アも対象にしている。②ボツリヌス治療後の患者を対象にしている。③FDG 投与時に臥位にて頭部固定をしており、感覚トリックの関与が疑われる。本研究では、これらの因子の関与を除外するために、ボツリヌス治療を受けていない特発性痙性斜頸のみを対象とし、座位にて FDG を投与し定常状態になるまで座位を保持した。その結果、痙性斜頸群では、小脳、レンズ核、一次運動野、視床外側腹側核での糖代謝亢進がみられた。本研究は大脳基底核—視床—大脳皮質ループや小脳—視床—大脳皮質ループの異常を裏付ける結果であった。

E. 結論

対象患者をボツリヌス未治療の特発性痙性斜頸に限局し、感覚トリックが入力されないようにFDG-PETを撮影した。その結果、基底核や運動野、小脳、視床（外側腹側核）では糖代謝亢進がみられ、大脳基底核—視床—大脳皮質ループや小脳—視床—大脳皮質ループの異常が示唆された。

F. 健康危険情報

（国民の生命・健康に重大な影響を及ぼす情報として厚生労働省に報告すべきものについて把握した過程、内容、理由を記載する。またその情報源の詳細。）

なし

G. 研究発表

1. 論文発表

1. Baba T, Takeda A, Kikuchi A, Nishio Y, Hosokai Y, Hirayama K, Hasegawa T, Sugeno N, Suzuki K, Mori E, Takahashi S, Fukuda H, Itoyama Y. Association of olfactory dysfunction and brain. Metabolism in Parkinson's disease. Mov Disord. 26: 621-628, 2011.
2. Kikuchi A, Baba T, Hasegawa T, Sugeno N, Konno M, Takeda A. Differentiating Parkinson's disease from multiple system atrophy by [^{123}I] meta-iodobenzylguanidine myocardial scintigraphy and olfactory test. Parkinsonism Relat Disord. 17:698-700, 2011.
3. Hasegawa T, Konno M, Baba T, Sugeno N, Kikuchi A, Kobayashi M, Miura E, Tanaka N, Tamai K, Furukawa K, Arai H, Mori F, Wakabayashi K, Aoki M, Itoyama Y, Takeda A. The AAA-ATPase VPS4 Regulates Extracellular Secretion and Lysosomal Targeting of α-Synuclein. PLoS ONE. 6:e29460, 2011.

2. 学会発表

1. 菊池昭夫、長谷川隆文、小林理子、菅野直人、馬場 徹、今野昌俊、岡村信行、古本祥三、谷内一彦、田代 学、工藤幸司、糸山泰人、武田 篤. 多系統萎縮症における[^{11}C]BF-227 PET の経時的変化. 第 52 回日本神経学会総会（2011 年 5 月 18 日、名古屋）
2. 長谷川隆文、今野昌俊、馬場徹、菅野直人、菊池昭夫、武田篤. 培養細胞系を用いたα-synuclein プリオン仮説の分子病態解析. 第 84 回日本生化学会大会（2011 年 9 月 22 日、京都）
3. 長谷川隆文、今野昌俊、馬場徹、菅野直人、菊池昭夫、若林孝一、武田篤. MVB sorting 機構はα-synuclein 分泌・ライソゾーム移行に関与する. 第 5 回パーキンソン病・運動障害疾患コングレス（2011 年 10 月 7 日、東京）
4. 菊池昭夫、長谷川隆文、小林理子、菅野直人、馬場 徹、今野昌俊、岡村信行、古本祥三、谷内一彦、田代 学、工藤幸司、糸山泰人、武田 篤. 多系統萎縮症における[^{11}C]BF-227 PET の経時的変化. 第 5 回パーキンソン病・運動障害疾患コングレス（2011 年 10 月 8 日、東京）
5. Hasegawa T, Baba T, Kobayashi M, Konno M, Sugeno N, Kikuchi A, Takeda A. Role of TPPP/p25 on alpha-synuclein-mediated oligodendroglial degeneration and the protective effect of SIRT2 inhibition in a cellular model of multiple system atrophy. The Movement Disorder Society's 15th International Congress of Parkinson's Disease and Movement Disorders (Jun 7 2011, Toronto)

H. 知的財産権の出願・登録状況（予定含む）

（特許取得・実用新案登録・その他）

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

Torsin A の細胞内動態

分担研究者　豊島　至　国立病院機構あきた病院副院長

研究要旨

TorsinA の変異が DYT1 ジストニアをもたらす。これまでは変異ジストニアは細胞内分布異常をきたすとされた。今回は TorsinA を蛍光タンパク質でラベルし細胞内分布を検討した。TorsinA は明瞭な核膜・小胞体分布を示したが変異体との差は見られずこれまでの報告を確認できなかった。

A. 研究目的

TorsinA の 302/303 のグルタミン酸が失われることが DYT1 ジストニアの本態であることが Ozelius らにより 1997 年に明らかになった。以来、TorsinA については AAA+タンパク質であり核膜と小胞体に分布し、これに由来するシナプス小胞が変異により少なくなるため、DYT1 ジストニアが発症すると考えられるようになった。今回はその仮説の検証のはじめとして、TorsinA を 2 種の蛍光タンパク質でラベルし細胞内動態追跡を試みた。

B. 研究方法

Tor1A を新たにクローニングし、302/303 のグルタミン酸の欠失する変異を作成した。TorsinA と AcGFP 、DsRed-monomer (Clontech) の 2 種の蛍光タンパク質との融合タンパク質をそれぞれ作成し Cos7 細胞と SHSY5Y 細胞で発現させた。小胞体を 2 種の ER マーカータンパク質で同時に発現させ蛍光顕微鏡で観察した。

C. 研究結果および考察

AcGFP で作成した融合タンパク質は核周囲に集積し、小胞体には微量に分布するだけであった。DsRed-monomer との融合タンパク質は核膜と小胞体に分布した。この分布パターンは変異のあるなしで有意な差は得られなかった。

E. 結論

TorsinA の蛍光タンパク質ラベルは明瞭な核膜・小胞体分布を示したが変異体との差は見られずこれまでの報告を確認できなかった。

G. 研究発表

1. 論文発表
   なし
2. 学会発表
   なし

H. 知的財産権の出願・登録状況

なし

厚生労働科研費補助金（ジストニアの診断及び治療法の更なる推進に関する研究事業）
（分担）　研究報告書

ジストニア診療とケアマニュアルの作成

（分担）　研究者　堀内正浩　川崎市立多摩病院神経内科部長
聖マリアンナ医科大学神経内科准教授

要旨：ジストニア患者を啓蒙する目的で、平易な文章で書かれた「ジストニア診療とケアマニュアル」を作成した。

A．研究目的
ジストニア患者を啓蒙する目的で「ジストニア診療とケアマニュアル」を作成した。

B．研究方法
本書は以下の目次からなる。①ジストニアとは、どんな病気ですか？、②どのようにして診断されますか？、③どうして起こるのでしょう？、④どんな人がなるのですか？、⑤ジストニアは命にかかわる病気ですか？、⑥どんな治療がありますか？、⑦ジストニア症状別 Q&A　(a)眼瞼痙攣、(b)痙攣性発声障害、(c)痙性斜頸、(d)書痙、(e)音楽家のジストニア、(f)下肢ジストニア、(g)スポーツにかかわるジストニア、(h)遅発性ジストニア、(i)ジストニアを起こす疾患、⑧ジストニア患者へのアドバイス、コラム（「NPO 法人ジストニア友の会」の概要)、⑨関連サイト。

C．研究結果
「診断と治療社」の御厚意により出版された。

D．考察
後半の Q&A は堀内が回答したが、質問は主に患者から伺ったものである。このため「医師が伝えたいこと」ではなく、「患者が知りたいこと」に近いと考える。

E．患者向けに平易な文章で書かれた啓蒙書も必要だと考えた。

III. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
堀内正浩		梶　龍兒	ジストニア診療とケアマニュアル	診断と治療社	東京	2011	
目崎高広	[I 総論編] 第 1 章 ボツリヌス治療総論.	目崎高広	ボツリヌス治療実践マニュアル	診断と治療社	東京	2012	2-4
目崎高広	[II マニュアル編] 第 1 章 治療前準備.	目崎高広	ボツリヌス治療実践マニュアル	診断と治療社	東京	2012	6-9
目崎高広	[II マニュアル編] 第 3 章 痙性斜頸.	目崎高広	ボツリヌス治療実践マニュアル	診断と治療社	東京	2012	46-79
武田朋美, 目崎高広	[II マニュアル編] 第 4 章 5 上下肢筋の超音波図譜.	目崎高広	ボツリヌス治療実践マニュアル	診断と治療社	東京	2012	172-183
目崎高広	A 型ボツリヌス毒素 (BTX-A)療法.	山口徹，北原光夫，福井次矢	今日の治療指針 私はこう治療している	医学書院	東京	2012	750-751
野村芳子	トゥレット症候群 Tourette syndrome	井村裕夫 福井次矢 辻省次	症候群ハンドブック Syndrome	中山書店		2011	P27-28
野村芳子	レット症候群 Rett syndrome	井村裕夫 福井次矢 辻省次	症候群ハンドブック Syndrome	中山書店		2011	P130-131
野村芳子	Pantothenate kinase 2 欠損症	水野美邦 近藤智善	よくわかるパーキンソン病のすべて 改	永井書店		2011	P312-316
野村芳子	Wilson 病	水野美邦 近藤智善	よくわかるパーキンソン病のすべて 改	永井書店		2011	P317-321
野村芳子	瀬川病	水野美邦 近藤智善	よくわかるパーキンソン病のすべて 改	永井書店		2011	P322-326
野村芳子	Rett 症候群 - 本症にみるジストニアについて -		Rett 症候群にみるジストニア			2011	印刷中

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kaji R.	New and emerging indications of botulinum toxin therapy.	Parkinsonism Relat Disord.	17 Suppl 1	S25-7	2011
Miyazaki Y, Sako W, Asanuma K, Izumi Y, Miki T, Kaji R.	Efficacy of zolpidem for dystonia: a study among different subtypes.	Front Neurol.	3	58	2012
Torii Y, Akaike N, Harakawa T, Kato K, Sugimoto N, Goto Y, Nakahira S, Kohda T, Kozaki S, Kaji R, Ginnaga A.	Type A1 but not type A2 botulinum toxin decreases the grip strength of the contralateral foreleg through axonal transport from the toxin-treated foreleg of rats.	J Pharmacol Sci.	117(4)	275-85	2011
Lim EC, Bhidayasiri R, Rosales RL, Kaji R.	Practical management of botulinum toxin therapy. Introduction.	Parkinsonism Relat Disord.	17 Suppl 1	S1-2	2011
Koshiba S, Tokuoka H, Yokoyama T, Horiuchi E, Ichinose H, Hasegawa K	Biopterin levels in the cerebrospinal fluid of patients with PARK8 (I2020T).	J Neural Transm	118	899-903	2011
Kawase Y, Hasegawa K, Horiuchi E, Ikeda K	Olfactory dysfunction in Parkinson's disease: benefits of quantitative odorant examination	Intern J General Med	3	181-185	2010
長谷川一子	パーキンソン病の臨床診断および鑑別診断ー臨床症状から	綜合臨床	59	2404-2412	2010
長谷川一子	比較的まれな認知症疾患：診断と治療のポイント　ハンチントン病	Clinical Neuroscience	29	346-348	2011
長谷川一子	抗パーキンソン病薬　塩酸アマンタジン	Clinical Neuroscience	29	570-571	2011
長谷川一子	パーキンソン病の治療はできるだけ早く始める方がよい　Yes	MDSJ letters	4	1-3	2011
長谷川一子	ハンチントン病：UPDATE	MDSJ letters	4	4-9	2011

長谷川一子，斎木栄資，伊藤和則，前田哲也	１日１回錠の有用性とプラミペキソール徐放錠の可能性について	Parma Medica	29	79-83	2011
長谷川一子	視床手	脊椎脊髄ジャーナル	24	697-699	2011
長谷川一子，徳田隆彦，服部信孝，中野今治	パーキンソン病の現状と将来	Human science	24	4-12	2011
小柴梢子，徳岡宏文，横山照夫，堀内恵美子，長谷川一子，一瀬宏	神経疾患患者の脳脊髄駅における還元型プテリジンレベルの測定：臨床資料中の還元型プテリジン測定のポストカラム酸化法の応用.	ビタミン	85	63-69	2011
Nakamura K, Enomoto H, Hanajima R, Hamada M, Shimizu E, Kawamura Y, Sasaki T, Matsuzawa D, Sutoh C, Shirota Y, Terao Y, Ugawa Y.	Quadri-pulse stimulation (QPS) induced LTP/LTD was not affected by Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene.	Neurosci Letts	487	264-267	2011
Shirota Y, Hanajima R, Hamada M, Terao Y, Matsumoto H, Tsutsumi R, Ohminami S, Furubayashi T, Ugawa Y.	Inter-individual variation in the efficient stimulation site for magnetic brainstem stimulation.	Clin Neurophysiol	122	2044-2048	2011
Hanajima R, Terao Y, Shirota Y, Ohminami S, Nakatani-Enomoto S, Okabe S, Matsumoto H, Tsutsumi R, Ugawa Y.	Short-interval intracortical inhibition in Parkinson's disease using anterior-posterior directed currents.	Exp Brain Res	214	317-321	2011
Hirose M, Mochizuki H, Groiss SJ, Tanji Y, Nakamura K, Nakatani-Enomoto S, Enomoto H, Nishizawa M, Ugawa Y.	On-line effects of quadripulse transcranial magnetic stimulation (QPS) on the contralateral hemisphere studied with somatosensory evoked potentials and near infrared spectroscopy.	Exp Brain Res	214	577-586	2011
Hanajima R, Terao Y, Nakatani-Enomoto S, Okabe S, Shirota Y, Oominami S, Matsumoto H, Tsuji S, Ugawa Y.	Triad stimulation frequency for cortical facilitation in cortical myoclonus.	Mov Disord	26	685-690	2011
Arai N, Müller-Dahlhaus F, Murakami T, Bliem B, Lu MK, Ugawa Y, Ziemann U.	State-dependent and timing-dependent bidirectional associative plasticity in the human SMA-M1 network.	J Neurosci	31	15376-15383	2011

Nakatani-Enomoto S, Hanajima R, Hamada M, Mochizuki H, Kobayashi S, Enomoto H, Sugiura Y, Matsumoto H, Furubayashi T, Terao Y, Sato F, Ugawa Y.	Some evidence supporting the safety of quadripulse stimulation (QPS).	Brain Stimul	4	303-305	2011
Kikuchi S, Mochizuki H, Moriya A, Nakatani-Enomoto S, Nakamura K, Hanajima R, Ugawa Y.	Ataxic Hemiparesis: Neurophysiological Analysis by Cerebellar Transcranial Magnetic Stimulation.	Cerebellum	(in press)		
Groiss SJ, Ugawa Y.	Cerebellar Stimulation in Ataxia.	Cerebellum	(in press)		
Tsutsumi R, Hanajima R, Hamada M, Shirota Y, Matsumoto H, Terao Y, Ohminami S, Yamakawa Y, Shimada H, Tsuji S, Ugawa Y.	Reduced interhemispheric inhibition in mild cognitive impairment.	Exp Brain Res	(in press)		
Matsumoto H, Hanajima R, Terao Y, Hashida H, Ugawa Y.	Neurophysiological analysis of the cauda equina in POEMS syndrome.	Neurol Sci	(in press)		
玉川　聡	知っておきたいボツリヌス療法 用量・濃度と治療効果との関係	Modern Physician	Vol. 31 No. 7	852-853	2011
堀内正浩、長谷川泰弘、 真木二葉、柳澤俊之	外傷後ジストニア：自経 14 例の検討	神経治療	28 巻 2 号	177-182	2011
堀内正浩、長谷川泰弘	頸部外傷後ジストニアの 3 例	神経内科	75 巻 5 号	509-514	2011
堀内正浩	知っておきたいボツリヌス療法 (治療のコツと未来への展望) 1, 患者にどう説明するか	Modern Physician	31 巻 7 号	845-848	2011
堀内正浩	知っておきたいボツリヌス療法 (治療のコツと未来への展望) 2, 患者はなぜ怖がる？ 医師はなぜ嫌がる？	Modern Physician	31 巻 7 号	849-851	2011

Baba T, Takeda A, Kikuchi A, Nishio Y, Hosokai Y, Hirayama K, Hasegawa T, Sugeno N, Suzuki K, Mori E, Takahashi S, Fukuda H, Itoyama Y.	Association of olfactory dysfunction and brain. Metabolism in Parkinson's disease.	Mov Disord	26	621-628	2011
Kikuchi A, Baba T, Hasegawa T, Sugeno N, Konno M, Takeda A.	Differentiating Parkinson's disease from multiple system atrophy by [^{123}I] meta-iodobenzylguanidine myocardial scintigraphy and olfactory test.	Parkinsonism Relat Disord	17	698-700	2011
Hasegawa T, Konno M, Baba T, Sugeno N, Kikuchi A, Kobayashi M, Miura E, Tanaka N, Tamai K, Furukawa K, Arai H, Mori F, Wakabayashi K, Aoki M, Itoyama Y, Takeda A.	The AAA-ATPase VPS4 Regulates Extracellular Secretion and Lysosomal Targeting of α-Synuclein.	PLoS ONE	6	e29460	2011
坂本　崇	世界のボツリヌス毒素製剤	モダンフィジシャン	31（7）	800-802	2011
坂本　崇	眼瞼痙攣・片側顔面痙攣	神経内科	75（5）	484-488	2011
坂本　崇	痙縮のボツリヌス治療	神経治療学	28（5）	528	2011
Ohta E, Kawakami F, Kubo M, Obata F	LRRK2 directly phosphorylates Akt1 as a possible physiological substrate: impairment of the kinase activity by Parkinson's disease-associated mutations	FEBS Lett	585(14)	2165-2170	2011
Mezaki T	Ultrasound-guided injection of botulinum toxin into the obliquus capitis inferior muscle.	Basal Ganglia	1	135-136	2011
Mezaki T	Reduced serum ceruloplasmin levels in cervical dystonia.	Eur J Neurol	in press	in press	2012
目崎高広	効かない患者　ー Primary Non-responder.	モダンフィジシャン	31	863-865	2011
目崎高広	効かなくなった患者 ーSecondary Non-responder.	モダンフィジシャン	31	866-868	2011

目崎高広	ボツリヌス毒素は心に効くか.	モダンフィジシャン	31	881-883	2011
目崎高広	キマイラートロイの木馬ー未来戦略.	モダンフィジシャン	31	884-886	2011
目崎高広	ボツリヌス毒素の治療への応用.	Brain Nerve	63	785-794	2011
目崎高広	ジストニアの病態と治療.	臨床神経	51	465-470	2011
目崎高広	攣縮性斜頸に対するボツリヌス療法の長期予後.	神経内科	75	489-496	2011
藤本健一，村田美穂，服部信孝，近藤智善	大規模患者調査で明らかになった日本における Parkinson 病薬物治療の実態；Parkinson 病患者の服薬状況および疾患・治療に対する意識調査	Brain and Nerve	63	255-265	2011
藤本健一	パーキンソン病に対する遺伝子治療	脳外誌	20	87-92	2011
藤本健一	Dopamin dysregulation syndrome	神経内科	74	7-13	2011
藤本健一	抗パーキンソン病薬の導入時期	内科	107	817-820	2011

Ⅳ．研究成果の刊行物・別刷

Parkinsonism and Related Disorders 17 (2011) S25–S27

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New and emerging indications of botulinum toxin therapy

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Keywords:
Botulinum toxin
Therapy
A1
A2
Pain
Epilepsy
Spasticity

A B S T R A C T

Botulinum neurotoxin (BoNT) is composed of the heavy chain with the receptor-binding site and the translocation domain and the light chain with endopeptidase activity that cleaves the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) complex, an essential molecule for membrane fusion. Its extraordinarily high toxicity depends on the affinity of the receptor-binding site to the receptor located inside the synaptosome. The membrane fusion mechanism is important not only in neurotransmitter release at the nerve terminals but also in the expression of pain receptors on the cell surface. Based on these mechanisms, BoNT is increasingly used for varieties of conditions including cosmetic uses, muscle hyperactivity, hyperhydrosis, pain, overactive bladder and epilepsy. It will become a major arm of neuromodulating treatments for neurological diseases. A part of this toxin, such as the heavy chain, may become a novel drug-delivery system for neurodegenerative diseases.

1. Advances in botulinum toxin research

Botulinum neurotoxins (BoNTs) are produced by anaerobic bacteria of the *Clostridium* group and are the most potent toxins known to date [1]. There are seven serotypes of BoNTs, indicated by letters from A to G. Each toxin is composed of a heavy (H, 100 kDa) and a light chain (L, 50 kDa) linked by a disulphide bond and non-covalent interactions. The carboxy terminus of the heavy chain (HC) binds with extraordinary specificity to nerve terminals. Following receptor-mediated endocytosis and acidification of the endosome, the amino-terminal portion of the heavy chain (HN) translocates the L chain across the vesicular membrane into the cytosol. The L chain acts as a Zn^{2+}-dependent endopeptidase to cleave essential protein components of the neurotransmitter release machinery, the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins. This disrupts Ca^{2+}-triggered fusion of synaptic vesicles (SVs) with the plasma membrane [2].

The receptors of BoNTs have been clarified recently: serotype B BoNT binds to synaptotagmin II[3] and serotype A to SV2 [4], both of which are located on the inner surface of the synaptosome. BoNT also recognises the ganglioside moiety (trisialoganglioside, GT1b) on the surface of the cell membrane, which determines the target selectivity [3]. These findings explain the activity-dependent action of the toxin: BoNTs affect the synapses most active in releasing the neurotransmitters because they can access the synapses or neuromuscular junctions with the receptors inside the vesicle. It has been known that the action of BoNTs is optimised when the muscles are activated immediately following the injection [5]. This action is in contrast with the neurolytic therapies, such as phenol injections, which affect all the nerve endings irrespective of the activities, resulting in unwanted weakness of the injected muscles. By contrast, BoNTs abolish only twitching muscles in case of hemifacial spasms. This is relevant with other involuntary movements or spasticity, where active engagement in the affected movement or posture is encouraged after injections, to attain the maximum benefit of BoNTs.

The potency of the toxin is mostly due to its very high affinity to the receptors. The receptor-binding capability of the heavy chain is now being explored for development of the drug-delivery system to neurons after replacing the L chain with other moieties [6]. Such an attempt may be fruitful for the development of drugs for amyotrophic lateral sclerosis, if the L chain is substituted by neurotrophic factors.

Types A, B and F toxins have been used for clinical settings in the past [7]. Currently, types A and B are marketed. Among type A toxins, four subtypes (A1–A4) exist, and all the marketed toxins are from subtype A1. Recently, type A2 toxin has been used in animals [8] and showed greater potency in producing weakness and less spreading into uninjected muscles than conventional A1 toxin. It was also shown that type A toxins affects central synapses, and subtype A2 has less central actions than A1 because of the less retrograde transport of the toxin to the spinal cord [9].These findings may lead to a BoNT preparation used for larger muscles, such as those in the lower extremities in patients with spasticity.

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2. Clinical indications

Indications of BoNTs have been constantly expanded in the past decade.

BoNTs' most popular use is for cosmetic purposes. It is widely accepted that wrinkles on the face go away almost indefinitely after the injection, but the exact mechanism is still elusive.

2.1. Muscle hyperactivity

By far the most important use in neurological diseases is for muscle hyperactivity, including dystonia and spasticity. Focal dystonias, such as blepharospasm and cervical dystonia, are the best indications among dystonias. Task-specific dystonias including writer's or musician's cramp are less optimal [10] because of the unwanted weakness for the tasks. Larger doses are required for treating truncal or lower-extremity dystonias, and new preparations, such as the A2 subtype, might be relevant. Generalised or segmental dystonias are treated more efficaciously by surgical manoeuvres, such as deep-brain stimulation of bilateral GPi.

Hemifacial spasms are also good indication of BoNTs, and decompression surgeries are becoming obsolete as the first-line treatment. The dose required is usually less than that in blepharospasm, and the injection interval is longer.

Spasmodic dysphonia, a dystonia involving vocal-cord muscles, is also a superb indication of BoNT. A special injection technique for this is needed.

Spasticity is probably one of the most prevalent and important health problems in developed nations. Up to 65% of the patients who survived stroke suffer from it. Cost of care for those patients far exceeds 2,000,000,000,000 yen or 20 billion US dollars per year in Japan. Until 2004, a few randomised controlled trials have reported some promising results in support of reduced muscle tone following BoNT injections [11]. Further research incorporating larger sample sizes, rigorous methodology, measurement of upper-limb function and functional outcomes was essential. Since then, there have been several large-scale clinical trials for upper-limb spasticity showing functional improvements [12]. A recent study in the post-stroke lower-limb spasticity also reported markedly significant improvements in the modified Ashworth scale [13]. Functional improvements were only attained by repeated injections. By now, uses in spasticity in upper and lower-limbs have been approved in UK, France, Germany and Japan, and use for upper-limb was approved by the Food and Drug Administration (FDA) in USA.

Interestingly, patients with upper-limb spasticity often improve their motor disturbance after BoNT injection and rehabilitation almost permanently, without the need for further injections. This is unlike those with hand dystonia, who need repeated injections to maintain the benefit. It is argued that BoNT may enhance spinal synaptic reorganisation directly by its central action or indirectly through alteration of muscle afferents [14]. Another possibility is that release of the affected hand into active movements may reverse anomalous interhemispheric inhibition from the unaffected cortex to the affected.

Because the sudomotor sympathetic fibres are also cholinergic, BoNTs have been used for controlling hyperhydrosis, which can occur either after skin incisures or without any known causes.

2.2. Pain

A breakthrough in the clinical application of BoNT is its use for controlling pain and migraine. BoNT was shown to decrease the expression of pain-sensitive vanilloid receptors (e.g., transient receptor potential cation channel subfamily V member 1, TRPV1), which are up-regulated in sensitised sensory neurons [15]. This is because those receptors are expressed to the cell membrane through the fusion mechanism mediated by the SNARE complex, the substrate of BoNTs.

It was accidentally found that BoNT injection into corrugator muscle for removing skin furrows brought about a decrease in the number of migraine attacks. Since then, a number of clinical trials with a small number of cases and modest doses have resulted in equivocal results for migraine. Recently, clinical trials with larger number of cases and doses of BoNT have successfully reduced the number of attacks [16–18], followed by its approval in UK and USA.

Intractable pain or complex regional pain syndrome is another important indication recently added. Patients with these conditions present with oedematous, painful and immobile limb with skin areas with allodynia, or abnormally induced pain after light touch. Repeated injections into these areas subcutaneously result in gradual improvement of allodynia and pain, followed by decreased oedema and increased mobility. It was also found that post-stroke pain including thalamic pain also responds to subcutaneous BoNT injections made into areas with allodynia [19].

2.3. Overactive bladder (OAB)

Urinary problems are very common in the elderly. Many people are affected by urinary urgency, which can be highly bothersome. Urgency is the cornerstone symptom of overactive bladder (OAB), commonly occurring in conjunction with urinary frequency and nocturia. Once other medical causes of similar symptoms have been excluded, first-line OAB management comprises fluid-intake advice and bladder training, supplemented by antimuscarinic drugs, if necessary. BoNTs are currently explored as an alternative therapy [20,21]. The injection into the inner surface of the bladder was shown to down-regulate the expression of TRPV1 and muscarinic Ach receptors, which trigger destrusors. Despite the technical difficulties, this technique will be widely used for these patients in the near future.

2.4. Epilepsy

Experimental pieces of evidence suggest that BoNT suppresses glutamate release in the central nervous system (CNS). Because of its activity-dependent action, BoNT may be used for managing intractable epilepsies [22,23]. Abnormal excitation at the epileptic foci is associated with large glutamate-induced excitatory postsynaptic potentials (EPSPs) that drive cortical neurons for lateral spread. BoNT would selectively suppress these active neurons, leaving the rest of the neurons unaffected. It would therefore be expected that BoNT suppresses neurons at the foci, while the rest of the neurons function normally. This method may become a substitute for surgical resections of the affected brain tissue. The largest problem would be the drug-delivery, and stereotactic device and cerebrospinal fluid (CSF) injections are now being contemplated.

In conclusion, BoNT is increasingly used for varieties of conditions including cosmetic uses, muscle hyperactivity, hyperhydrosis, pain, OAB and epilepsy. It will become a major arm of neuromodulating treatment for neurological diseases.

References

[1] Montecucco C, Schiavo G, Pantano S. SNARE complexes and neuroexocytosis: how many, how close? Trends Biochem Sci 2005;30:367–72.
[2] Schiavo G, Matteoli M, Montecucco C. Neurotoxins affecting neuroexocytosis. Physiol Rev 2000;80:717–66.
[3] Nishiki T, Kamata Y, Nemoto Y, Yoshida A, Sato K, Sekiguchi M, et al. Identification of protein receptor for *Clostridium botulinum* type B neurotoxin in rat brain synaptosomes. J Biol Chem 1994;269:10498–503.
[4] Dong M, Yeh F, Tepp WH, Dean C, Johnson EA, Janz R, et al. SV2 is the protein receptor for botulinum neurotoxin A. Science 2006;312:592–6.

[5] Kim HS, Hwang JH, Jeong ST, Lee YT, Lee PK, Suh YL, et al. Effect of muscle activity and botulinum toxin dilution volume on muscle paralysis. Dev Med Child Neurol 2003;45:200–6.
[6] Fahrer J, Plunien R, Binder U, Langer T, Seliger H, Barth H. Genetically engineered clostridial C2 toxin as a novel delivery system for living mammalian cells. Bioconjug Chem 2010;21:130–9.
[7] Mezaki T, Kaji R, Brin MF, Hirota-Katayama M, Kubori T, Shimizu T, et al. Combined use of type A and F botulinum toxins for blepharospasm: a double-blind controlled trial. Mov Disord 1999;14:1017–20.
[8] Akaike N, Ito Y, Shin MC, Nonaka K, Torii Y, Harakawa T, et al. Effects of A2 type botulinum toxin on spontaneous miniature and evoked transmitter release from the rat spinal excitatory and inhibitory synapses. Toxicon 2010; 56:1315–26.
[9] Torii Y, Kiyota N, Sugimoto N, Mori Y, Goto Y, Harakawa T, et al. Comparison of effects of botulinum toxin subtype A1 and A2 using twitch tension assay and rat grip strength test. Toxicon 2011;57:93–9.
[10] Simpson DM, Gracies JM, Graham HK, Miyasaki JM, Naumann M, Russman B, et al. Assessment: botulinum neurotoxin for the treatment of spasticity (an evidence-based review): report of the therapeutics and technology assessment subcommittee of the American academy of neurology. Neurology 2008; 70:1691–8.
[11] Simpson DM, Alexander DN, O'Brien CF, Tagliati M, Aswad AS, Leon JM, et al. Botulinum toxin type A in the treatment of upper extremity spasticity: a randomized, double-blind, placebo-controlled trial. Neurology 1996;46: 1306–10.
[12] Kaji R, Osako Y, Suyama K, Maeda T, Uechi Y, Iwasaki M. Botulinum toxin type A in post-strokeupper limb spasticity. Curr Med Res Opin 2010;26:1983–92.
[13] Kaji R, Osako Y, Suyama K, Maeda T, Uechi Y, Iwasaki M. Botulinum toxin type A in post-strokelower limb spasticity: a multicenter, double-blind, placebo-controlled trial. J Neurol 2010;257:1330–7.
[14] Caleo M, Antonucci F, Restani L, Mazzocchio R. A reappraisal of the central effects of botulinum neurotoxin type A: by what mechanism? J Neurochem 2009;109:15–24.
[15] Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, Ferrer-Montiel A. Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 2004;279:25665–72.
[16] Diener HC, Dodick DW, Aurora SK, Turkel CC, DeGryse RE, Lipton RB, et al. OnabotulinumtoxinA for treatment of chronic migraine: results from the double-blind, randomized, placebo-controlled phase of the PREEMPT 2 trial. Cephalalgia 2010;30:804–14.
[17] Aurora SK, Dodick DW, Turkel CC, DeGryse RE, Silberstein SD, Lipton RB, et al. OnabotulinumtoxinA for treatment of chronic migraine: results from the double-blind, randomized, placebo-controlled phase of the PREEMPT 1 trial. Cephalalgia 2010;30:793–803.
[18] Dodick DW, Turkel CC, DeGryse RE, Aurora SK, Silberstein SD, Lipton RB, et al. OnabotulinumtoxinA for treatment of chronic migraine: pooled results from the double-blind, randomized, placebo-controlled phases of the PREEMPT clinical program. Headache 2010;50:921–36.
[19] Brin MF. Development of future indications for BOTOX. Toxicon 2009;54:668–74.
[20] Sahai A, Dowson C, Khan MS, Dasgupta P. Repeated injections of botulinum toxin-Afor idiopathic detrusor overactivity. Urology 2010;75:552–8.
[21] Campbell JD, Gries KS, Watanabe JH, Ravelo A, Dmochowski RR, Sullivan SD. Treatment success for overactive bladder with urinary urge incontinence refractory to oral antimuscarinics: a review of published evidence. BMC Urol 2009;9:18.
[22] Antonucci F, Bozzi Y, Caleo M. Intrahippocampal infusion of botulinum neurotoxin E (BoNT/E) reduces spontaneous recurrent seizures in a mouse model of mesial temporal lobe epilepsy. Epilepsia 2009;50:963–6.
[23] Kang JS, Krakow K, Roggendorf J, Steinmetz H, Hilker R. Botulinum toxin treatment of epilepsia partialis continua. Mov Disord 2009;24:141–3.

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Efficacy of zolpidem for dystonia: a study among different subtypes

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Although there are some newly developed options to treat dystonia, its medical treatment is not always satisfactory. Zolpidem, an imidazopyridine agonist with a high affinity on benzodiazepine subtype receptor BZ1 (ω1), was found to improve clinical symptoms of dystonia in a limited number of case reports. To investigate what subtype of dystonia is responsive to the therapy, we conducted an open label study to assess the efficacy of zolpidem (5–20 mg) in 34 patients suffering from miscellaneous types of dystonia using the Burke–Fahn–Marsden Dystonia Rating Scale (BFMDRS). Patients were entered into the study if they had been refractory to other medications as evaluated by BFMDRS (no change in the previous two successive visits). After zolpidem therapy, the scores in the patients as a whole were decreased from 7.2 ± 7.9 to 5.5 ± 5.0 ($P = 0.042$). Patients with generalized dystonia, Meige syndrome/blepharospasm, and hand dystonia improved in the scale by 27.8, 17.8, and 31.0%, respectively, whereas no improvement was found in cervical dystonia patients. Overall response rate among patients were comparable to that of trihexyphenidyl. Zolpidem may be a therapeutic option for generalized dystonia, Meige syndrome, and hand dystonia including musician's. Drowsiness was the dose-limiting factor.

Keywords: generalized dystonia, Meige syndrome, hand dystonia, zolpidem

INTRODUCTION

Dystonia is a syndrome of sustained muscle contractions causing twisting and repetitive movements or abnormal postures (Fahn et al., 1998). Although there are several options to treat dystonia, its medical treatment is notoriously difficult and often unsuccessful. Zolpidem, an imidazopyridine agonist with a high affinity to benzodiazepine subtype receptor BZ1 (ω1; Holm and Goa, 2000), is reported to improve basal ganglia disease including Parkinson's disease (Daniele et al., 1997) and various types of dystonia (Evidente, 2002; Garretto et al., 2004; An et al., 2008; Park et al., 2009) Despite these case reports, zolpidem has not been tested in a large number of patients with various subtypes of dystonia. Here we report two dystonia patients who improved remarkably by oral zolpidem therapy, and assessed treatment outcome of zolpidem in 34 medically intractable patients suffering from miscellaneous types of dystonia, in order to determine what subtypes of dystonia are good candidates for zolpidem trial.

MATERIALS AND METHODS

PATIENTS

Dystonia patients were selected, not in randomized, nor controlled design, from those seen at Tokushima University Hospital and Takeda General Hospital, Japan. The diagnosis of primary dystonia was made according to standard criteria (Albanese et al., 2006): Major exclusion criteria were the presence of brain lesion in basal ganglia detected by 1.5 T magnetic resonance image and the past history of antipsychotics administration. We enrolled 34 patients with dystonia, who were treated with trihexyphenidyl (4–12 mg/day), clonazepam (0.5–3 mg/day), baclofen (15–60 mg/day), and others (9 generalized dystonia; 10 Meige syndrome/blepharospasm; 7 cervical dystonia; 8 hand dystonia). All patients were refractory to further dose increases of oral medications other than zolpidem. Their doses were unchanged if continued in the zolpidem trial. Of all, 23 patients were resistant to botulinum toxin type A (OnabotulinumtoxinA: 50–200 IU, 0.5–8 ml) injections. The refractoriness was evidenced by the lack of improvement in the Burke–Fahn–Marsden Dystonia Rating Scale (BFMDRS) in the last two visits. All focal dystonia patients (Meige syndrome/blepharospasm, cervical dystonia, and hand dystonia) did not spread to multiple body parts during 1 year follow up. One patient underwent palidal stimulation before entry. Their clinical characteristics are summarized in **Table 1**. Their mean age was 48.8 ± 15.8 years; mean disease duration was 5.2 ± 5.1 years. Zolpidem was started at 10 mg/day (once a day in the evening), later increased or decreased in dosage (5–20 mg/day: once or twice a day in the morning and evening) depending on the tolerability and the benefit. The mean dosage of zolpidem was 11.2 ± 5.12 mg.

ASSESSMENTS

All patients were assessed before and 1 month after zolpidem administration using BFMDRS, including the Dystonia Movement Scale (Part I) and Disability Scale (Part II; Burke et al., 1985).

We defined the global improvement as follows; more than 40% improvement in BFMDRS as "remarkable improvement," less than 40% improvement as "mild improvement," and no change in the scale as " no improvement."

Table 1 | Patients' summary.

	Generalized dystonia ($n=9$)	Meige/blepharospasm ($n=10$)	Cervical dystonia ($n=7$)	Hand dystonia ($n=8$)	Total ($n=34$)
Gender (male/female)	3M/6F	6M/4F	7M/0F	5M/3F	21M/13F
Age	38.3 ± 19.4	60.6 ± 9.6	45.7 ± 14.4	48.4 ± 10.1	48.8 ± 15.8
Duration (years)	4.6 ± 6.8	3.6 ± 3.2	6.0 ± 4.9	7.4 ± 5.2	5.2 ± 5.1
BFMDRS: before	15.8 ± 10.0	6.2 ± 5.4	2.4 ± 1.1	2.9 ± 2.0	7.2 ± 7.9
BFMDRS: after	11.4 ± 5.7	5.1 ± 3.0	2.4 ± 1.1	2.0 ± 0.9	*5.5 ± 5.0
Zolpidem (mg/day)	12.2 ± 6.2	12.0 ± 4.8	10 ± 0	8.8 ± 5.1	10.9 ± 4.8
BTX	6	10	6	1	26

$P = 0.041$ vs before administration (t-test).

Standard protocol approvals, registrations, and patient consents

This study was approved by JSPS Grants-in-Aid for Scientific Research (No. 21390269), and informed consent was obtained from all patients.

Data analysis

Statistical analyses were made using t-test, results were considered significant at a level of $P < 0.05$.

RESULTS

CASE REPORTS

Case 1

A 36-years-old man, who was a clarinet player, had 1-year history of cramps during the performance. His physical condition and mental condition was normal, and there were no neurologic abnormalities. At the age 35, he noticed an abnormal cramp on the left little finger during clarinet performance. The symptoms gradually worsened over time, finally he became no longer able to play the clarinet in the concert. He had been on medications with trihexyphenidyl up to 12 mg/day and clonazepam (1–3 mg/day) with no benefits.

At the age of 36-years-old, we tried zolpidem on him, which improved his symptoms dramatically to the extent that he had no problems in the performance. He took 10 mg of zolpidem before playing the clarinet, and found the beneficial effect within 30 min, its durations of action being about 3 h. One year later, he was still using zolpidem 10 mg once or twice a day for occasional concert.

Case 2

A-20-years-old woman, who was a softball player, had 1-year history for lower limbs dystonia. Her physical condition and mental condition was normal, and there were no neurologic abnormalities except for dystonic symptoms on the bilateral lower limbs. At the age 19, she noticed an abnormal inversion of the left ankle during walking. The symptoms gradually worsened, and she developed difficulty in walking because of her lower limbs muscle hyperactivity. Her dystonic symptoms did not change with or without shoes. She was tried medication with trihexyphenidyl (up to 12 mg/day), baclofen (up to 30 mg/day), and gabapentin, with no effect. At age 20, she became unable to walk, or to bend her knees and ankles. She was referred to us with a diagnosis of lower limb dystonia (**Figure 1**).

We treated her with zolpidem oral monotherapy with a dose up to 20 mg/day. Three days after the therapy, she found it easy to bend her right knee and could stand without any help. She could walk on day 7, and finally she could climb up and down stairs on day 14. One year later, she was still on zolpidem, with continued benefit.

Effects of zolpidem in miscellaneous types of dystonia

Table 1 depicts summary of the patients. BFMDRS in total dystonia patients were significantly decreased from 7.2 ± 7.9 to 5.5 ± 5.0 ($P = 0.041$).

As for subtypes of dystonia, the scale decreased on the average in generalized, Meige syndrome/blepharospasm, and hand dystonia (**Table 1**). After zolpidem, 3 of 9 generalized dystonia (33%), 2 of 10 Meige syndrome/blepharospasm (20%), and 3 of 8 hand dystonia patients (38%) improved in the motor subscale of BFMDRS (generalized dystonia; 29–75% improvement, Meige syndrome/blepharospasm; 33–39% improvement, hand dystonia; 33–67% improvement), whereas cervical dystonia patients did not. Overall, the present study showed that 8 of 34 dystonia patients (24%) responded to zolpidem.

Adverse effects associated with zolpidem were drowsiness, amnesia, and abnormal behavior (somnambulism). Moderate or severe drowsiness occurred in eight patients (three cases of responders and five non-responders), and transient amnesia occurred in four patients (two responders and two non-responders).

DISCUSSION

Here we described the outcome of zolpidem trial in patients with miscellaneous types of dystonia, whose symptoms had been refractory to other medications. In all dystonia patients, 24% of the patients responded to zolpidem, and remarkable improvements were found particularly in generalized and hand dystonias. No improvement was found in cervical dystonia. Despite the different outcome measures and clinical protocols, the present data are comparable to the efficacy of trihexyphenidyl in a previous study reporting improvements in 44% for generalized dystonia patients, 63% for Meige syndrome/blepharospasm, and 28% for focal dystonia patients (Jabbari et al., 1989).

Our result has a limitation that the design was not a randomized controlled trial. Indeed this is a pivotal study so that the conclusion regarding efficacy of zolpidem should be cautious and other studies are needed to replicate our results, It is

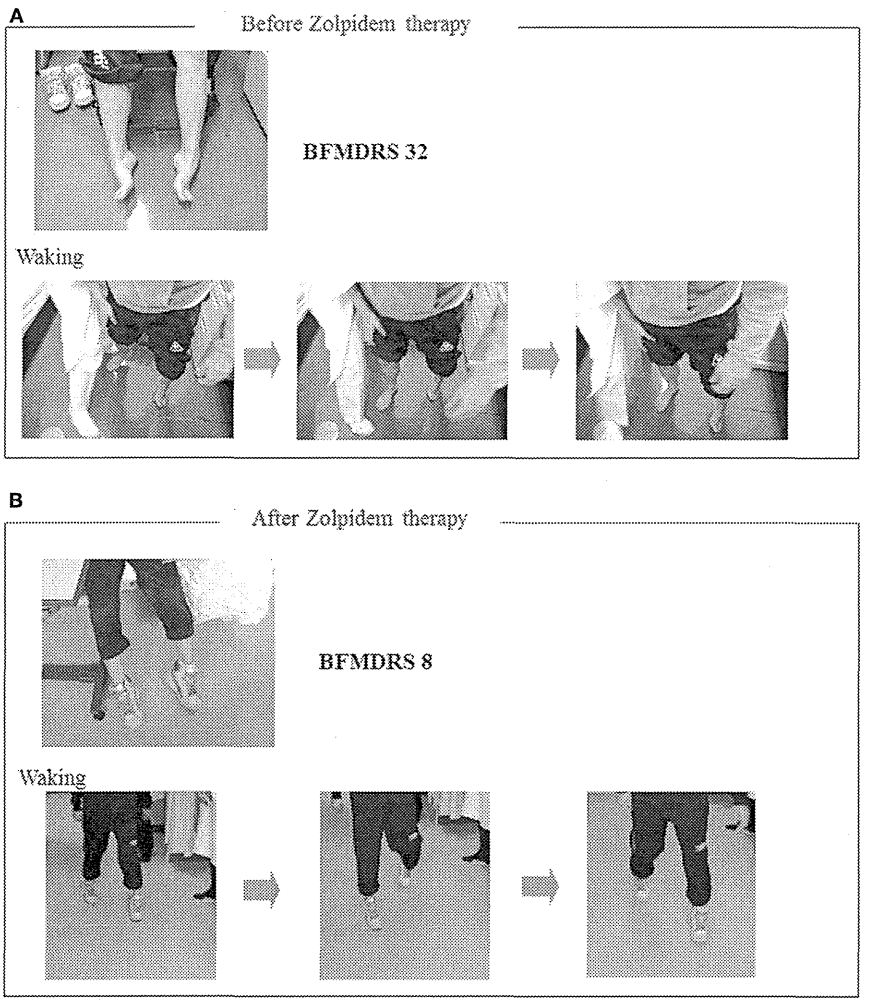

FIGURE 1 | (A) It is before treatment. She could not walk without help because of left dominant lower limbs dystonic spasm. **(B)** We started zolpidem 20 mg/day monotherapy, and at the day 14, she could walk without any support, despite the persisting inversion of the left ankle.

however unlikely that the beneficial effects are entirely placebo-based, because the patients had been equally tried on other medications with no benefit before enrollment. Moreover, improvement in the scale of the whole patients was significant. We therefore consider that zolpidem is a useful option for treating dystonia.

It was reported that some of adult onset primary focal dystonia patients spread proximally or contralaterally or become generalized within several years of symptom onset (Weiss et al., 2006). For that reason, we assessed all patients using BFMDRS, one of the major clinical dystonia scales for generalized dystonia, in this study. It would be desirable to evaluate on the scale suitable for each types of dystonia in future trials, with divided subtypes, being randomized, blinded, and placebo-controlled.

For the patients with generalized dystonia, Meige syndrome/blepharospasm, and hand dystonia, mild to remarkable improvements (29–75% improvement in BFMDRS) were observed, whereas no significant changes were found for cervical dystonia after zolpidem (**Figure 2**). Despite the small number of cases, blepharospasm was also refractory. Even within the same subtype, responsiveness to zolpidem considerably varied among patients.

We used zolpidem 5–20 mg/day for the patients with dystonia, and drowsiness was tolerated for most of the subjects. Eight out of 34 subjects complained relatively persistent drowsiness (3 cases of responders and 5 of non-responders). No correlation between drowsiness and effects to dystonia syndrome was found. It is however possible that doses used in this study may not be large enough to obtain the maximal benefit, because the previous studies used the doses up to 50–70 mg/day (Garretto et al., 2004; Young et al., 2008).

Focal hand dystonia (writer's cramp and other occupational cramps) is a primary dystonia produced by the excessive co-contraction of antagonistic muscles of the hand and forearm

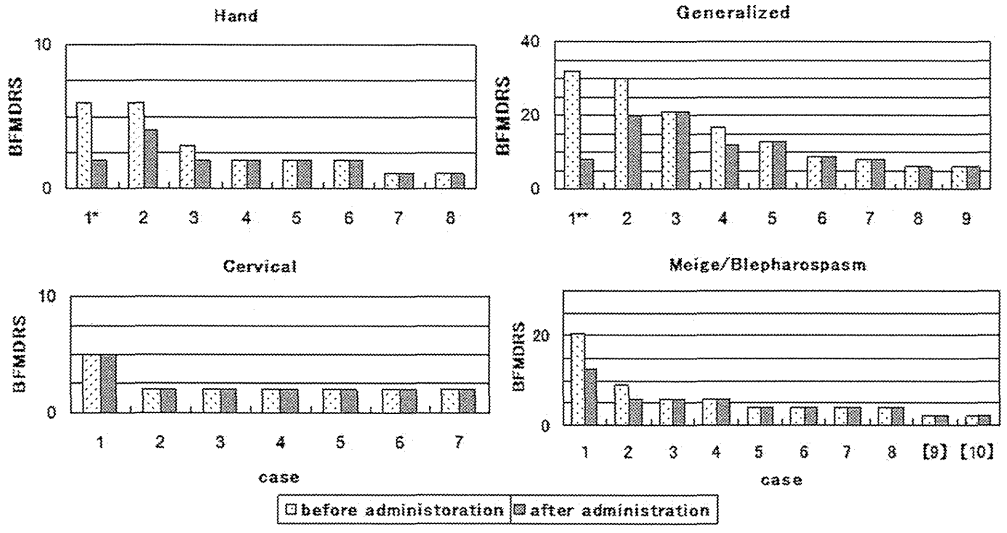

FIGURE 2 | Before and 1 month after zolpidem administration, patients with patients with generalized dystonia, Meige syndrome/ blepharospasm, cervical dystonia, and hand dystonia experienced improvement in the motor subscale of Burke–Fahn–Marsden Dystonia Rating Scale (BFMDRS). *Case 1; **Case 2; [9][10], blepharospasm.

(Sheehy and Marsden, 1982). In our study, 38% of the hand dystonia patients improved after zolpidem. In past study, botulinum toxin treatment of hand dystonia showed less favorable benefits than cervical dystonia or blepharospasm (Karp et al., 1994). Musicians' cramp or dystonia of other highly skilled performance are even more difficult to obtain the satisfactory outcome. Zolpidem is worth being tried on such patients as Case 1 in our study.

Zolpidem is an imidazopyridine agonist with a high affinity on the benzodiazepine site of $GABA_A$ receptors containing $\alpha 1$ subunit in combination with $\beta 2$ and $\gamma 2$ subunits (McKernan and Whiting, 1996; Sanna et al., 2002), equivalent to $\omega 1$ subtypes, present in interneurons in all brain areas including the hippocampus, the cortex, and the cerebellar Purkinje cells (McKernan and Whiting, 1996). Recently a high density of zolpidem binding sites was found in the thalamus (Licata et al., 2009) and the subthalamic nucleus (Chen et al., 2007), and possibly the globus pallidus (Duncan et al., 1995; Chen et al., 2004). After binding to these sites, zolpidem could enhance inhibitory pathways in the basal ganglia motor loop, accounting for the clinical improvement in dystonia.

Lack of responsiveness in cervical dystonia is unexplained in the present study. Intriguingly, the clinical improvement in cervical dystonia after globus pallidus internus deep brain stimulation (GPi-DBS) was reported to be less satisfactory than other subtypes (Starr et al., 2006; Lee et al., 2007). It might be possible that GPi-DBS and zolpidem could have similar mechanisms of action.

In conclusion, zolpidem may be a useful option for treating generalized/hand dystonias, and Meige syndrome who do not respond to botulinum toxin or oral medications.

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SUPPLEMENTARY MATERIAL

The Movies S1–S5 for this article can be found online at http://www.frontiersin.org/Movement_Disorders/10.3389/fneur.2012.00058/abstract

REFERENCES

Albanese, A., Barnes, M. P., Bhatia, K. P., Fernandez-Alvarez, E., Filippini, G., Gasser, T., Krauss, J. K., Newton, A., Rektor, I., Savoiardo, M., and Valls-Solè, J. (2006). A systematic review on the diagnosis and treatment of primary (idiopathic) dystonia and dystonia plus syndromes: report of an EFNS/MDS-ES task force. *Eur. J. Neurol.* 13, 433–444.

An, J. Y., Kim, J. S., Kim, Y. I., and Lee, K. S. (2008). Successful treatment of the Meige syndrome with oral zolpidem monotherapy. *Mov. Disord.* 23, 1619–1621.

Burke, R. E., Fahn, S., Marsden, C. D., Bressman, S. B., Moskowitz, C., and Friedman, J. (1985). Validity and reliability of a rating scale for the primary torsion dystonias. *Neurology* 35, 73–77.

Chen, L., Savio, C. C., and Yung, W. H. (2004). Electrophysiological and behavioral effects of zolpidem in rat globus pallidus. *Exp. Neurol.* 186, 212–220.

Chen, L., Xie, J. X., Fung, K. S., and Yung, W. H. (2007). Zolpidem modulates GABA(A) receptor function in subthalamic nucleus. *Neurosci. Res.* 58, 77–85.

Daniele, A., Albanese, A., Gainotti, G., Gregori, B., and Bartolomeo, P. (1997). Zolpidem in Parkinson's disease. *Lancet* 349, 1222–1223.

Duncan, G. E., Breese, G. R., Criswell, H. E., McCown, T. J., Herbert, J. S., Devaud, L. L., and Morrow, A. L. (1995). Distribution of [3H] zolpidem binding sites in relation to messenger RNA encoding the $\alpha 1$, $\beta 2$ and $\gamma 2$ subunits of GABAA receptors in rat brain. *Neuroscience* 64, 1113–1128.

Evidente, V. G. (2002). Zolpidem improves dystonia in Lubag or X-linked dystonia-parkinsonism syndrome. *Neurology* 26, 662–663.

Fahn, S., Bressman, S. B., and Marsden, C. D. (1998). Classification of dystonia. *Adv. Neurol.* 78, 1–10.

Garretto, N. S., Bueri, J. A., Rey, R. D., Arakaki, T., Nano, G. V., and Mancuso, M. (2004). Improvement of

blepharospasm with zolpidem. *Mov. Disord.* 19, 967–968.

Holm, K. J., and Goa, K. L. (2000). Zolpidem: an update of its pharmacology, therapeutic efficacy and tolerability in the treatment of insomnia. *Drugs* 59, 865–889.

Jabbari, B., Scherokaman, B., Gunderson, C. H., Rosenberg, M. L., and Miller, J. (1989). Treatment of movement disorders with trihexyphenidyle. *Mov. Disord.* 4, 202–212.

Karp, B. I., Cole, R. A., Cohen, L. G., Grill, S., Lou, J. S., and Hallett, M. (1994). Long-term botulinum toxin treatment of focal hand dystonia. *Neurology* 44, 70–76.

Lee, J. Y., Deogankar, M., and Rezai, A. (2007). Deep brain stimulation of globus pallidus internus for dystonia. *Parkinsonism Relat. Disord.* 13, 261–265.

Licata, S. C., Jensen, J. E., Penetar, D. M., Prescot, A. P., Lukas, S. E., and Renshaw, P. F. (2009). A therapeutic dose of zolpidem reduces thalamic GABA in healthy volunteers: a proton MRS study at 4 T. *Psychopharmacology (Berl.)* 203, 819–829.

McKernan, R. M., and Whiting, P. J. (1996). Which GABAA-receptor subtypes really occur in the brain? *Tends Neurosci.* 19, 139–143.

Park, I. S., Kim, J. S., An, J. Y., Kim, Y. L., and Lee, K. S. (2009). Excellent response to oral zolpidem in a sporadic case of the myoclonus dystonia syndrome. *Mov. Disord.* 24, 2172–2173.

Sanna, E., Busonero, F., Talani, G., Carta, M., Massa, F., Peis, M., Maciocco, M., and Biggio, G. (2002). Comparison of the effects of zaleplon, zolpidem, and triazolam at various GABA(A) receptor subtypes. *Eur. J. Pharmacol.* 451, 103–110.

Sheehy, M. P., and Marsden, C. D. (1982). Writer's cramp: a focal dystonia. *Brain* 105, 461–480.

Starr, P. A., Turner, R. S., Rau, G., Lindsey, N., Heath, S., Volz, M., Ostrem, J. L., and Marks, W. J. Jr. (2006). Microelectrode-guided implantation of deep stimulators into the globus pallidus internus for dystonia: techniques, electrode locations, and out comes. *J. Neurosurg.* 104, 488–501.

Weiss, E. M., Hershey, T., Karimi, M., Racette, B., Tabbal, S. D., Mink, J. W., Paniello, R. C., and Perlmutter, J. S. (2006). Relative risk of spread of symptoms among the focal onset primary dystonias. *Mov. Disord.* 21, 1175–1181.

Young, J., Kim, J. S., Kim, Y. I., and Lee, K. S. (2008). Successful treatment of the Meige syndrome with oral zolpidem monotherapy. *Mov. Disord.* 23, 1619–1620.

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Full Paper

Type A1 but Not Type A2 Botulinum Toxin Decreases the Grip Strength of the Contralateral Foreleg Through Axonal Transport From the Toxin-Treated Foreleg of Rats

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Abstract. The adverse effects of botulinum LL toxin and neurotoxin produced by subtype A1 (A1LL and A1NTX) are becoming issues, as the toxins could diffuse from the toxin-treated (ipsilateral) to contralateral muscles. We have attempted to produce neurotoxin from subtype A2 (A2NTX) with an amino acid sequence different from that of neurotoxin subtype A1. We measured the grip strength on the contralateral foreleg as an indicator of toxin spread from the ipsilateral to contralateral muscles. Doses of 0.30 log U or above of A1LL and A1NTX reduced the contralateral grip strength, whereas a dose of 0.78 log U of A2NTX was required to do so. We investigated the route of toxin spread using denervated, colchicine-treated, and antitoxin-treated rats. A1LL was transported via axons at doses higher than 0.30 log U and via both axons and body fluid at about 0.80 log U or a higher dose. Interestingly, A2NTX was transported via body fluid at about 0.80 log U or a higher dose, but not via axons to the contralateral side. It was concluded that A1LL and A1NTX decreased the grip strength of the toxin-untreated foreleg via both axonal transport and body fluids, while A2NTX was only transported via the body fluid.

Keywords: botulinum toxin, grip strength (rat), neurotomy, colchicine, axonal transport

Introduction

Botulinum toxins have been researched and developed for use as important therapeutic agents for neurological disorders such as blepharospasm, hemifacial spasm, various dystonias, and overactive bladder (1 – 3). The toxins are protein complexes containing a 150-kDa neurotoxin (NTX) and nontoxic components. Type A protein complexes, called progenitor toxins, have molecular weights of 900 (LL toxin), 500 (L toxin), or 300 (M toxin) kDa (4). LL and L toxins have nontoxic components exhibiting hemagglutinin (HA) activity, whereas the nontoxic components of M toxin have no HA activity. The NTX component consists of heavy (100 kDa) and light (50 kDa) chain components held together by a disulfide bond (5). The heavy chain contains the translocation (N-termi-

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