行政院國家科學委員會補助專題研究計畫成果報告
※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※
※ 肉毒桿菌素對肌膜激痛點治療效果之探討 (1/2) ※
※ The Study of Therapeutic Effect of Botulinum Toxin ※
※ Type A on Myofascial Trigger Point (1/2) ※ ※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：□個別型計畫  □整合型計畫
計畫編號：NSC 89－2314－B－006－179－
執行期間：89 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：官大紳
共同主持人：黃英儒

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

執行單位：國立成功大學醫學院復健學科

中華民國九十年五月十一日
The concept of multiple small loci in a myofascial trigger point (MTrP) has been wildly accepted based on recent animal and human studies. Recording of spontaneous electrical activity (SEA) from many active loci is an important objective finding to identify an MTrP in human or myofascial trigger point (MTrS) in rabbit. Therefore, Botulinum toxin type A (BTX-A), which inhibits muscle contraction by blocking the release of acetylcholine from peripheral nerve endings, may inactivate MTrP activity. This study is designed to assess the effect of BTX-A on SEA prevalence in rabbit MTrS in order to confirm the role of excessive acetylcholine release on the pathogenesis of MTrP. In our first year experiment, Eighteen New Zealand rabbits were divided into three groups (a single bolus of BTX-A 5u, BTX-A 10u, and BTX-A 15u) to delineate the dose effect of BTX-A on the SEA. Another 10 rabbits were grouped as receiving multiple point injection to delineate the different injection technique effect. It was found that injection of BTX-A 15u or BTX-A 10u, but not BTX-A 5u, significantly reduced the prevalence of
SEA. However, there was no significant difference in the effectiveness between the injection dose of 10u and 15u. No significant difference between single bolus injection and multiple point injection were noted, although there was a tendency that multiple point injection might maintain the SEA decreasing effect much longer than single injection. This study demonstrated the suppressive effect of BTX-A on SEA prevalence in an MTrP region. The effect of BTX-A on SEA in human MTrPs would be explored in our second year experiment. Prevalence of SEA in the MTrP region may be an useful objective indicator to evaluate the therapeutic effectiveness of BTX-A injection to treat MTrPs.

Key Words: Botulinum toxin type A, myofascial pain syndrome, myofascial trigger point, spontaneous electrical activity.

Background and Objective

Myofascial pain syndrome (MPS) is characterized by the existence of painful myofascial trigger point (MTrP). An MTrP is a highly localized hyperirritable spot in a palpable taut band of skeletal muscle fibers. It has been recommended that during MTrP injection the needle should be inserted into multiple sites in the entire region in order to eliminate tenderness in the entire MTrP region. Based on these observations, Hong had proposed a model of multiple small sensitive loci in an MTrP region. Hong and Torigoe developed an animal model and found it very useful in studying pathophysiology of MTrPs. The most sensitive spot to elicit a local twitch response (LTR) in a taut band in rabbit muscle was defined as a myofascial trigger spot (MTrS), which corresponds to the human MTrP in many aspects. Simons and colleagues found spontaneous and continuous low-amplitude action potentials (10 to 50 μV, occasionally up to 80 μV) in human MTrPs, which were similar to the observation of Hubbard and Berkoff. To be distinguished from the intermittent spike activity (100 to 600 μV, biphasic, initially negative), this continuous low-amplitude activity was originally defined as spontaneous electrical activity (SEA). The minute locus from which SEA can be recorded is defined as an active locus of an MTrP. Based on both human and animal studies, it has been further confirmed that SEA is actually endplate noise as described by electromyographers. Since Botulinum toxin type A (BTX-A) can inhibit muscle contraction by blocking the release of acetylcholine from peripheral nerves, it should block SEA, and thus, might inhibit MTrP or MTrS activity.

The introduction of BTX-A into clinical use started from early 1980s. Through binding irreversibly to presynaptic cholinergic nerve terminals, BTX-A blocks the exocytosis of the neurotransmitter, acetylcholine, and then inhibits muscle contraction. BTX-A has been approved by the Food and Drug Administration (FDA) in United States for the management of strabismus, blepharospasm, and hemifacial spasm in patients 12 years of age or older. However, there are only few studies regarding to the management of MPS with BTX-A, their sampled size were small, and most of the therapeutic effects were evaluated by subjective parameters, not by objective parameters.

Twenty-eight New Zealand rabbits (weight 3 ~ 5 Kg) were used for this study. They were divided into four groups: 6 rabbits in Group I injected with a single bolus of BTX-A 5u, 6 rabbits in Group II injected with a single bolus of BTX-A 10u, 6 rabbits in Group III injected with a single bolus of BTX-A 15u, and 10 rabbits in Group IV received multiple EMG-determined injections, each with a small dose of BTX-A into an active locus. An MTrS in the biceps femoris muscle of a rabbit was located and the prevalence of SEA before injection was assessed. A single bolus of BTX-A was injected into the MTrS in one side of the biceps femoris muscle for the rabbits of Group I-III. For the multiple injections in Group IV rabbits, a small dose of BTX-A
(1–2u) was injected into any locus in the MTrS region when SEA (EPN) was recorded from that locus. Control study (without BTX-A injection) was performed on the other side of biceps femoris muscle of the same animal by applying the same procedure as the experimental side except that no BTX-A was injected. For both experimental and control sides, the SEA (EPN) prevalence was assessed before injection and 3 weeks, 6 weeks, and 9 weeks after BTX-A injection.

The purpose of this study includes: (1) to assess the effect of BTX-A on SEA in rabbit MTrS. If effective, it would indicate that excessive acetylcholine release is a factor in the pathogenesis of MTrPs, (2) to develop an objective indicator of the effectiveness of BTX-A in the treatment of MTrPs. Our hypothesis is that BTX-A is capable to decrease the occurrence of SEA (EPN) in an MTrS region, which is an objective characteristics of an MTrS (in rabbits) or an MTrP (in human subjects). The results of three single doses of BTX-A and two injection techniques were compared.

Results and Discussion

Our study revealed that injection of BTX-A to an MTrP region could reduce the prevalence of SEA, which might be an objective evidence of therapeutic effect of BTX-A on MPS. Since SEAs were recorded more often from an MTrP region than from non-MTrP sites, SEA can be an objective indicator for the existence of an MTrP, and probably may be used as a reference for the degree of MTrP activity. This important finding supports Simons’ conclusion that SEA is abnormal endplate potentials due to excessive leakage of acetylcholine.

Our study showed that the effect on the decrease of SEA prevalence was significantly more in Groups II (BTX-A 10u) or III (BTX-A 15u) than in Group I (BTX-A 5u). We found no significant difference in the effects on SEA prevalence between Groups II and III (BTX-A 10u and 15u). This might be due to the small muscle volume of rabbit biceps femoris. However, it was apparent that the effect on the decrease in SEA prevalence was dose-dependent.

The multiple point injection strategy has been used empirically based on efficacy in an early clinical study. Since the size of the denervation field is largely determined by the dose and volume, multiple injections with a smaller volume for each point in the affected muscle might contain the better biologic effects of BTX-A within the boundaries of the target muscle. Unfortunately, we were unable to find any significant difference among single bolus injection with BTX-A 10u, BTX-A 15u, and multiple injections. Since the muscle volume of rabbit biceps femoris were small, and there were always around 5–6 active loci in an MTrS region, the total volume of BTX-A used for multiple injections (each active locus injected with 2u of BTX-A) was almost equal to that of BTX-A 10u and BTX-A 15u. It might be the reason why multiple injections did not provide more advantage than the single bolus injection procedure. However, we expected this SEA-guided multiple point injection would be more efficient for MPS patients, since the usual dose for single bolus injection for human muscle was 50–100u, which is higher than the dose needed for this multiple injection procedure.

Self-Assessment

In conclusion, injection of BTX-A significantly reduced and sometimes eliminated the prevalence of SEA in an MTrS region of rabbit skeletal muscle. This may be an useful objective indicator to evaluate the therapeutic effectiveness of BTX-A injection to treat MTrPs. However, it is necessary to further establish the correlation between the SEA prevalence in an MTrP region and the pain intensity of that MTrP, when applying the results of animal study to human study.
**References**


---

**Fig. 1.** Mean value of occurrence of spontaneous electrical activity (SEA) among different groups of botulinum toxin type A injection (BTX-A 5u, BTX-A 10u, BTX-A 15u, BTX-A multiple point injection).
# 國立成功大學國科會專題計畫下出席國際會報告

(參考格式)

<table>
<thead>
<tr>
<th>報告人姓名</th>
<th>官大緯</th>
<th>服務機構及現職</th>
<th>國立成功大學醫學院復健學科系所</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>時間、會場</th>
<th></th>
<th>計畫編號</th>
<th>89-2314-B-006-179-</th>
</tr>
</thead>
<tbody>
<tr>
<td>自 90 年 9 月 9 日至 90 年 9 月 13 日</td>
<td>美國、俄勒岡州、波特蘭港市</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>會議名稱</th>
<th>(中文) 第五屆肌筋膜疼痛與纖維肌痛症世界大會</th>
<th>(英文) Fifth World Congress on Myofascial Pain and Fibromyalgia</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>發表論文題目</th>
<th>(中文)肉毒桿菌素對於兔子骨髄肌上肌筋膜肌痛點裡自發性電位活動的效應</th>
<th>(英文) THE EFFECTS OF BOTULINUM TOXIN ON THE SPONTANEOUS ELECTRICAL ACTIVITY IN MYOFASCIAL TRIGGER SPOTS OF RABBIT SKELETAL MUSCLE.</th>
</tr>
</thead>
</table>

報告內容應包括下列各項：
一、參加會議經過
二、與會心得
三、考察參觀活動（無事項活動者省略）
四、建議
五、檔案資料名稱及內容
六、其他
出席國際會議報告

第五屆肌筋膜疼痛與纖維肌痛症世界大會

國立成功大學醫學院
醫學系復健學科講師 官大紳

一、參加會議經過

第五屆肌筋膜疼痛與纖維肌痛症世界大會(The 5th World Congress on Myofascial Pain and Fibromyalgia: 簡稱 MYOPAIN'01)於2001年9月9日至9月13日在美國俄勒岡州的波特蘭市(Portland)舉行。這個國際性會議每隔三年舉辦一次，已先後在美國、丹麥、與義大利舉辦過四屆，對於整合肌肉軟組織疼痛在生理學、神經醫學、疼痛醫學、風濕免疫學，以及復健醫學方面的研究進展，具有重要的影響地位。本部前主任洪春仁教授在肌筋膜疼痛症激痛點(Myofascial Trigger Point)方面研究卓然有成，近幾年來更是積極指導部內同仁從事這方面的研究，因此我與陳若佟醫師此次皆有關於激痛點方面的論文要發表。另外，本人剛剛於今年七月底結束为期一年在美國德州大學與 Dr. Russell的研究，Dr. Russell 是纖維肌痛症(Fibromyalgia)世界知名的大師，他本身是 Journal of Musculoskeletal Pain 的主編，也是主辦此次大會 International Myopain Society 的主席。在他的指導之下，我也有兩篇關於 Fibromyalgia 的論文要發表，而且是以口頭報告方式發表，使得此次會議對我來說特別具有意義。

開幕當天(9月9日)，在簡單而隆重的開幕式後，由義大利的學者 Leonardo Vecchiet 做 President’s address，講題是 Muscle pain and aging。會後在 welcome reception 裡，遇到去年在 San Francisco 認識的紐約大學退休教授 Fischer，他在 myofascial pain syndrome 也是知名的學者，其獨創的 segmental sensitization 理論，以及特殊的 myofascial trigger
point 的注射方法，更使他获邀担任 Physical Medicine and Rehabilitation Clinics of North America 雜誌 1997 年二月號的單期主编。自從去年相互認識以來，我們即經常透過 e-mail 彼此聯絡。雖然他的論點和洪教授、及洪的老師 Dr. Simons 對於 myofascial trigger point 的見解不大相同，且彼此仍舊在論戰當中，但是 Dr. Fischer 總是毫不吝惜地把他的論文與著作送給我，令我深受感動！此次異地再度相逢，自是格外欣喜，Dr. Fischer 邀我共進晚餐，席間也認識了來自加拿大的知名復健大師 Dr. Dubo，彼此也交換了研究心得。

9月10日的會議主題是 Neurobiology of central sensitization，討論者多為生理學與神經學方面的學者。不同於以往的是，此次大會將重點擺在與疼痛有關的基礎神經生理學，與較先進的基因學，令與會者感到新鮮，卻又感到些許的陌生。下午是論文的口頭報告發表，主要是各個學術單位關於 myofascial pain 與 fibromyalgia 的治療成果報告；晚上則還有另一場專題演講，關於 5-HT 接受拮抗劑，與生長激素應用在 Fibromyalgia 的研究成果發表。

9月11日，會議的主題是 Regional pain syndrome。這一天本來對我是別具意義的，因為這是我第一次在國際的學術會議上做口頭報告方式的論文發表。在整個大會主持選的22篇口頭報告論文當中，Dr. Russell 所指導我完成的論文就佔了兩篇；而我獲得國科會計劃補助所完成的論文，也在今天的中午到下午的時段，以壁報的方式來展示發表，總計此次大會裡我共有三篇論文發表，Dr. Russell 相當滿意我的成就。然而，當我們在吃早餐的時候，飯店的人員特別把電視機搬了出來，看到紐約世貿大樓著火的畫面，接著是一架飛機對著大樓迎面撞上，在熊熊的烈焰當中，兩棟世界聞名、紐約地標的摩天大樓竟然整個倒塌下去，全場的人都被這驚悚的畫面所震懾住！這是一個多麼大的消息！恐怖份子攻擊美國！America under attack！世貿大樓不見了！四架飛機墜毀了！多少人的性命在這傾刻之間化為烏有？我們好像都已經可以嗅到，一股巨大的風暴即將到來！以後的人類歷史，勢必因爲今天的恐怖分子攻擊事件，產生極大的影響與變化！後世的子孫孫孫，也就應該會牢牢地記住今天這個日子：2001年9月11日！

我的口頭報告被排在下午2點整，兩篇論文的題目是：Discrimination of fibromyalgia patients from normal controls using the levels of cerebrospinal chemicals。以及 Correlations of clinical variables with cerebrospinal chemical levels among fibromyalgia patients and healthy normal controls。這兩篇論文所探討的重點，是將 Dr. Russell 多年來在 fibromyalgia 所做的研究，做一歸納性的研究。Fibromyalgia syndrome
是一個全身性疼痛的症候群，它常伴隨著疲勞、失眠、全身酸痛、憂鬱、焦慮、排便與膀胱障礙等多種症狀。雖然它多半是以肌肉疼痛為主要表徵，然而多年來的研究仍舊無法在肌肉組織裡找到任何病灶性的證據。近年來學界已逐漸將研究重點擺向中樞神經方面，各種與疼痛傳導有關的神經介質（nociceptive neurotransmitters），包括：serotonin、substance P、5 hydroxyindole acetic acid...，都已被證明在 fibromyalgia 患者的身上有不正常的數值發生。我的第一篇研究，是將 Dr. Russell 所收集的 300 多位 fibromyalgia 患者腦脊髓液(CSF)裡的各種神經介質，運用多變異項、複迴歸的統計方式，歸納出一個可以區分出 fibromyalgia 患者與正常人的神經介質多項式方程式。第二篇的研究主題，則是將臨床上用來分析 fibromyalgia 的十幾項臨床指標，運用因素分析(factor analysis)的方法，將這些臨床指標归纳分类为四个因素；再將這四個因素與第一篇研究所建立出来的方程式，做相關性的分析，以驗證這個方程式的區分 fibromyalgia 患者與正常人的準確度。口頭報告完後，共有四位聽者提出問題討論，顯然大家對於這個議題感到很有興趣。

我得到國科會計劃補助發表的論文，也被安排在同一天下午以壁報的方式發表，題目是：The effects of botulinum toxin on the spontaneous electrical activity in myofascial trigger spots of rabbit skeletal muscle。這是運用洪教授在 myofascial trigger point 所建立的理論基礎，拿來驗證肉毒桿菌素(botulinum toxin)治療 myofascial pain syndrome 的效果。肉毒桿菌素才剛剛引進台灣，藥價非常貴，其藥理的治療機轉，非常適合拿來治療 myofascial trigger point，但是其臨床真正的治療效果，仍有待我們進一步深入的研究探討。利用這次國際性的會議，能有機會與國外的學者交換 botulinum toxin 治療的經驗，真是獲益良多。

完成論文的報告，緊張的心情總算獲得緩解。大會在這天晚上安排了渡輪晚宴，坐著豪華的郵輪，沿著波特蘭市邊的河流，享用著豐盛的晚餐與音樂，的確是一次很美的體驗。在筵席上我認識了來自韓國高麗大學的教授 Dr. Kang，他也曾在 Dr. Russell 那裡研究了一年半，我們可說是有師兄弟的關係，又同樣是來自亞洲對 myofascial pain 有興趣的學者，所以聊起來特別感到親切，也建立了良好而深刻的友誼，相約共同為亞洲的肌肉疼痛努力！

9 月 12 日的會議主題是 Chronic widespread pain，討論的範圍集中在 fibromyalgia 及其所伴隨的相關症狀。下午還是論文的口頭報告，還有一場關懷世界各地受刑人被凌虐的報告，也讓我們見識到另一層面的“痛”了。
9月13日的會議主題是兩場 myofascial pain 與 fibromyalgia 的 workshop。Myofascial pain 的主講者是來自加拿大的 Dr. Gunn，他本身也是華人，看起來還很年輕，卻已是七十多歲了！剛從華盛頓大學教授退休。他演講的用詞非常幽默，長惹得全場哈哈大笑！不過他對觀念的闡述，卻是相當清晰而深入，實在是一位令人敬佩的大師！

由於911事件之故，美國全國機場都閉關，所有班機都取消！大家都為著如何回家而傷透腦筋，整個大會就在這種慌亂的氣氛下結束！

（後記：我們原訂從西雅圖回台灣的班機被取消，要等七天上才排得到位子！幸好得到洪教授之助，租到車子開十七小時開到洛杉磯，再等了三天，才排到機位順利回到台灣！）

二、與會心得

本次大會總計有來自世界各個國家，約300多位學者參加，是一次成功而又盛大的會議。從同一個主題，聽取不同國家對其研究的心得與經驗，不僅在知識層面上獲得啓發，更親身體會到肌肉疼痛醫學已在世界各地蓬勃地發展壯盛起來；讓人有知己知彼，互相學習成長的喜悅。

在肌筋膜疼痛症激痛點的學術領域上，本部在前主任，目前也是美國加州大學爾灣校區復健科副教授的洪章仁醫師領導下，其研究水準可說是獨步全球，世所公認。在洪教授的指導之下，我們積極地從事肌筋膜激痛點的動物實驗，並與本校的病理部、解剖學科、與醫學工程研究所展開充分的合作。基於洪教授的激痛點多發性敏感點的理論基礎之上，我們繼續探討激痛點與神經肌肉間終板的關聯性，及其它們所發展出的治療方法，初步所獲得的一些重要的研究成果。這次能在此世界性的會議上發表，我們的努力也獲得了大家的肯定。而我有幸能得到 Dr. Russell 的指導，將研究的觸角更擴展到 fibromyalgia 的領域裡，兩者皆是肌肉疼痛症的重要課題，相信將來必能在此一領域的研究裡有所進展！

在本次大會中，我們也遇到了來自國內的學者：如台大醫院復健科的劉榮倫醫師，以及世界各國專研疼痛理論與治療的學者，如：Dr. Russell, Dr. Fischer, Dr. Gunn, Dr. Kanf...，對於將來在研究上學術的討論，與經驗的交流，實在助益良多！
三、建議事項

1. 吸取各國的經驗，並聯合各相關學科之專家學者，致力從事肌肉疼痛症之基礎性與臨床性研究，以趕上世界潮流。

2. 聯合各相關醫學團隊，並呼籲衛生主管機關，以及社會大眾，重視肌膜疼痛症的發生與預防治療，以減少病患的痛苦並增進其生活品質。

四、攜回資料名稱及內容

本次大會各研討會議程與論文摘要，包括：

- Final Program and Abstracts of Plenary Sessions of Fifth World Congress on Myofascial Pain and Fibromyalgia。
The Effect of Botulinum Toxin on the Spontaneous Electrical Activity in Myofascial Trigger Spots of Rabbit Skeletal Muscle

Ta-Shen Kuan, MD, MS, Shu-Min Chen, MD, Jo-Tong Chen, MD,
Chi-Hsien Chien PhD*, Chang-Zern Hong, MD**.

Department of Physical Medicine and Rehabilitation, Department of Anatomy*
College of Medicine, National Cheng Kung University, Tainan, Taiwan, R.O.C.;
Department of Physical Medicine and Rehabilitation,
University of California Irvine, California, U.S.A**.

Supported by National Science Council (Taiwan) Grant NSC-89-2314-B-006-179.

Correspondence to: Ta-Shen Kuan, MD, MS, Department of Physical Medicine and Rehabilitation, National Cheng Kung University Hospital, No.138, Sheng-Li Road, Tainan, 704, Taiwan, R.O.C.
TEL: 011-886-6-276-6606 (O), 011-886-6-264-2789 (H)
FAX: 011-886-6-276-6106
E-mail: kuantashen@hotmail.com

RUNNING HEAD: Botox on SEA in Myofascial Trigger Spot
Meeting presentation: Part of the material was contained within a presentation at 2000 Annual Meeting of Rehabilitation Medicine Association, Republic of China. December 16-17, 2000. Taipei, Taiwan.
The Effect of Botulinum Toxin on the Spontaneous Electrical Activity in Myofascial Trigger Spots of Rabbit Skeletal Muscle

ABSTRACT

Objective: To assess the effect of Botulinum toxin type A (BTX-A) on spontaneous electrical activity (SEA) prevalence in rabbit myofascial trigger spot (MTrS), equivalent to a human myofascial trigger point (MTrP), in order to confirm the role of excessive acetylcholine release on the pathogenesis of MTrP and to develop an objective indicator of the effectiveness of BTX-A in the treatment of MTrPs.

Design: Eighteen adult New Zealand rabbits were divided into three groups receiving a single bolus of BTX-A 5u (Group I), BTX-A 10u (Group II), and BTX-A 15u (Group III) respectively over an MTrS region in one side of biceps femoris muscle. Another 10 rabbits were grouped as Group IV receiving multiple point injection in an MTrS where SEAs were found. Control study was performed on the other side of biceps femoris muscle by applying the same procedure except that no BTX-A was injected. The SEA prevalence in an MTrS region was assessed before injection and 3 weeks, 6 weeks, and 9 weeks after BTX-A injection.

Results: It was found that injection of BTX-A 15u or BTX-A 10u, but not BTX-A 5u, significantly reduced the prevalence of SEA. However, there was no significant
difference in the effectiveness between the injection dose of 10u and 15u. No significant difference between single bolus injection and multiple point injection was noted, although there was a tendency that multiple point injection might maintain the SEA decreasing effect much longer than single injection.

**Conclusions:** This study demonstrated the suppressive effect of BTX-A on SEA prevalence in an MTrS region. This supports the theory that SEA may be abnormal endplate potentials due to excessive leakage of acetylcholine. Prevalence of SEA in the MTrP region may be a useful objective indicator to evaluate the therapeutic effectiveness of BTX-A injection to treat MTrPs.

**Key Words:** Botulinum toxin type A, Myofascial pain syndrome,

Myofascial trigger point, Spontaneous electrical activity.
INTRODUCTION

Myofascial pain syndrome (MPS) is one of the most common muscle pain problems noted in clinical practice. It is characterized by the existence of a painful myofascial trigger point (MTrP). An MTrP is a highly localized hyperirritable spot in a palpable taut band of skeletal muscle fibers\textsuperscript{1,2}. Important clinical characteristics of MTrPs include referred pain and a local twitch response (LTR). An LTR, a brisk contraction of a group of muscle fibers in a taut band containing a trigger point, can be identified either by palpation or by vision when an MTrP is mechanically stimulated by snapping palpation or needling\textsuperscript{3}.

Taut bands, similar to those in human muscle, have been identified by finger palpation in rabbit biceps femoris\textsuperscript{4}. When a sensitive site in a taut band of rabbit skeletal muscle was stimulated mechanically with a needle or by a blunt metal probe (snapping or tapping), LTRs could be elicited\textsuperscript{4}. Rabbit LTRs are similar to human LTRs both in the characteristic of a visible muscle twitching and in their electromyographic (EMG) appearance\textsuperscript{5}. The most sensitive spot to elicit an LTR in a taut band in rabbit muscle was defined as a myofascial trigger spot (MTrS), which corresponds to the human MTrP in many aspects\textsuperscript{4}. When the rabbit is awake and its MTrS is squeezed, the rabbit usually responds to the noxious stimulation as if it suffers
pain or discomfort. Based on these observations, Hong and Torigoe developed an animal model and found it very useful in studying pathophysiology of MTrP\(^4\). It has been recommended that during MTrP injection the needle should be inserted into multiple sites in the entire region in order to eliminate tenderness in the entire MTrP region\(^1\). Hong had also proposed a model of multiple small sensitive loci in an MTrP region\(^6,7\). Similar to human MTrP, the multiple loci hypothesis could also be applied in this animal model based on the fact that multiple needle insertions into an MTrS could elicit multiple LTRs\(^4\).

Simons and colleagues found spontaneous and continuous low-amplitude action potentials (10 to 50 µV, occasionally up to 80 µV) in human MTrPs\(^8,10\), which were similar to the observation of Hubbard and Berkoff\(^11\). To be distinguished from the intermittent spike activity (100 to 600 µV, biphasic, initially negative), this continuous low-amplitude activity was originally defined as spontaneous electrical activity (SEA)\(^8,10\). The minute locus from which SEA can be recorded is defined as an active locus of an MTrP\(^12\). Based on both human and animal studies\(^8,10,12,13\), it has been further confirmed that SEA is actually abnormal endplate noise as described by electromyographers\(^14\). The association between SEA and MTrS or MTrP is now much clearer\(^9,11,13\). Since Botulinum toxin type A (BTX-A) can inhibit muscle contraction by blocking the release of acetylcholine from peripheral nerves\(^15\), it should block SEA,
and thus, might inhibit MTrP or MTrS activity.

The introduction of BTX-A into clinical use started in the early 1980's. Through binding irreversibly to presynaptic cholinergic nerve terminals, BTX-A blocks the exocytosis of the neurotransmitter, acetylcholine, and then inhibits muscle contraction. This chemically denervated skeletal muscle remains paralyzed until the motor nerve supplying it sprouts new axons and forms new synaptic contacts to re-establish the neuromuscular junction. Thus, the muscle-paralyzing effectiveness of BTX-A generally lasts 3 to 4 months. Toxin potency is expressed in mouse units, 1 unit (u) representing the estimated median lethal dose (LD₅₀) for 18-20 g female Swiss-Webster mice, which is equal to approximately 0.4 ng of BTX-A. BTX-A has been approved by the Food and Drug Administration (FDA) in the United States for the management of strabismus, blepharospasm, and hemifacial spasm in patients 12 years of age or older. Furthermore, the results of clinical investigation support the use of BTX-A in the management of torticollis, cerebral palsy and many focal dystonic and nondystonic disorders of muscle spasm. However, there are only a few studies regarding the management of MPS with BTX-A. The sample size was small, and most of the therapeutic effects were evaluated by subjective parameters, not by objective parameters.

The purpose of this study includes: (1) to assess the effect of BTX-A on SEA
in rabbit MTrS. If effective, it would indicate that excessive acetylcholine release is a factor in the pathogenesis of MTrPs, (2) to develop an objective indicator of the effectiveness of BTX-A in the treatment of MTrPs. We used an animal model to study the role of BTX-A on SEA in the MTrS region. Our hypothesis is that BTX-A is capable of decreasing the occurrence of SEA in an MTrS region, which may be an objective indicator of an MTrS (in rabbits) or an MTrP (in human subjects). The results of three single doses of BTX-A and two injection techniques (single bolus vs. multiple point injection) were also compared.
MATERIAL AND METHODS

General Design

Twenty-eight New Zealand rabbits (weight 3 ~ 5 Kg) were used for this study. They were divided into four groups: 6 rabbits in Group I injected with a single bolus of BTX-A 5u, 6 rabbits in Group II injected with a single bolus of BTX-A 10u, 6 rabbits in Group III injected with a single bolus of BTX-A 15u, and 10 rabbits in Group IV received multiple EMG-determined injections, each with a small dose of BTX-A (about 1–2u) into an active locus (Fig. 1). An MTrS in the biceps femoris muscle of a rabbit was located and the prevalence of SEA before injection was assessed. A single bolus of BTX-A was injected into the MTrS in one side of the biceps femoris muscle for the rabbits of Group I–III. For the multiple injections in Group IV rabbits, a small dose of BTX-A (1–2u) was injected into any locus in the MTrS region when SEA was recorded from that locus. A control study to rule out the needling effect on SEA was performed on the other side of biceps femoris muscle of the same animal by applying the same procedure as the experimental side except that no BTX-A was injected. For both experimental and control sides, the SEA prevalence was assessed before injection and 3 weeks, 6 weeks, and 9 weeks after BTX-A injection. All the procedures were in accordance with our college’s committee on animal experimentation.
Animal Preparation

The rabbit was first anesthetized with an intramuscular injection of Ketamine 0.05mg/gBW. Subsequent intravenous injections of Thiopentone Sodium at 0.0lg/ml were given every 20–30 minutes to maintain the anesthetic level. The anesthetic level was controlled so that most of the spinal reflexes were preserved. The rabbit was fixed on an immobilization board which was placed on a heating pad to maintain a constant body temperature. The rabbit was also monitored for heart rate and respiration to avoid overdose of anesthetics. The skin of the lateral thigh was incised to expose the biceps femoris muscle and it was separated posteriorly from the semimembranosus muscle. This made it possible to slip a finger beneath the muscle for pincer palpation of the muscle fibers when searching for taut bands.

Identification of a Myofascial Trigger Spot (MTrS)

The biceps femoris muscle was grasped between the fingers and was palpated by gently rubbing to find a taut band. The fibers of the taut band are unmistakably firmer in consistency than the surrounding muscle such that the band would be snapped between the fingers and was felt like a clearly delineated “rope” of muscle fibers roughly 2–3 mm or more in diameter. The location along the band where snapping palpation produced the most vigorous localized twitch response was
identified as the myofascial trigger spot (MTrS).

**Electromyographic Recordings**

A 2-channel Viking IV EMG unit (Nicolet, Madison, WI) was used to assess the SEA prevalence. The high-cut frequency filter was set at 1,000 Hz and the low-cut at 100 Hz. The gain was generally set at 20 μV per division for recordings. At the usual sweep speed of 10 ms per divisions, one screen presented 100 ms of record. Intramuscular electrical activity was recorded using 25-mm, disposable, monopolar Teflon-coated EMG needle electrodes for EMG recording. The needle electrode used to search SEA (active recording electrode) was connected to channel 1 of the preamplifier box of the EMG unit. The control needle electrode, which was inserted into a normal muscle tissue (non-taut band, non-MTrS), was connected to channel 2. A clip used for the surface electrode was attached onto the nearby skin. It served as the common reference electrode by connecting it to both channels through a "Y" connector. The ground electrode was clipped to another site of nearby skin. Room temperature was maintained at 21 ± 1 °C.

**Assessment of SEA prevalence**

To search an active locus (SEA locus) where SEA could be recorded, the
active recording electrode was inserted at an angle of approximately $45^\circ \sim 60^\circ$ into the surface of the muscle, and parallel to the direction of the muscle fibers into the region of the MTrS. After the initial insertion to a point just short of the depth of the MTrS, the needle was advanced very slowly. To prevent the needle from 'grabbing' the tissue and releasing the tissue suddenly to advance in a large jump, each advance was made through the least possible distance (usually 1–2 mm for one advancement) by simultaneously rotating the needle to facilitate smooth entry through the muscle tissue. Large advances should be avoided because of the minute size of an active locus and the likelihood of inducing a* rabbit LTR instead of finding a locus of SEA. After 8 advancements of the needle in each track, the needle was pulled out and was reinserted into the next track in the muscle, 1 mm next to the previous track. Eight tracks were investigated in one MTrS region (Figure 2). When the needle approached an active locus, the continuous distant electrical activity was heard. A site was an active locus when SEA was identified if 1). endplate noise- like potentials persisted continuously for more than three screens (300 ms); 2). the potentials had an amplitude of $> 10 \, \mu V$ (which was more than twice the instrumentation noise level of $4 \, \mu V$ that was observed in control recordings taken at the beginning and upon completion of each track); and 3). the adjacent control channel was not recording potentials greater than instrumentation noise level. Once the active locus was localized, the needle remained there without
further movement, and the SEA was stored for later analysis. Then, the investigator continued searching for another SEA. The total number of SEAs found in 8 needling tracks was recorded.

**Preparation of BTX-A**

Before use, BTX-A was reconstituted with sterile, unpreserved normal saline (0.9% sodium chloride for injection) into the required concentration of 100 units/ml. The diluent (normal saline) was injected gently, and the powder mixed with it by gentle and slow rotation of the vial to produce a clear, colorless solution. Violent agitation and bubbling might denature BTX-A and should be avoided. If the diluent was not pulled into the vial by a vacuum, the vial must be discarded. After reconstitution, BTX-A might be stored in a refrigerator (2–8°C) for up to 4 hours before use.

**Injection of BTX-A**

After pre-injection assessment of SEA prevalence, rabbits in Group I–III received a single bolus injection of BTX-A into the MTrS region with a dosage of 5u, 10u, and 15u, respectively. Group IV rabbits were injected with BTX-A by multiple injection procedure. To perform this procedure, a specialized hypodermic needle electrode (Allergan, Irvine, CA), connected to EMG recording channel and also
connected to a syringe containing BTX-A, was used for both SEA searching and BTX-A injection. This needle was moved gently to search for SEA using the same technique for SEA searching noted above. Approximately 1~2u of BTX-A was injected through this needle whenever an SEA was found. To rule out the effect of needling on SEA, the biceps femoris muscle in the contralateral limb was served as a control study. The same procedures for MTrS identification and SEA searching were performed, but no injection was given to this control muscle.

After BTX-A injection, the wounds were sutured, and the rabbit was kept alive. Similar procedures for SEA searching in MTrS region were performed 3, 6, and 9 weeks after BTX-A injection.

**Data Analysis**

The prevalence of SEA (total number of SEA noted during these 8-needle-track searches) in different groups (BTX-A 5u, BTX-A 10u, BTX-A 15u, BTX-A multiple point injection) were recorded for analysis. Data were also grouped by time interval as pre-injection group, post-injection 3-week group, post-injection 6-week group, and post-injection 9-week group. Differences of SEA prevalence between time intervals were evaluated by Wilcoxon Matched Pairs test. Mann-Whitney U test was applied to analyze differences among groups with different dosage. Statistical
significance was confirmed if $P < 0.05$. 
RESULTS

The results of our study revealed that BTX-A decreased the prevalence of SEA in an MTrS region of rabbit. Five rabbits (one in Group I, one in Group III, three in Group IV) expired during the experiment course, mostly around 6 weeks after BTX-A injection. In Group I (BTX-A 5u injection) (Fig. 3), there was no significant difference between the pre-injection SEA prevalence and post-injection SEA prevalence 3 weeks, 6 weeks, or 9 weeks after injection (P>0.05). However, the most remarkable effects of the decrease in SEA were noted during the first 3 weeks after BTX-A injection. Regarding Groups II–IV (Figs. 4–6), significant differences (P<0.05) between pre-injection SEA prevalence and post-injection SEA prevalence at 3 weeks, 6 weeks, or 9 weeks were found. The most prominent effects of the decrease in SEA were also noticed in the first 3 weeks after BTX-A injection in these three groups. Most of the SEA prevalence in Group II (BTX-A 10u) and Group III (BTX-A 15u) began to increase again 3 weeks after injection (Fig. 4–5), while that of Group IV (multiple injections) mostly did not start to increase until 6 weeks after injection (Fig. 6). If we took the mean value of SEA prevalence in each time interval, the effects of the decrease in SEA seemed to persist much longer in Groups IV than the other groups (Fig. 7). Significant differences (P<0.05) between mean values of pre-injection SEA
prevalence and post-injection SEA prevalence at 3 weeks, 6 weeks, or 9 weeks were also found in Groups II–IV, but not in Group I. However, there was no statistically significant difference in SEA prevalence among Group II (BTX-A 10u), Group III (BTX-A 15u), and Group IV (BTX-A multiple injections). The SEA prevalence of Group I (BTX-A 5u) was significantly different from that of Group II, Group III, and Group IV.

In the control (contralateral) sides, there were no significant differences among data (SEA prevalence) obtained at different time points in all groups (Fig. 3–6). This might suggest that there was little effect of needling on SEA prevalence.
DISCUSSION

Acquadro and Borodic first described the therapeutic effectiveness of BTX-A on myofascial pain syndrome (MPS) patients based on a study of only two patients\textsuperscript{23}. Cheshire et al\textsuperscript{18} conducted a randomized, double-blind, placebo-controlled study of BTX-A in patients with chronic MPS involving cervical paraspinal and shoulder girdle muscles. Measured by visual analog scales, verbal descriptions of pain intensity and unpleasantness, palpable muscle firmness, and pressure pain thresholds, four of six patients experienced significant reduction in pain of at least 30% following BTX-A, but not saline, injections. Wheeler et al recruited thirty-three patients and divided them randomly to receive either 50 or 100 units of BTX-A, or normal saline\textsuperscript{24}. Patients were re-evaluated over a 4-month period and then offered a second injection of 100 units of BTX-A. All three groups showed significant treatment effects as measured by a decline in the scores on the Neck Pain and Disability Visual Analogue Scale and an increase in the pressure algometer scores. However, no statistically significant benefit of BTX-A over placebo was demonstrated in this study. With the high incidence of patients who were asymptomatic after a second injection, it was suggested that further research is needed to determine whether higher dosage and sequential injections in a larger cohort might show a BTX-A effect. Porta\textsuperscript{26} recruited 40 patients with MPS and
20 patients with tension-type headache. Each patient received injection of either BTX-A or methylprednisolone, and their pain was evaluated by standard visual analogue scale at baseline, 30 and 60 days after injection. He concluded that BTX-A produced a more prolonged pain relief than methylprednisolone. The above mentioned studies were mostly measured by patients’ subjective symptoms. Larger sample-sized studies with objective indicators to assess the therapeutic effectiveness of BTX-A on MPS patients are still lacking.

Since SEAs were recorded more often from an MTrP region than from non-MTrP sites\textsuperscript{4,13}, SEA can be an objective indicator for the existence of an MTrP, and probably may be used as a reference for the degree of MTrP activity. Our study revealed that injection of BTX-A into an MTrS region could reduce the prevalence of SEA, which might be objective evidence of a therapeutic effect of BTX-A on MPS. This important finding also supports Simons’ conclusion that SEA is abnormal endplate potentials due to excessive leakage of acetylcholine\textsuperscript{8}. It had been documented that the waveforms of SEA are closely similar to the endplate potentials\textsuperscript{14}. Wiederholt\textsuperscript{27} demonstrated that EMG activity (similar to SEA) was probably endplate potentials based on electrophysiological, histological, and pharmacological studies. Previous animal studies\textsuperscript{28,29} indicated that EMG activity (similar to SEA) could not be recorded from a normal endplate but only from an abnormal endplate which had been
disturbed mechanically or biochemically. A recent histological study on rabbit skeletal muscle revealed that small nerve fibers were found in the vicinity of the needle recording site with iron deposition. Therefore the SEA locus in an MTrS region is probably related to abnormal endplate potentials in the motor endplate region. The abnormal endplate potentials (SEA) could induce sensitization of nociceptors and cause pain in the MTrP region.

Our study showed that the decrease of SEA prevalence was significantly more in Groups II (BTX-A 10u) or III (BTX-A 15u) than in Group I (BTX-A 5u). Previous animal studies have demonstrated that BTX-A can produce a gradient of denervation in a given muscle and that both the magnitude of denervation and the extent of the gradient were dose dependent. In our study, we found no significant difference in the effects on SEA prevalence between Groups II and III (BTX-A 10u and 15u). This might be due to the small muscle volume of rabbit biceps femoris. However, it was apparent that the decrease in SEA prevalence was dose-dependent. It is the matter of concern to establish the optimal therapeutic dose for each individual muscle. Over dosage should be avoided to eliminate the side effects of BTX-A injection. The principle side effect of BTX-A overdose is weakness in the muscle injected. Muscular weakness and even death were noted in an animal study with BTX-A dose beyond 15u. Complication of dysphagia in the treatment of spasmodic torticollis by BTX-A
had also been reported\textsuperscript{33}.

The multiple point injection strategy has been used empirically based on efficacy in an early clinical study\textsuperscript{34}. Not only the injection sites, but also the diffusion of BTX-A to contiguous muscles may affect the effectiveness of BTX-A injection in clinical practice. Since the size of the denervation field is largely determined by the dose and volume, multiple injections with a smaller volume for each point in the affected muscle might contain the better biologic effects of BTX-A within the boundaries of the target muscle\textsuperscript{35}. In the treatment of adult onset spasmodic torticollis, Borodic et al\textsuperscript{35} found that multiple point injection strategy appeared superior to the single injection per muscle technique with respect to pain, posture deformity, range of motion, and improvement in activity endurance. Measured by tarsal joint forces, Childers et al\textsuperscript{36} demonstrated that injection at motor end-plates of dogs targeted by EMG potentiated the effects of BTX-A. However, the clinical effects with only single bolus injection into the motor point appeared to be worse than multiple injections in the management of blepharospasm\textsuperscript{37}. The fact that the distribution of neuromuscular junctions in the muscle is diffuse should be kept in mind\textsuperscript{37}. Our study tried to analyze the effects of BTX-A on SEA prevalence in MTrSs between single bolus injection and multiple point (SEA guided) injection. Unfortunately, we were unable to find any significant difference among single bolus injection with BTX-A 10u, BTX-A 15u, and
multiple injections. Since the muscle volume of rabbit biceps femoris were small, and there were always around 5~6 active loci in an MTrS region, the total volume of BTX-A used for multiple injections (each active locus injected with 1~2u of BTX-A) was almost equal to that of BTX-A 10u and BTX-A 15u. It might be the reason why multiple injections did not provide more advantage than the single bolus injection procedure. However, we expected that SEA-guided multiple point injection would be more efficient for MPS patients, since the usual dose for single bolus injection for human muscle was 50~100u, which is higher than the dose needed for the multiple injection procedure.

In conclusion, injection of BTX-A significantly reduced the prevalence of and sometimes eliminated SEA in an MTrS region of rabbit skeletal muscle. Since SEA appears to be characteristic of the MTrP of human subjects, injection of BTX-A into human MTrPs would be expected to inactivate the MTrPs and relieve the clinical symptoms associated with it. It is suggested that SEA prevalence may be served as an objective indicator to assess the therapeutic effects of BTX-A on human's MTrPs in the future study. However, it is necessary to further establish the correlation between the SEA prevalence in an MTrP region and the pain intensity of that MTrP, when applying the results of animal study to human study.
REFERENCES


26. Porta M. Botulinum toxin type A injections for myofascial pain syndrome and

27. Wiederholt WC. ‘End-plate noise’ in electromyography. Neurology 1970; 20:214-
24.


29. Liley AW. An investigation of spontaneous activity at the neuromuscular junction

30. Hong CZ, Chen JT, Chen SM, Kuan TS. Sensitive loci in a myofascial trigger point
region are related to sensory nerve fibers [abstract]. Am J Phys Med Rehabil
1997;76:172.

diffusion and muscle fiber response after therapeutic botulinum A toxin injections.


32. Shaari CM, Sanders I. Quantifying how location and dose of botulinum toxin

treatment of spasmodic torticollis: Dysphagia and regional toxin spread. Head


Figure Legends

Fig. 1. General design.

Fig. 2. Search for a SEA locus an MTrS region in rabbit biceps femoris.

Fig. 3. The occurrence of spontaneous electrical activity (SEA) before and after botulinum toxin type A (BTX-A) 5u injection.

* Mean SEA prevalence of control (contralateral) side.

Fig. 4. The occurrence of spontaneous electrical activity (SEA) before and after botulinum toxin type A (BTX-A) 10u injection.

* Mean SEA prevalence of control (contralateral) side.

Fig. 5. The occurrence of spontaneous electrical activity (SEA) before and after botulinum toxin type A (BTX-A) 15u injection.

* Mean SEA prevalence of control (contralateral) side.

Fig. 6. The occurrence of spontaneous electrical activity (SEA) before and after botulinum toxin type A (BTX-A) multiple point injections.

* Mean SEA prevalence of control (contralateral) side.

Fig. 7. Mean value of occurrence of spontaneous electrical activity (SEA) among different groups of botulinum toxin type A injection (BTX-A 5u, BTX-A 10u, BTX-A 15u, BTX-A multiple point injection).
Assessment of Pre-injection SEA prevalence

Group I
Single bolus of BTX-A 5u Injection

Group II
Single bolus of BTX-A 10u injection

Group III
Single bolus of BTX-A 15u injection

Group IV
Multiple small doses of BTX-A injection

Assessment of Post-injection SEA prevalence
3 weeks after BTX-A injection

Assessment of Post-injection SEA prevalence
6 weeks after BTX-A injection

Assessment of Post-injection SEA prevalence
9 weeks after BTX-A injection

Fig. 1. General design
Fig. 2. Search for an SEA locus in an MTrS region in rabbit biceps femoris muscle.
Fig. 3. The occurrence of spontaneous electrical activity (SEA) before and after botulinum toxin type A (BTX-A) 5u injection.

* Mean SEA prevalence of control (contralateral) side.
Fig. 4. The occurrence of spontaneous electrical activity (SEA) before and after botulinum toxin type A (BTX-A) 10u injection.

* Mean SEA prevalence of control (contralateral) side.
Fig. 5. The occurrence of spontaneous electrical activity (SEA) before and after botulinum toxin type A (BTX-A) 15u injection.
* Mean SEA prevalence of control (contralateral) side.
Fig. 6. The occurrence of spontaneous electrical activity (SEA) before and after botulinum toxin type A (BTX-A) multiple point injections.

* Mean SEA prevalence of control (contralateral) side.
Fig. 7. Mean value of occurrence of spontaneous electrical activity (SEA) among different groups of botulinum toxin type A injection (BTX-A 5u, BTX-A 10u, BTX-A 15u, BTX-A multiple point injection).