55 ± 0.6	**0.044 ***
Vehicle control	3.1 ± 0.64	2.3 ± 0.66	2.65 ± 0.49	2.95 ± 0.6	3.05 ± 0.6	**0.004 ***
Mean diff. (95%CI)	0	-0.75 (-1.01, -0.49)	-0.9 (-1.04, -0.76)	-0.95 (-1.23, -0.67)	-0.5 (-0.74, -0.26)	
p-value	**NA**	**<0.001***	**<0.001***	**<0.001***	**<0.001***	

Values presented as mean ± SD. and mean difference (95% Confident interval). p-value corresponds to Paired t-test (between treatments) and Repeated measurement ANOVA (within treatment)

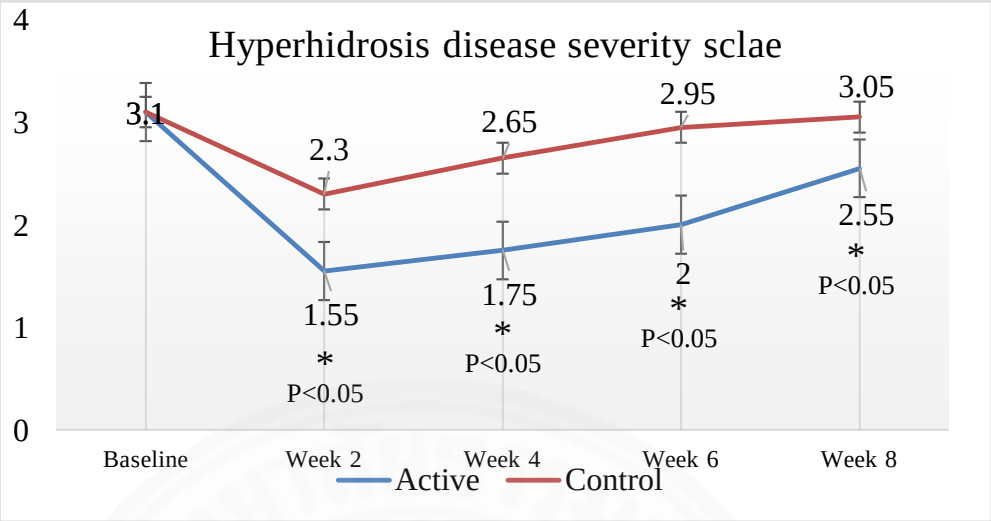


Figure 7.7 Hyperhidrosis Disease Severity Scale

7.5 Dermatology life quality index (DLQI)

There were significant improvements for the question 1, 2, 3, 4, 5, 7 and 10 before the 4th and 8th week of follow up. For the question 1, "Over the last week, how itchy, sore, painful or stinging has your skin been?" the answer for "a lot" was significantly changed from 90% to 0% and 5%, respectively, while the answer for "a little" was significantly changed from 0% to 100% and 75%, respectively.

For the question 2, "Over the last week, how embarrassed or self-conscious have you been because of your skin?", the answer for "a lot" was significantly changed from 65% to 5% and 10% respectively; whereas, the answer for "a little" was significantly changed from 35% to 95% and 90%, respectively.

For the question 3, "Over the last week, how much has your skin interfered with you going shopping or looking after your home or garden?", the answer for "a lot" was significantly changed from 95% to 0% and 0% respectively, whilst, the answer for "a little" was significantly changed from 5% to 100% and 100%, respectively.

For the question 4, "Over the last week, how much has your skin influenced the clothes you wear?", the answer for "a lot" was significantly changed from 90% to 0% and 10% respectively, and the answer "a little" was significantly changed from 5% to 100%, and 90%, respectively.

For the question 5, “Over the last week, how much has your skin affected any social or leisure activities?”, the answer for “a lot” was significantly changed from 100% to 0% and 5% respectively, and the answer “a little” was significantly changed from 0% to 100% and 95%, respectively.

For the question 7, “Over the last week, has your skin prevented you from working or studying?”, the answer for “no” was significantly changed from 90%, to 100% and 95% respectively. For an additional question, “if "no", over the last week how much has your skin been a problem at work or studying?”, the answer “not at all” was significantly changed from 100% to 100% and 80%, respectively.

Finally, for the question 10, “Over the last week, how much of a problem has the treatment for your skin been, for example by making your home messy, or by taking up time?”, the answer for “a lot” was significantly changed from 65% to 0% and 10% respectively, and the answer for “a little” was significantly changed from 35% to 100% and 90%, respectively.

Meanwhile, other questions showed no answers of significant changes for improvement.

Table 7.6 Dermatology quality of life index (DQLI) result

Question	**Before**	**2nd week**	**4th week**	**6th week**	**8th week**	**P-value**
1. Over the last week, how itchy, sore, painful or stinging has your skin been?	2.10±0.31	1.15±0.37	1.00±0.00	1.30±0.47	1.60±0.50	**<0.001**
Very much	2 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	18 (90.0)	3 (15.0)	0 (0.0)	6 (30.0)	12 (60.00	
A little	0 (0.0)	17 (85.0)	20 (100.0)	14 (70.0)	8 (40.0)	
Not at all	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
2. Over the last week, how embarrassed or self-conscious have you been because of your skin?	1.65±0.49	1.05±0.22	1.05±0.22	1.05±0.22	1.10±0.31	**<0.001**

Very much	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	13 (65.0)	1 (5.0)	1 (5.0)	1 (5.0)	2 (10.0)	
A little	7 (35.0)	19 (95.0)	19 (95.0)	19 (95.0)	18 (90.0)	
Not at all	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
3. Over the last week, how much has your skin interfered with you going shopping or looking after your home or garden?	1.95±0.22	1.05±0.22	1.00±0.00	1.00±0.00	1.00±0.00	**<0.001**
Very much	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	19 (95.0)	19 (95.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A little	1 (5.0)	1 (5.0)	20 (100.0)	20 (100.0)	20 (100.0)	
Not at all	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Not relevant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
4. Over the last week, how much has your skin influenced the clothes you wear?	2.00±0.32	1.10±0.31	1.00±0.00	1.00±0.00	1.10±0.31	**<0.001**
Very much	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	18 (90.0)	2 (10.0)	0 (0.0)	0 (0.0)	2 (10.0)	
A little	1 (5.0)	18 (90.0)	20 (100.0)	20 (100.0)	18 (90.0)	
Not at all	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Not relevant	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
5. Over the last week, how much has your skin affected any social or leisure activities?	2.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.05±0.22	**<0.001**
Very much	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	20 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	
A little	0 (0.0)	20 (100.0)	20 (100.0)	20 (100.0)	19 (95.0)	
Not at all	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Not relevant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
6. Over the last week, how much has your skin made it difficult for you to do any sport?	1.05±0.69	0.95±0.51	0.95±0.51	1.00±0.56	1.00±0.56	0.231
Very much	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

A lot	2 (10.0)	2 (10.0)	2 (10.0)	3 (15.0)	3 (15.0)	
A little	14 (70.0)	15 (75.0)	15 (75.0)	14 (70.0)	14 (70.0)	
Not at all	3 (15.0)	3 (15.0)	3 (15.0)	3 (15.0)	3 (15.0)	
Not relevant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
7. Over the last week, has your skin prevented you from working or studying?						
Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
No	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	
Not relevant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
If "No", over the last week how much has your skin been a problem at work or studying?	2.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.20±0.41	**<0.001**
A lot	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A little	20 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (20.0)	
Not at all	0 (0.0)	20 (100.0)	20 (100.0)	20 (100.0)	16 (80.0)	
8. Over the last week, how much has your skin created problems with your partner or any of your close friends or relatives?	0.10±0.45	0.05±0.22	0.05±0.22	0.05±0.22	0.05±0.22	0.406
Very much	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A little	0 (0.0)	1 (5.0)	1 (5.0)	1 (5.0)	1 (5.0)	
Not at all	19 (95.0)	19 (95.0)	19 (95.0)	19 (95.0)	19 (95.0)	
Not relevant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
9. Over the last week, how much has your skin caused any sexual difficulties?	0.20±0.52	0.15±0.37	0.15±037	0.15±0.37	0.15±0.37	0.406
Very much	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A little	2 (10.0)	3 (15.0)	3 (15.0)	3 (15.0)	3 (15.0)	
Not at all	17 (85.0)	17 (85.0)	17 (85.0)	17 (85.0)	17 (85.0)	
Not relevant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

10. Over the last week, how much of a problem has the treatment for your skin been, for example by making your home messy, or by taking up time?	1.65±0.49	1.05±0.22	1.00±0.00	1.00±0.00	1.05±0.22	**<0.001**
Very much	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	13 (65.0)	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	
A little	7 (35.0)	19 (95.0)	20 (100.0)	20 (100.0)	19 (95.0)	
Not at all	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Not relevant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

Question	**Before**	**2 week**	**4 week**	**6 week**	**8 week**	**P-value**
Overall DLQI score	**1.40±0.50**	**0.90±0.31**	**0.90±0.31**	**0.95±0.22**	**1.00±0.00**	<0.001
Very much	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A lot	8 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
A little	12 (60.0)	18 (90.0)	18 (90.0)	19 (95.0)	20 (100.0)	
Not at all	0 (0.0)	2 (10.0)	2 (10.0)	1 (5.0)	0 (0.0)	

Friedman Test, Post Hoc: Wilcoxon Signed Ranks Test (P-value<0.005)

7.6 Patient's satisfaction

The investigator used the questionnaires to evaluate patient's satisfaction at 2nd, 4th, 6th and 8th week of follow up, with the score of -4 to +4 (-4: markedly worsen than baseline, -3: moderate worsen than baseline, -2: mild worsen than baseline, -1: minimal worsen than baseline, 0: no difference from baseline, +1: minimal improvement from baseline, +2: minimal improvement from baseline, +2: mild improvement from baseline, +3: moderate improvement from baseline, +4: markedly improvement from baseline.

The result showed a significant satisfaction on the BTX-A treated side compared with the vehicle control treated side, with the 2nd, 4th, 6th, 8th week follow up of 2.75±0.44 vs 0.90±0.45; 2.25±0.55 vs 0.55±0.51; 2.00±0.56 vs 0.35±0.49; and 1.55±0.51 vs 0.15±0.37, with the mean difference of 1.85 (1.56-2.14), 1.70 (1.36-2.04), 1.65 (1.31-1.99), and 1.40 (1.12-1.68) respectively, with statistical significance ($p<0.001$).

Table 7.7 Patient satisfaction score

Treatment	Week 2	Week 4	Week 6	Week 8	P-value
BTX_A Treated	2.75±0.44[1,2,3]	2.25±0.55[1,4]	2.00±0.56[2,5]	1.55±0.51[3,4,5]	**<0.001**
Vehicle Control	0.90±0.45[1,2,3]	0.55±0.51[1,4]	0.35±0.49[2]	0.15±0.37[3,4]	**<0.001**
Mean diff. 95% CI	1.85 (1.56-2.14)	1.70 (1.36-2.04)	1.65 (1.31-1.99)	1.40 (1.12-1.68)	
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	

Mann-Whitney U Test, Friedman Test, Post Hoc: Wilcoxon Signed Ranks Test (P-value<0.005)

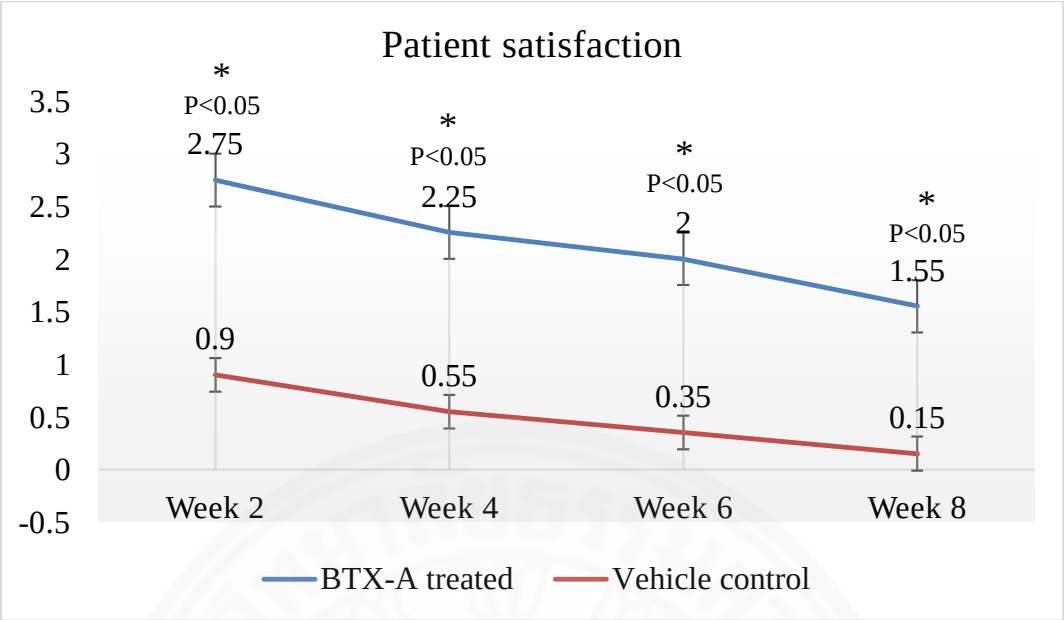


Figure 7.8 Patient satisfaction score

7.7 Safety

Every participant had no side effects (rash, irritation, itching, burning, scale, hyperpigmentation, hypopigmentation, and eruption) from the BTX-A and the vehicle control treated sides.

CHAPTER 8
DISCUSSION

The US FDA has currently certified the use of botulinum toxin intradermal injection for treatment of axillary hyperhidrosis (2). Nonetheless, pains are main treatment side effects due to multiple injection points. Hence, this study was designed to investigate the topical formulation of BTX-A with multilamellar liposomal beaded capsule for the painless delivery of BTX-A through the skin, which is significantly effective in PAH treatment.

A previous randomized controlled trial of subcutaneous injection of BTX-A 50 U per axillar in patients with excessive axillary sweating revealed the efficacious results of treatment with BTX-A. When compared to the placebo-treated patients, those with BTX-A statistically exhibited significantly greater improvement in the physical component summary score at 16 weeks, confirmed by gravimetry and minor's iodine starch test (47). However, pains and other complications such as bruising were still the barriers to this injection for the delivery of BTX-A to targeted skin sites as well as the safety and effectiveness evaluation of this painless, topical application of BTX-A for PAH treatment. Similarly, the previous study of a randomized, double blinded, vehicle-controlled trial of 200 U of BTX-A combined with transport peptide molecule to bind the toxin in a non-covalent manner also showed the effectiveness of the topically applied BTX-A in PAH treatment. The decreased severity of PAH up to 4 weeks, was verified by minor's IST with few local adverse events and no systemic events (22). Thus, this study confirmed the efficacy of pharmaceutical enhancing skin penetration using multilamellar liposomal beaded capsule of topical form with lower dose, 30 U of BTX-A. Moreover, it was interestingly demonstrated that the lower dose of BTX-A with more frequent application could significantly improve the treatment outcomes of PAH, compared to the previous study of one time applied topical 200 U BTX-A with transport peptide molecule.

According to the study, topical BTX-A in multilamellar liposomal beaded capsule cream reduced the mean Tewameter measurement of sweat production compared to the vehicle control side in total follow up, with statistical significance

($P<0.05$). The panel assessment of the compared photography of minor's iodine starch test with baseline showed that BTX-A in multilamellar liposomal beaded capsule cream decreased the mean assessment value compared to the vehicle control side in total follow up, with statistical significance ($p<0.05$). In the meantime, there was a statistically significant difference between the BTX-A treated and the vehicle treated groups ($p<0.05$) by HDSS score. As well, DLQI score showed the improvement in 8 from 10 choices, with statistical significance ($p<0.05$).

Regarding drug delivery, the enhancement of BTX-A was performed through the skin by multilamellar liposomal beaded capsule which could carry both water soluble and lipid soluble drug. The lipid soluble drug must be loaded during liposomal formation (active drug loading), while the water soluble drug could be done after drug formation (passive drug loading). Nonetheless, BTX-A is water soluble drug with large molecule size and considered as highly resistant to transdermal delivery, thus the needling injection should still be necessary. In this study, BTX-A was passively loaded and delivered through epidermal papillae and ductular portion to the sweat gland.

Interestingly, sweat production was reduced in the vehicle control treated side, but certainly not related to local application of a vehicle. According to the study by Heckmann et al (46), patients were treated with the BTX-A injection in one axillae and the placebo in the other. At 2 weeks, there was a significant difference in the mean rate of sweat production in both axillae, but greater for the one treated with BTX-A injection. It was suggested that the axilla injected with the placebo benefitted from the systemic spread of BTX-A injection in the contralateral axilla. This hypothesis may be confirmed by the observation of clinical changes from the activity of distant sweating following the BTX-A administration. In another study, however, when BTX-A was injected into just one axilla, there was not any improvement of the contralateral axilla in the gravimetric measurements of sweat (79). It was similarly plausible that the down-regulation of autonomic nervous system occurred and may explain the decreased sweating in areas distant to treatment, just as the contralateral axillae. Nevertheless, the final follow up at 8^{th} week illustrated the minimal increase of Tewameter and HDSS in both axillae. This effect explained the compensatory hyperhidrosis and identified that

the sweat glands of one area could become hyperactive to compensate for hypo- or anhidrosis (80). The exact mechanism for this effect should require further study.

The results of this study is distinctive from previous findings by demonstrating that the minimal dose of topical BTX-A cream can improve PAH treatment following the statistical significant improvement of the BTX-A treated side confirmed by Tewameter, HDSS, and iodine starch test with the panel assessment compared to the placebo-treated side.

Whilst, there are some limitations to this study, including small sample size, short observation period, and lack of standardization of BTX-A concentration treatment dosage. Meanwhile, there are several variables resulting in sweat production; namely, the environment such as temperature and humidity, seasonal conditions, and physiologic and psychological conditions of each patient. These variables could affect the testing for the assessment of patient sweating, like the starch-iodine test. Importantly, most of the patients are females even though the hyperhidrosis incidence is equal in both genders, probably due to the fact that females seek medical help for those problems more than males.

CHAPTER 9
CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusion

The results in the 30U of BTX-A inversion with multilamellar liposomal beaded capsule cream could yield the effective treatment outcomes of PAH compared to the vehicle control side, evaluated by Tewameter, clinical grading of improvement by Minor's IST, HDSS and DLQI. Tewameter demonstrates the statistically significance improvement of the BTX-A treated than the vehicle control treated sides. The clinical grading and HDSS score of improvement in BTX-A treated groups show the statistically significant improvement from baseline up to 8 weeks ($P < 0.01$). Ultimately, DLQI illustrates the significantly improved quality of life and greater patient satisfaction outcomes with no serious side effects.

9.2 Recommendations

The BTX-A inversion with multilamellar liposomal beaded capsule cream could be potentially efficacious as a non-invasive topical method for PIH treatment, using minimally effective concentration of botulinum toxin type A.

Moreover, the amount of BTX-A concentration, number of applying day, amount of concentration in daily application, different mode of drug delivery, treatment interval and number of treatment sessions, and longer duration of follow up are suggestive to verify the efficacy of topical BTX-A formulation.

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APPENDICES

APPENDIX A

CASE RECORD FORM

Case Record Form

ข้อมูลทั่วไปของผู้ป่วย (Patient demographic Information)

1. เพศ □ ชาย □ หญิง

2. วัน/เดือน/ปีเกิด ……/……/……

3. อายุ……ปี……เดือน.

4. อาชีพ □ 1. ข้าราชการ □ 2. พนักงานบริษัท □ 3. ประกอบกิจการส่วนตัว
□ 4. แม่บ้าน □ 5. นักเรียน/นักศึกษา □ 6. อื่นๆ (โปรดระบุ) .....

5. โรคประจำตัว □ 1. ไม่เป็น □ 2.เป็น (ระบุโรค……)

5.1. การรักษาที่เคยได้รับ

……

5.2. การรักษาที่ได้รับในปัจจุบัน

……

6. ประวัติแพ้ยา, อาหาร หรือสารเคมี □ ไม่มี □ มี (ระบุ……)

7. อาหารเสริมหรือยาที่ใช้เป็นประจำ □ ไม่มี □ มี (ระบุ……)

8. มีประวัติภาวะเหงื่อออกมากผิดปกติในครอบครัว (ตอบได้มากกว่า 1 ข้อ)
□ บิดา □ มารดา □พี่น้อง(ระบุ……)

9. ประวัติการรักษาภาวะเหงื่อออกมากผิดปกติที่รักแร้

9.1. ประวัติการรักษาโดยใช้ยากิน/ยาทา

1. ชื่อยา……ระยะเวลาที่ใช้……
□ ปัจจุบันใช้อยู่ □ หยุดใช้มาเป็นระยะเวลา……
2. ชื่อยา……ระยะเวลาที่ใช้……
□ ปัจจุบันใช้อยู่ □ หยุดใช้มาเป็นระยะเวลา……

9.2. ประวัติการรักษาภาวะเหงื่อออกมากผิดปกติที่รักแร้ด้วยเลเซอร์ □ ไม่เคย □ เคยระบุ……(ครั้งล่าสุดที่ทำ……เดือน)

9.3. ประวัติการใช้เลเซอร์กำจัดขนบริเวณรักแร้ □ ไม่เคย □ เคย (ครั้งล่าสุดที่ทำ……เดือน)

9.4. ประวัติการรักษาภาวะเหงื่อออกมากผิดปกติที่รักแร้ด้วยการผ่าตัด □ ไม่เคย □ เคย ระบุ……

9.5. ประวัติการใช้โรลออน, สเปรย์ดับกลิ่นตัว ภายในสามเดือนที่ผ่านมา □ ไม่ใช่ □ ใช่

9.6. งดการใช้โรลออน, สเปรย์ดับกลิ่นตัวมาก่อนเริ่มทำวัย □ ไม่ใช่ □ ใช่ (ระบุ……วัน)

10. หญิงตั้งครรภ์, สงสัยว่าตั้งครรภ์ หรือให้นมบุตร □ ไม่ใช่ □ ใช่

11. การทำงานในสถานที่ที่มีอากาศร้อน □ ไม่ใช่ □ ใช่ ระบุ……(วัน/สัปดาห์)

12. การอาบน้ำอุ่น □ ไม่ใช่ □ ใช่ ระบุ……(ครั้ง/สัปดาห์)

13. การออกกำลังกาย □ ไม่ออก □ ออก ระบุ……(ครั้ง/สัปดาห์)

14. มีการใช้ห้องอบซาวน่า หรือห้องอบไอน้ำเป็นประจำ □ ไม่ใช่ □ ใช่ ระบุ……(ครั้ง/สัปดาห์)

15. ท่านมีความเครียดในการทำงานหรือชีวิตส่วนตัว □ ไม่เครียด □ ปานกลาง □ เครียด

APPENDIX B

EXPERT PANEL ASSESSMENT

No_____

□W2 □W4 □W6 □W8

Expert panel Assessment: Treatment Result □ 1 □ 2 □ 3

แบบประเมินผลการรักษาเมื่อเปรียบเทียบก่อนและหลังการรักษาจากรูปภาพการทำ Iodine-starch Test

โปรดทำเครื่องหมายในช่องที่ตรงกับความเห็นของท่ามากที่สุด

ด้านขวา

	แย่ลงมากที่สุด	แย่ลงมาก	แย่ลงปานกลาง	แย่ลงเล็กน้อย	ไม่เปลี่ยนแปลง	ดีขึ้นเล็กน้อย	ดีขึ้นปานกลาง	ดีขึ้นมาก	ดีขึ้นมากที่สุด
	-4	-3	-2	-1	0	+1	+2	+3	+4
สัปดาห์ที่ 2									
สัปดาห์ที่ 4									
สัปดาห์ที่ 6									
สัปดาห์ที่ 8									

ด้านซ้าย

	แย่ลงมากที่สุด	แย่ลงมาก	แย่ลงปานกลาง	แย่ลงเล็กน้อย	ไม่เปลี่ยนแปลง	ดีขึ้นเล็กน้อย	ดีขึ้นปานกลาง	ดีขึ้นมาก	ดีขึ้นมากที่สุด
	-4	-3	-2	-1	0	+1	+2	+3	+4
สัปดาห์ที่ 2									
สัปดาห์ที่ 4									
สัปดาห์ที่ 6									
สัปดาห์ที่ 8									

APPENDIX C

HYPERHIDROSIS DISEASE SEVERITY SCALE

Hyperhidrosis Disease Severity Scale	
"How would you rate the severity of your hyperhidrosis?"	
❑ 1.	My sweating is never noticeable and never interferes with my daily activities
❑ 2.	My sweating is tolerable but sometimes interferes with my daily activities
❑ 3.	My sweating is barely tolerable and frequently interferes with my daily activities
❑ 4.	My sweating is intolerable and always interferes with my daily activities

APPENDIX D

SIDE EFFECT

แบบสอบถามความ<u>อาการข้างเคียง</u>หลังการรักษา

No_____

โปรดทำเครื่องหมายในช่องที่ตรงกับความเห็นของท่ามากที่สุด

☐W2 ☐W4 ☐W6 ☐W8

อาการ	รักแร้**ขวา**					รักแร้**ซ้าย**				
	ไม่มี	มีเล็กน้อย	มีปานกลาง	มีมาก	มีมากสุด	ไม่มี	มีเล็กน้อย	มีปานกลาง	มีมาก	มีมากสุด
	0	+1	+2	+3	+4	0	+1	+2	+3	+4
ผื่น/ผด										
คัน										
ปวด										
แสบ										
บวม										
ตุ่มขุมขนอักเสบ										
ผิวแห้ง										
สีผิวหนังเข้มขึ้น										
ผิวขุยสะเก็ด										
ผิวไวต่อสิ่งกระตุ้น										

อาการผิดปกติอื่นๆ(โปรดระบุ).....

APPENDIX E

DERMATOLOGY QUALITY LIFE INDEX (DQLI)

จุดประสงค์ของแบบสอบถามนี้ เพื่อประเมินว่า ผื่นผิวหนังทำให้เกิดปัญหากับคุณมากน้อยเพียงใดในช่วงหนึ่งสัปดาห์ที่ผ่านมา? **กรุณาตอบคำถามโดยทำเครื่องหมาย ✔ ลงในช่องทางขวามือ (ขอความกรุณาตอบคำถามทุกข้อ)**		
1. ช่วงสัปดาห์ที่ผ่านมา คุณมีเหงื่อที่ออกบริเวณรักแร้ในขณะพัก มากน้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	
2. ช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้ทำให้คุณรู้สึกอับอาย, ขาดความมั่นใจ มาก น้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	
3. ในช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้ทำให้คุณมีปัญหาในการออกจากบ้านไปจับจ่ายซื้อสินค้า, ดูแลบ้าน หรือดูแลสวน มากน้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	□ ไม่มีความเกี่ยวข้อง
4. ช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้ของคุณ มีผลกระทบต่อการเลือกเสื้อผ้าที่จะสวมใส่ มากน้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	□ ไม่มีความเกี่ยวข้อง
5. ช่วงสัปดาห์ที่ผ่านมา ปริมาณเหงื่อที่ออกบริเวณรักแร้ของคุณ มีผลกระทบต่อการเข้าสังคม หรือต่อกิจกรรมในยามว่าง มากน้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	□ ไม่มีความเกี่ยวข้อง
6. ช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้มีผลกระทบต่อการเล่นกีฬาการออกกำลังกายของคุณ มากน้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	□ ไม่มีความเกี่ยวข้อง
7. ช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้มีผลทำให้คุณขาดงานหรือขาดเรียนหรือไม่	มี□ ไม่มีเลย □	□ ไม่มีความเกี่ยวข้อง
ถ้า “ไม่มี” ในช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้ทำให้มีคุณมีปัญหาในการทำงาน หรือ การเรียน มากน้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	
8. ช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้ของคุณ ได้สร้างปัญหาให้กับคู่ครอง หรือญาติหรือเพื่อนสนิท มากน้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	□ ไม่มีความเกี่ยวข้อง
9. ช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้ทำให้คุณมีปัญหาในการมีเพศสัมพันธ์ มากน้อยเพียงใด	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	□ ไม่มีความเกี่ยวข้อง
10. ช่วงสัปดาห์ที่ผ่านมา เหงื่อที่ออกบริเวณรักแร้ก่อให้เกิดปัญหาแก่คุณมากน้อยเพียงใด เช่น ทำให้มีการเปรอะเปื้อนในบ้าน, การรักษาทำให้เสียเวลา เป็นต้น	มาก □ ปานกลาง □ เล็กน้อย □ ไม่มีเลย □	□ ไม่มีความเกี่ยวข้อง

BIOGRAPHY

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