おわりに

虚弱は低栄養状態，サルコペニア，精神状態の悪化を含む概念であったが[1]，近年では，認知機能低下や社会的支援の不足，多剤併用などを含めた多次元的な概念として考えられている[15]．またサルコペニアも同様に，筋量や筋力の低下だけではなく，ADLの低下をその概念に含め，加齢以外にも疾病や不活動，低栄養などさまざまなリスク因子が同定されるなど[2]，エビデンスの集積とともにパラダイムシフトが進んでいる．虚弱とサルコペニアの予防と改善に際しては，栄養状態や運動機能に関する検査，また生理・生化学的検査や身体活動量やADLに関する調査などを含めた包括的な評価と，多面的なアプローチが必要といえる．

高齢化が急速に進む日本の社会において，高齢者の健康維持・増進は極めて重要な課題である．虚弱やサルコペニアは高齢者におけるADLやQOLの低下要因であり，寝たきりや廃用症候群の原因ともなることから，虚弱やサルコペニアの予防と改善は，高齢者の健康長寿を考える場合には不可欠といえる．そのためのエビデンスを集積する研究として，疾患そのものだけでなく，栄養，運動などの生活習慣から遺伝的素因までを含めた学際的な長期縦断疫学研究の進展が望まれる．

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骨格筋幹細胞―最新基礎知見を踏まえて

Muscle satellite cells: Possible role in sarcopenia

橋本 有弘*
Hashimoto Naohiro

抄録▶加齢依存的筋量減少症（サルコペニア）の発症機序については，まだ不明の点が多く，科学的証拠に基づいた予防法・治療法の開発は，今後の大きな課題である．最近，サルコペニアの発症に骨格筋に存在する組織幹細胞「筋サテライト細胞」が関与している可能性が議論され，サルコペニアにおける筋幹細胞の役割が注目されている．最新の知見を踏まえて，サルコペニアにおける筋サテライト細胞の役割について解説する．

Key Words 筋サテライト細胞，筋再生，サルコペニア，筋線維

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はじめに

加齢に伴って骨格筋量が減少する傾向は，広く知られており，それ自体は必ずしも疾患とはいえない．しかし，筋量の減少が著しい場合には，運動機能の低下が著しく，日常的な生活や活動を行うことが困難になる．日常生活に支障をもたらすほどの筋量減少は，「サルコペニア（sarcopenia，筋肉減少症）」と呼ばれ，加齢に伴って発症する疾患として定義されるに至った[1]．著しい筋量の減少は，高齢者の「生活の質（QOL）」を規定する要因であり，かつ高齢者の転倒事故の主な原因のひとつであると考えられている[2]．しかし，サルコペニアの発症機序については，まだ不明の点が多く，科学的証拠（scientific evidence）に基づいた予防法・治療法の開発は，今後の大きな課題である．最近，サルコペニアの発症に骨格筋に存在する組織幹細胞「筋サテライト細胞」が関与している可能性が議論され，サルコペニアにおける筋幹細胞の役割が注目されている．

骨格筋（横紋筋）は，成人においても高い再生能力を保持していることが知られている．近年，従来再生しないと考えられてきた心筋組織においても再生が報告されているが，実験的に証明された横紋筋の再生能力は，飛び抜けて高い．マウスやラットの骨格筋に物理的傷害あるいは化学的傷害を与えた場合，おおむね2～3週間後には再生筋線維が形成され，4～8週間後にはほぼ完全に組織修復が終了する．このような骨格筋の再生能力は，トレーニングやリハビリテーションによる筋力増進の機序と密接に関わっていると考えられている．

本稿では，運動機能の低下に直接関係する骨格筋の筋サテライト細胞について議論するが，その内容は骨格に結合していない横紋筋についてもあてはまると考えて差し支えない．

骨格筋の構造と筋サテライト細胞の特性

骨格筋の機能を担っている最終分化細胞は，

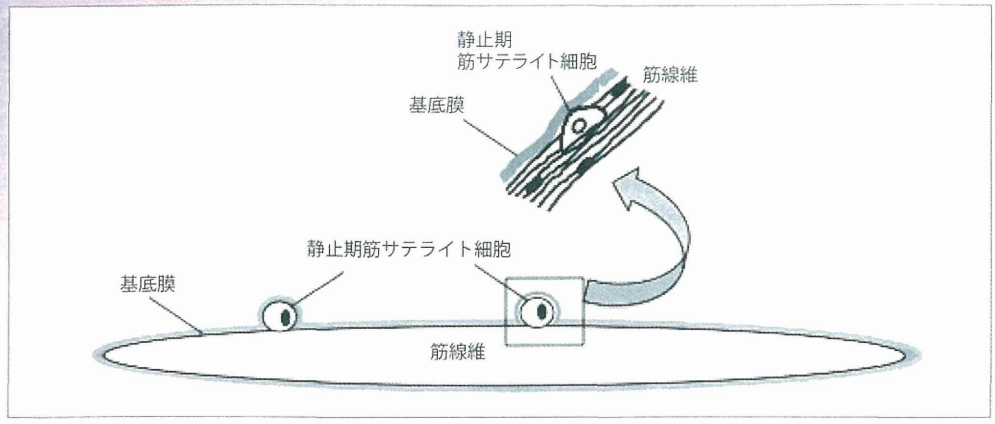

図1　筋線維とサテライト細胞

筋線維と筋サテライト細胞は，基底膜によって包み込まれており，非筋細胞と区画化されている．

「筋線維(myofiber)」と呼ばれる巨大な多核細胞である．筋線維は，未分化な筋細胞(筋芽細胞，筋前駆細胞)が細胞融合することによって形成される．骨格筋組織は，この筋線維が束状に詰め込まれた袋にたとえることができ，その両端は腱によって骨につながれている．病理学的解析によって，筋線維の細胞膜上には単核の細胞が接着していることが知られていた．これが骨格筋組織の特異的な幹細胞，「筋サテライト細胞」である．筋サテライト細胞は，M-cadherinなどの接着分子を介して筋線維と接着している．また，筋線維と筋サテライト細胞は基底膜によって包み込まれており，線維芽細胞や血球系細胞などの非筋細胞とは空間的に明確に区画化されている(図1)．すなわち，筋サテライト細胞は基底膜と筋線維の細胞膜に挟まれて存在しており，この場所は幹細胞の維持に必要な条件を満たす特殊な場所「ニッシェ（niche)」であると考えられ，「satellite cell position」とも呼ばれている．筋組織に含まれる筋サテライト細胞数は，極めて少なく，高々全核数の1～5%あるいはそれ以下である．

筋サテライト細胞を識別することのできるさまざまなマーカー・タンパク質が発見され，動態解析に用いられている．なかでもペアード・ホメオボックス転写因子Pax7は，最も広く用いられている．しかし，いずれのマーカーに関してもすべての筋サテライト細胞が発現しているわけではないという批判的報告がある．また，筋サテライト細胞特異的な表面抗原として，マウスではVCAM，ヒトではNCAM（CD56)という接着分子が同定され，筋サテライト細胞の分離に用いられている．しかし，VCAMはヒト筋サテライト細胞，NCAMはマウス筋サテライト細胞のマーカーとはならない．マウス筋サテライト細胞に関する解析結果を，単純にヒト筋サテライト細胞に外挿することの危険性には留意しなければならない．

筋再生機序と筋サテライト細胞

成体筋組織中に存在する筋サテライト細胞は，休止期にあって分裂増殖しない，あるいは極めてゆっくり細胞周期を進行しているものと考えられている．骨格筋が重篤な傷害(実験的な筋傷害がこれにあたる)を受けた場合，損傷部域の筋サテライト細胞は，筋線維とともに死滅する．一方，同じ筋組織中に含まれる損傷を受けていない筋線維上の筋サテライト細胞は，筋損傷によって生じる未知の刺激によって活性化され，細胞分裂を開始する．筋サテライト細胞は，

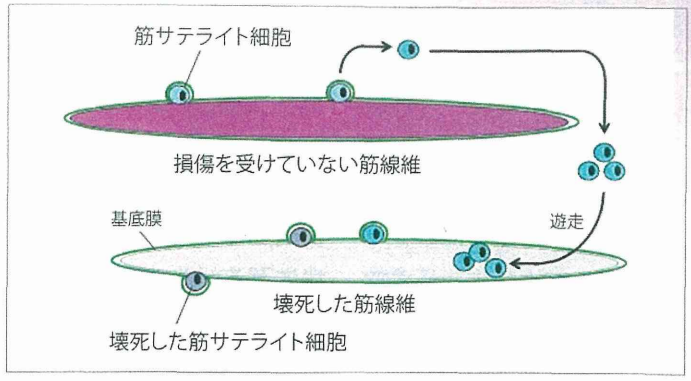

図2　筋再生における筋サテライト細胞の役割
骨格筋が損傷を受けると，筋サテライト細胞は活性化され，細胞分裂を繰り返しつつ，損傷箇所まで遊走し，筋線維を再生する．

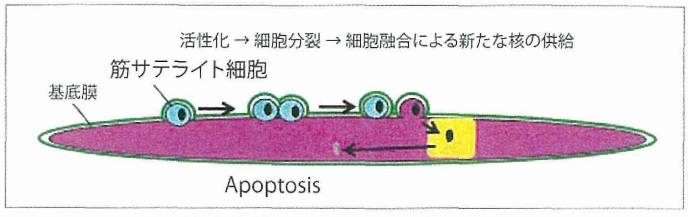

図3　筋線維核のターンオーバー・サイクル
筋サテライト細胞は，筋線維へ新たな核を供給し，筋線維の核数が減少し，筋萎縮に至ることがないように恒常性を担保している．

さらに分裂を繰り返しつつ，MyoD陽性の筋前駆細胞(筋芽細胞)となり，損傷箇所まで遊走する．筋芽細胞は，残存する基底膜を通り抜け，部分的に壊死した残存筋線維と融合して再生するか，あるいは他の筋芽細胞と融合して新たな筋線維をつくる(図2)．これまでに蓄積された病理学的，分子生物学的解析の結果は，再生筋線維のほとんどが筋サテライト細胞に由来するものであることを示している．

実験的筋再生の結果は，骨格筋および筋サテライト細胞の潜在的な筋再生能力を明瞭に示している．しかし，実験的に示された筋サテライト細胞の潜在的再生能力は，必ずしも生理的役割を反映しているとは限らない可能性がある．

筋サテライト細胞の生理的機能と筋線維の維持

私たちは，休止期にあると思われていた「satellite cell position」にあるラット筋サテライト細胞の約15％が細胞分裂周期を回っていることを示す知見を得ている(梅田，橋本，未発表)．すなわち，筋再生時に限らず，筋サテライト細胞は，常時，一定頻度で活性化されている．しかし，正常筋組織を組織学的に検索しても，筋サテライト細胞(あるいは筋前駆細胞)が分裂増殖して3個以上の細胞集団(クラスター)を形成している組織像は，ほとんどみられない．したがって，正常筋組織における筋サテライト細胞の細胞増殖は，1～2回の分裂で終わる単発的な現象であり，分裂によって生じた子孫細胞は，直ちに筋線維と融合してしまうものと推定される．それゆえ，私たちは，「筋サテライト細胞の本来の役割は，(筋組織の再生ではなく)恒常的に低頻度で細胞分裂することによって筋線維に新たな核を供給し，筋線維の機能を維持することにあ

る」という仮説を提唱してきた(図3).

筋線維は，数百以上の核を含む，巨大な多核細胞である．組織病理学的解析の結果から，筋線維は，「細胞」として置き換わることはないと考えられてきた．しかし，筋線維の個々の核には寿命があり，ラット骨格筋では1週間に1～2%の核がターンオーバーしていることが示唆されている[3]．これは，上記の仮説を支持する．一方，最近，遺伝子改変マウスを用いて，細胞の運命を前向きにたどる「cell lineage tracing」という解析方法を用いて，実験的筋損傷後の再生筋線維の核は，筋サテライト細胞に由来する[4]が，生理的な刺激を与えただけでは，成体マウスの筋サテライト細胞から筋線維に新たな核が供給されることはないことが示唆された．

筋線維核の増減は，現象としては報告されているが，その機構については明らかになっていない．何を原因として，どのような機序で筋線維核が増減するのか，新たな核はどのようにして供給されるのかなど，未解決の課題が残されている．

筋再生能力の加齢変化とサルコペニア

老化マウスあるいはラットの骨格筋に損傷を与えて実験的に再生を誘導した場合，筋組織は若い個体の場合と同様に，ほぼ完全に再構築される．しかし，筋再生過程を詳細に検討すると，老化個体では再生過程に明らかな遅延が認められる[5]．

このような加齢に伴う筋再生能力の低下は，サルコペニアの発症と，どのように関わっているのであろうか．サルコペニア筋においては，廃用性筋萎縮にみられるような筋線維細胞内の構造異常は少なく，筋線維の縮小(横断面積の縮小)および筋線維数の減少が認められる[6]，といわれている．しかし，筋線維の壊死や損傷がサルコペニア発症の原因であることを示す知見は得られていない．すなわち，老化に伴う筋再生能力の低下によって筋線維の壊死が誘導され，それを再生が補いきれなくなる結果，サルコペニアが発症するとは考え難い．

サルコペニアの発症をもたらす要因としては，生理的要因(生体からみた内的要因)，環境要因(外的要因)などがあり，サルコペニアの発症に至る経路は多様である．筋量減少をもたらす筋線維の加齢変化として，筋線維の萎縮(径の縮小)，筋線維核数の減少および筋線維数の減少が報告されている．このような筋線維の加齢変化に対して，筋サテライト細胞は，細胞融合を介して抑制的に働くものと考えられる．「筋再生能力の低下」は，「加齢による筋サテライト細胞–筋再生系の機能低下」を示唆しており，その結果，加齢に伴う筋量減少が加速化されるのではないかと考えられる．

筋サテライト細胞の加齢変化

加齢に伴う筋線維の変化として，筋線維核数の減少が報告されている[7,8]．筋線維における核数の減少は，筋タンパク質の総合成量の低下をもたらすため，筋線維の縮小および筋量減少に直結すると考えられる．筋線維における核数の減少は，筋サテライト細胞から筋線維への核の供給が滞ることを示唆している．老化によって筋線維核のターンオーバー機構が破綻すれば，筋線維は矮小化し，筋量減少を導く可能性がある．

加齢に伴う筋サテライト細胞数の変化については，従来，相反する結果が報告されてきた．しかし，遺伝子改変マウスや優れた表面抗原マーカーが導入された結果，老化マウスでは筋サテライト細胞が1/2～1/3に減少することが複数の研究者によって確認された．

数的減少以外に，筋サテライト細胞には，加齢に伴う何らかの機能低下が生じているのだろうか．従来，初代培養の結果から高齢者由来筋細胞の増殖能は，低下していると結論されてい

た．しかし，私たちは，培養条件を至適化し，高齢者の筋サテライト細胞の増殖・分化能には，加齢による低下が認められないことを確認した(橋本，岡村ら，未発表)．マウスでの解析では，筋サテライト細胞の中に増殖特性の異なる2群が存在し[9,10]，それらの比率は加齢に伴って変化するという．ヒトにおいても，高齢者の筋サテライト細胞の中に，増殖・分化能の低下した細胞が存在する可能性があり，今後の検討課題である．

筋サテライト細胞の数的・質的加齢変化以上に，加齢に伴う筋組織内の微小環境(内分泌因子など)の変化が，筋再生能力の低下に大きな影響を与えうることが報告されている．老化ラット筋組織を若いラット骨格筋へ移植すると高い再生能力を示すこと[11]，老化マウス由来サテライト細胞を若いマウスの筋組織へ移植すると若いマウス由来のサテライト細胞と同程度の筋再生能力を示すこと(上住–池本，橋本，未発表)から，微小環境の加齢変化は，筋サテライト細胞の増殖・分化に対して抑制的に作用する可能性が高い．

マウスにおいては，加齢に伴う内分泌因子の低下が，筋サテライト細胞のNotchシグナル系の低下をもたらし，筋再生を抑制する，といわれている[12,13]．動物実験の結果は，『加齢に伴う筋組織内微小環境の変化が原因となって引き起こされる筋サテライト細胞の増殖・分化抑制』が，筋再生能力の低下の主な原因であり，加齢に伴う筋サテライト細胞の数的減少と質的変化は，筋再生能力の低下を加速化する可能性を示している．しかし，これらの結果をヒト骨格筋の加齢変化に外挿することが妥当であるか否かについては，今後の検討を待たねばならない．

動物モデルとヒト・サルコペニア

サルコペニアの発症機序を解明し，科学的証拠に基づいた予防法・治療法を開発するうえで，動物モデルは必須である．しかし，現時点では，サルコペニアの適切な動物モデルは確立されていない．老化マウスやラットにみられる筋量減少は，わずかであり，組織病理学的変化も極めて軽微である．一方，筋力が低下した高齢者の筋組織を解析すると，極めて著しい筋線維数の減少と脂肪化が認められる．また，マイクロアレイ解析の結果は，ヒト骨格筋とマウス骨格筋では，老化に伴って変化する遺伝子群にかなり大きな違いがあることを示している[14,15]．これらのことから，そもそも，60～70歳のヒトで発症する筋量減少という慢性的な加齢変化が，寿命が2～3年しかないマウスやラットでも再現されるだろうという前提自体に無理があるのではないかとも考えられる．まず，ヒト・サルコペニア筋の詳細な解析を行い，その結果を基盤として，「筋量減少」そのものではなく，サルコペニア発症に至る素過程を反映した現象(例えば，あるホルモンに対する応答変化など)に着目して動物モデルを構築することが，遠回りに見えて実は近道なのかもしれない．

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Geriatrics Gerontology INTERNATIONAL 1st Impact Factor in June 2010 Medline Indexed

Geriatr Gerontol Int 2012; **12:** *425–430*

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Polypharmacy as a risk for fall occurrence in geriatric outpatients

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Objective: To investigate the predictors of falls, such as comorbidity and medication, in geriatric outpatients in a longitudinal observational study.

Methods: A total of 172 outpatients (45 men and 126 women, mean age 76.9 ± 7.0 years) were evaluated. Physical examination, clinical history and medication profile were obtained from each patient at baseline. These patients were followed for up to 2 years and falls were self-reported to their physicians. The factors associated with falls were analyzed statistically.

Results: A total of 32 patients experienced falls within 2 years. On univariate analysis, older age, osteoporosis, number of comorbid conditions and number of drugs were significantly associated with falls within 2 years. On multiple logistic regression analysis, the number of drugs was associated with falls, independent of age, sex, number of comorbid conditions and other factors that were significantly associated in univariate analysis. A receiver–operator curve evaluating the optimal cut-off value for the number of drugs showed that taking five or more drugs was a significant risk.

Conclusion: In geriatric outpatients, polypharmacy is associated with falls. Intervention studies are needed to clarify the causal relationship between polypharmacy, comorbidity and falls. **Geriatr Gerontol Int 2012; 12: 425–430.**

Keywords: bone/musculo-skeletal, elderly, falls, geriatric medicine, internal medicine, polypharmacy.

Introduction

Previous studies have assessed the risk factors for falls in community-dwelling elderly,[1–3] but not in geriatric outpatients, and history of falls, physical ability and living environment were found to be predictors of falls. Outpatients have different characteristics from community-dwelling elderly, and previous studies have not assessed whether medical comorbidity and therapeutic drugs might be risk factors for falls. Falls in patients on medication are complicated, because some drugs, such as aspirin, can cause serious bleeding when they have injurious falls, and others, such as antihypertensive[4] and hypoglycemic[5,6] agents, can cause falls.

Previously, we reported that polypharmacy was associated with the tendency for falls using four indices of fall tendency in a cross-sectional setting in geriatric outpatients,[7] though that study did not evaluate fall occurrences, and also not in a longitudinal manner. Therefore, we aimed at investigating whether polypharmacy was predictive of fall occurrences in a prospective fashion. For this purpose, we followed geriatric outpatients for up to 2 years, and assessed whether polypharmacy is a risk for fall occurrence, together with other risks.

Accepted for publication 19 October 2011.

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doi: 10.1111/j.1447-0594.2011.00783.x

The validity of two novel indices of fall tendency, the 22 items fall risk index[8] and the 13 points simple screening test,[3] which were used in our previous study, have been confirmed in community-dwelling elderly, but not in geriatric outpatients. Therefore, in the present investigation, the association of these two indices with falls was also evaluated to confirm their validity in geriatric outpatients in a longitudinal study.

Methods

Patients

From 2006 to 2007, a total of 190 consecutive patients aged 65 years or older who were receiving treatment for chronic diseases, such as hypertension, dyslipidemia, diabetes and osteoporosis, who were seen every 2–4 weeks at the outpatient clinic of the Research Institute of Aging Science, Tokyo, were enrolled. All the patients were able to walk independently and their condition was stable. Patients who had acute illness or overt dementia were excluded. Anthropometric and medical information including past history of stroke, myocardial infarction, malignancy and prescribed drugs was obtained from each patient at baseline from the medical chart recorded by the physician in charge. However, 18 patients were excluded, because they were lost to follow up soon after enrolment and the medical information was not fully obtained. All prescribed drugs had not been changed in the included patients for at least 2 months before enrolment. The patients were followed up for 2 years.

Occurrence of falls

During the follow-up period, the patients and their family members responded to the annual questionnaire asking about the occurrence of falls within the past year. The questionnaire was repeated for 2 years.

Indices of fall tendency

After enrolment, the patients were examined for two indices to investigate the fall tendency. These were (i) a questionnaire of the 22 items portable fall risk index;[8] and (ii) the 13 points simple screening test to assess the fall tendency.[3]

Ethical consideration

The present study was approved by the Institutional Review Board of the Research Institute of Aging Science. We obtained written consent from all participants and/or their guardians.

Data analysis and statistical methods

Values are expressed as mean ± standard deviation. In order to analyze the relationship between falls and comorbidity or drugs, variables were compared using Student's *t*-test or χ^2-test as appropriate. Significant factors found in univariate analysis were included in multivariate logistic regression analysis to determine the association of falls with other variables. Receiver–operating curve (ROC) analysis was carried out to identify the optimal cut-off value of the number of drugs for predicting falls within 2 years. The value with the highest sum of sensitivity and specificity was used as the optimal cut-off value. Logistic regression analysis was carried out to assess the validity of the two indices of fall tendency, adjusted by age and sex. *P*-values <0.05 were considered statistically significant. Data were analyzed using JMP version 8.0.1 (SAS Institute, Cary, North Carolina, USA).

Results

Baseline medical information and two indices of fall tendency were evaluated in 172 patients (Table 1). Drugs prescribed in less than 5% of the patients are not shown. Because only patients who were in a stable condition and were able to walk independently were included, patients with Parkinson's disease, severe paresis or painful arthralgia were not included. Calcium channel blockers prescribed in the present study were all long-acting agents, and the prescribed aspirin dosage was 100 mg in all cases. Only a few patients were receiving insulin therapy, sulfonylureas, angiotensin converting enzyme inhibitors, β-blockers, α-blockers, non-steroidal anti-inflammatory drugs or anticoagulants. No patients were taking neuroleptics or antiparkinsonian drugs.

After 1 year, all patients, except for one who died of congestive heart failure, were followed up ($n = 171$, follow-up rate 99.4%). Falls occurred in 22 patients. Only a higher age was associated with falls within 1 year on univariate analysis (non-fallers: 76.4 ± 6.8 years, fallers: 81.0 ± 6.9 years, $P = 0.004$).

After another year (2 years after enrolment), one patient had died of lung cancer, and five patients were lost to follow up. A total of 165 patients were evaluated (follow-up rate 95.9%), and 10 patients had fallen during the second year; thus a total of 32 patients had fallen within 2 years. As shown in Table 2, higher age, osteoporosis, number of comorbid conditions and number of drugs were significant factors associated with falls. To determine the association of falls with these significant factors, multivariate logistic regression analysis was carried out, and as shown in Table 2, the number of drugs was the only factor that was significantly associated with falls within 2 years.

As polypharmacy was assumed to be a risk for falls within 2 years, the cut-off of the number of the drugs was analyzed. Figure 1 shows the ROC curves to define the optimal cut-off point in relation to falls within

Table 1 Characteristics and univariate analysis of association with fallers and non-fallers within 2 years and risk factors

Total		Non-fallers ($n = 133$)	Fallers ($n = 32$)	P-value (Fallers vs. Non-fallers)
Age (years)	77.0 ± 7.0	76.3 ± 6.9	80.0 ± 6.9	0.007
Body mass index (kg/cm^2)	22.7 ± 3.2	22.7 ± 3.3	22.7 ± 3.1	0.98
No. comorbid conditions	1.9 ± 1.1	1.8 ± 1.1	2.3 ± 0.9	0.009
No. drugs	3.2 ± 2.8	2.8 ± 2.7	4.9 ± 2.5	<0.0001
Female ($n = 122$)	–	72.9%	78.1%	0.66
Hypertension ($n = 106$)	–	62.4%	71.8%	0.41
Dyslipidemia ($n = 76$)	–	47.3%	40.6%	0.56
Diabetes ($n = 23$)	–	12.8%	18.8%	0.40
Osteoporosis ($n = 59$)	–	30.8%	56.3%	0.01
History of stroke ($n = 6$)	–	2.3%	9.4%	0.09
History of myocardial infarction ($n = 3$)	–	0.8%	6.3%	0.10
History of cancer ($n = 8$)	–	5.3%	3.1%	0.99
Calcium channel blocker ($n = 59$)	–	33.3%	46.9%	0.16
Angiotensin II receptor blocker ($n = 56$)	–	33.3%	37.5%	0.68
Statin ($n = 40$)	–	23.5%	28.1%	0.65
Aspirin ($n = 31$)	–	19.0%	24.1%	0.61
Bisphosphonate ($n = 9$)	–	4.6%	9.4%	0.38
H2-blocker ($n = 9$)	–	3.8%	12.1%	0.80
Proton pump inhibitor ($n = 11$)	–	5.3%	12.1%	0.23
Hypnotic ($n = 31$)	–	16.7%	28.1%	0.14

Values are expressed as mean ± SD ($n = 165$).

Table 2 Logistic regression analysis of association of falls within 2 years with age, sex, other significant factors found in univariate analysis, and polypharmacy

	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Age (/1 year)	1.08 (1.03–1.13)†	1.06 (0.99–1.13)	1.06 (0.99–1.13)
Sex (male = 0, female = 1)	1.39 (0.56–3.48)	0.98 (0.29–3.23)	0.75 (0.23–2.38)
Osteoporosis ($n = 0$, Y = 1)	3.12 (1.43–6.84)†	2.76 (0.92–7.38)	3.02 (0.96–6.15)
No. comorbid conditions (/disease)	1.63 (1.14–2.32)*	0.90 (0.55–1.47)	0.99 (0.62–1.56)
No. drugs (/drug)	1.29 (1.12–1.48)‡	1.30 (1.08–1.57)*	–
Five or more drugs ($n = 0$, Y = 1)	5.04 (2.25–11.3)‡	–	4.50 (1.66–12.2)†

*$P < 0.05$, †$P < 0.005$, ‡$P < 0.0005$. CI, confidence interval.

2 years: the area under the ROC was 0.731, and the optimal cut-off value of the number of drugs was five (sensitivity 0.576, specificity 0.788). Logistic regression analysis showed that taking five or more drugs was significantly associated with an increased risk of falls (odds ratio 4.5, 95% CI 1.7–12.2) after adjustment for age, sex, osteoporosis and number of comorbid conditions (Table 2).

Also, the association between falls and two indices of fall tendency was evaluated to confirm the validity of each index in geriatric outpatients. As both indices included the questionnaire asking whether patients were "taking five or more drugs," the number of drugs was excluded from this analysis because of duplication in the statistical model. As shown in Table 3, the 22 items fall risk index showed a tendency towards an association with falls within 2 years, odds ratio 1.12 (95% CI 1.00–1.26; $P = 0.05$), whereas the 13 points screening test was significantly associated with falls after adjustment for age, sex and other factors significantly associated in the univariate analysis. Therefore, these indices are considered to be good predictors of falls in geriatric outpatients, as has been shown in community-dwelling elderly subjects.

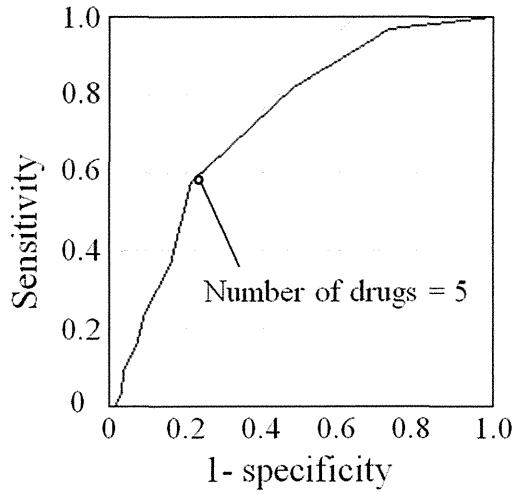

Figure 1 Receiver–operating curves to define optimal cut-off value of number of drugs at baseline in relation to falls within 2 years. Area under the curve was 0.731, optimal cut-off value of the number of drugs was five (sensitivity = 57.6%, specificity = 78.8%).

Discussion

The risk of falls has been assessed in community-dwelling elderly, and history of falls, physical ability and living environment were found to be predictors of falls. Also, in nursing home residents, cognitive function, gait disturbance and urinary incontinence are reported to be risk factors for falls,[9,10] and length of stay, disease condition, surgical procedures and some specific drugs are reported to be risk factors in hospital inpatients.[11,12]

Nevertheless, the risks in geriatric outpatients have not been sufficiently assessed, although assessment of fall risk in geriatric outpatients is important; their medical conditions or drugs might cause falls, and drugs, such as antiplatelet agents or anticoagulants, might cause critical bleeding after a fall. Also, physicians could prevent falls in their patients by giving advice during regular consultations, if risk factors are identified.

In our previous cross-sectional study assessing geriatric outpatients, polypharmacy was significantly correlated with indices of fall tendency, and the present follow-up study of geriatric outpatients showed the impact of polypharmacy on falls within 2 years. Statistical analyses showed that polypharmacy was a risk factor for falls, independent of age, sex and comorbidity.

Besides polypharmacy, several medications and comorbid conditions have been reported as risks for falls.[13–22] Among these, diabetes,[5,6] insomnia,[13] hypnotics,[13–15] antiarrhythmics[22] and antihypertensive agents[14] were not significantly associated with fall risk in the present study. Just 11 patients (45.9% of diabetic patients) were prescribed hypoglycemic agents, such as a sulfonylurea ($n = 8$) or insulin ($n = 3$), and the relatively low rate of prescription of hypoglycemic agents might have affected our result. Neither hypnotics nor antihypertensives were associated with falls. This result might be a result of the small sample size. Anti-arrhythmics were taken by just three patients (digoxin: $n = 2$, class IA anti-arrhythmic drug: $n = 1$). Other drugs, such as major tranquillizers,[14] antidepressants[17,18] and antiparkinsonian agents,[19,22] might increase fall risk; however, no patient used these drugs in the present study. In the present study, most of the patients were in a stable condition throughout the 2 years, though their drugs were changed gradually according to their medical conditions during the observation period. We only used the number of drugs at baseline for statistical analysis; however, the number of drugs increased from 3.2 ± 2.8 to 3.9 ± 3.0 during the 2 years. There were 17 patients whose number of drugs had been decreased, 70 patients not changed and 78 patients increased. The number of drugs after 2 years was also associated with falls ($P < 0.0005$). The optimal cut-off point for the number of drugs was again five (area under ROC curve 0.780, sensitivity 0.576, specificity 0.788). Furthermore, the changes in number of drugs were also associated with falls ($P < 0.05$), and the optimal cut-off point for the change in number of drugs was +1 (area under ROC curve 0.649, sensitivity 0.727, specificity 0.409).

Table 3 Logistic regression analysis of association between 2-year fall occurrences with two indices of fall tendency; 22 items fall risk index and 13 points simple screening test

	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Age (/year)	1.08 (1.03–1.15)**	1.06 (0.99–1.13)	1.06 (1.00–1.13)
Sex (male = 0, female = 1)	1.39 (0.56–3.48)	0.75 (0.23–2.43)	0.79 (0.24–2.56)
Osteoporosis ($n = 0$, Y = 1)	3.12 (1.43–6.84)**	2.56 (0.96–6.82)	2.61 (0.98–6.95)
No. comorbid conditions (/disease)	1.63 (1.14–2.32)*	1.24 (0.83–1.86)	1.32 (0.88–1.97)
Fall risk index (/item)	1.23 (1.11–1.37)***	1.12 (1.00–1.26)	–
Simple screening test (/point)	1.19 (1.06–1.33)**	–	1.14 (1.01–1.29)*

*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$. CI, confidence interval.

Consequently, polypharmacy, especially taking five or more drugs, should be considered a risk for falls.

There were several limitations of the present study. First, the falls were self-reported by the patients. Although all the patients had no overt dementia, they might have forgotten the incident of falling. We attempted to count the total fall occurrences in each patient; however, we could not differentiate the repeated falls in the second year from the fall occurrence in the first year. In fact, we asked 22 patients who reported falls in the first year about fall occurrence during the second year, but they did not accurately recall whether they experienced falls in the first or second year. Second, five patients were lost to follow up at 2 years for unknown reasons. The follow-up ratio was acceptable, although some of the patients might have fallen, have been no longer able to come to the clinic and moved to nursing homes. This might have slightly influenced the result. Also, the cause of falls in polypharmacy patients is not explained. Potentially inappropriate medications, which could cause adverse drug reactions, are usually seen in patients with polypharmacy, and falls might be the consequence of adverse drug reactions, such as dizziness, instability and light-headedness. Pathophysiological assessments and drug-reducing interventions are expected to elucidate the causal relationship.

Additionally, we showed that the 22-item fall risk index and its simple screening test were useful to predict falls in geriatric outpatients. Although both indices have been validated in community-dwelling elderly people, the present finding also showed their association with fall risk among geriatric outpatients. The difference of statistical significance between fall risk index and simple screening test might be a result of small sample size or the difference in the contribution of each item to total scores between the two indices. "Taking five or more drugs" accounts for only one item out of the 22-item fall risk index; in contrast, the same questionnaire accounts two points in the 13-point simple screening test. Because polypharmacy was a strong risk factor of falls in elderly outpatients in the present study, the proportion of polypharmacy in the scores might have caused the discrepancy. Taken together, it is likely that 13-point screening test was more suitable to our subjects who were taking several medicines.

In summary, the present study showed that geriatric outpatients with polypharmacy were at a high risk of falls, especially those receiving five or more drugs. Our finding might add new information for pharmacotherapy and geriatric research in elderly patients with chronic diseases. Intervention studies examining the effect of drug reduction for the prevention of falls are required in the future.

Acknowledgment

We thank Ms Fumie Tanaka for her excellent technical assistance. This study was financially supported by grants from the Ministry of Health, Labour and Welfare of Japan (H21-Chouju-Ippan-005, H22-Chouju-Shitei-009).

Disclosure statement

The authors declare no conflict of interest.

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has determined that the authors have no financial or any other kind of personal conflicts with this paper.

Author Contributions: Paul Regal designed the study, assessed patients, served on the consensus panel, analyzed the data, and wrote the article. Eileen Heatherington performed cognitive tests and was a panel member for consensus diagnosis of dementia.

Sponsor's Role: No sponsor.

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FACTORS ASSOCIATED WITH PROLONGED HOSPITAL STAY IN A GERIATRIC WARD OF A UNIVERSITY HOSPITAL IN JAPAN

To the Editor: We read with interest the article by Lakhan and colleagues,[1] which showed the high prevalence and worsening of geriatric syndrome during acute care hospitalization. Because falls, incontinence, impairment in activities of daily living (ADLs), and other geriatric syndrome components increase the care burden and limit discharge planning in acute care hospitals, geriatric syndrome might cause prolonged hospital stays. A prolonged hospital stay is one of the major determinants of medical cost and is thus a serious problem in geriatric medicine. Previous studies have shown that clinical events during hospitalization,[2,3] basic ADLs,[4] and nonmedical factors such as delayed transfer to a nursing facility or disagreement on the discharge plan among family members[5] are risk factors for prolonged hospital stay. Furthermore, because older adults have multiple comorbid conditions and are susceptible to adverse drug reactions (ADRs), these factors might be related to length of hospital stay. To test this hypothesis, the association between geriatric conditions such as geriatric syndrome, ADLs, and ADRs and prolonged hospital stay were comprehensively investigated using the database of the geriatric ward of the University of Tokyo Hospital from 1995 to 2010. The ethics committee of the Graduate School of Medicine, University of Tokyo approved this study.

All records of patients aged 65 and older from 1995 to 2010 were reviewed. Data on length of stay, acute hospitalization, ADRs, body mass index (BMI), number of diseases and drugs, geriatric syndrome, and Barthel Index were collected. Twenty-three components of geriatric syndrome such as falls, cognitive impairment, urinary incontinence, constipation, and insomnia were included in the analysis. Records lacking information on any of the variables were excluded. Cases of scheduled short-term hospitalization were excluded. Finally, the records of 1,616

Table 1. Characteristics of Study Patients and Analyses for Length of Hospital Stay (N = 1,616)

Characteristic	Value	Univariate Analysis (R or Hospital Stay, Days, Mean ± SD)	Standardized Regression Coefficient
Age, mean ± SD	78.3 ± 7.0	0.001	−0.099[d]
Sex, *n* (%)			
Female	778 (48.1)	26.8 ± 20.2	
Male	838 (51.9)	27.6 ± 24.6[a]	
Acute hospitalization, *n* (%)			
Yes	300 (18.5)	26.2 ± 21.0	
No	1,316 (81.5)	31.8 ± 28.2[a,d]	
Adverse drug reaction, *n* (%)			
Yes	190 (11.8)	26.4 ± 19.5	0.078[c]
No	1,426 (88.2)	33.3 ± 38.1[a,d]	
Body mass index, kg/m^2, mean ± SD	22.0 ± 4.1	−0.59[d]	−0.062[b]
Barthel Index (points out of 100), mean ± SD	83.1 ± 26.1	−0.178[d]	−0.13[d]
Number of diseases, mean ± SD	5.3 ± 2.3	1.43[c]	0.082[c]
Number of drugs, mean ± SD	6.8 ± 3.6	0.411[b]	–
Number of geriatric syndrome components, mean ± SD	4.6 ± 3.6	1.66[d]	0.19[d]

All data were collected soon after admission. For sex, acute hospitalization, and adverse drug reactions, a simple *t*-test was performed for univariate analysis, and values are expressed as mean ± standard deviation (SD).

[a]*P*-values are for comparison to female or no. Pearson correlation coefficients (R) are shown for the remaining factors in univariate analysis. All variables shown were included in stepwise regression analysis, and factors significantly associated were analyzed in multiple regression analysis (coefficient of determination = 0.32).

[b]$P < .05$.

[c]$P < .005$.

[d]$P < .001$.

patients were analyzed (mean age 78.3 ± 7.0, 52% male). All data were obtained soon after admission. Values are expressed as means ± standard deviations and were analyzed using JMP version 9.0.2 (SAS Institute, Inc., Cary, NC). $P < .05$ was considered statistically significant.

Mean length of stay was 27.3 ± 22.6 days (range 1–322 days). The results of univariate and multivariate analyses for length of stay are shown in Table 1. Multiple stepwise regression analysis showed that ADRs, number of diseases, and number of geriatric syndrome components were positively associated with longer hospital stay, whereas age, BMI, and Barthel Index were negatively associated. The number of geriatric syndrome components was significantly associated with hospital stay independent of number of diseases.

The present analysis demonstrated that geriatric factors such as ADRs, multiple diseases, low BMI, ADL dependence, and number of geriatric syndrome components were associated with longer hospital stay in a large group. The finding that ADRs are a risk for prolonged hospital stay is consistent with a previous report,[6] and ADL dependence has been reported as a risk in a smaller group.[4] Furthermore, the number of geriatric syndrome components and undernutrition were risk factors for prolonged hospital stay in a large-scale study. Frailty, which is also known to be a risk factor,[7] was not examined independently in the present study, but ADL dependence and undernutrition, both of which are major components of frailty, were found to be risk factors, so it is reasonable to assume that frailty was associated with length of hospital stay in the current cohort as well. The present study revealed that the accumulation of geriatric syndrome components was a risk factor for prolonged hospital stay independent of multiple diseases and, presumably, frailty. Thus, geriatric syndrome should be comprehensively managed during hospitalization. The reason for the negative association between age and length of stay is unclear, but the presence of young-old patients with disability or complicated conditions on the geriatric ward might have influenced the results.

In summary, the present study provides new insight into the significance of geriatric conditions in relation to prolonged hospital stay in older adults. ADL dependence, undernutrition, ADRs, and geriatric syndrome should be carefully assessed and interventions provided when caring for older inpatients.

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ACKNOWLEDGMENTS

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this paper.

Author Contributions: TK: acquisition of subjects and data analysis, interpretation of data, and drafting of manuscript. MA: coordinator of study concept and design, and study supervision. YK, KY, and HY: acquisition of subjects and data. ME: data analysis and interpretation of data. YO: study supervision.

Sponsor's Role: The sponsors had no role in the design, methods, data collections, analysis, and preparation of this paper.

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ACTIVITIES OF DAILY LIVING RATHER THAN DEPRESSIVE SYMPTOMS INCREASE THE RISK OF MORTALITY IN JAPANESE COMMUNITY-DWELLING ELDERLY PEOPLE: A 4-YEAR LONGITUDINAL SURVEY

To the Editor: The article entitled "Depressive Symptoms Increase the Risk of Mortality in Older Mexican Community-Dwelling Adults" by Piña-Escudero et al.[1] deeply impressed us. Although it has been shown that older adults with depressive symptoms (DSs) have fewer quality-adjusted life years than those with chronic medical conditions,[2] Piña-Escudero et al. in their 2-year longitudinal study, showed that DSs increase mortality risk regardless of multiple covariates such as medical conditions and disability in activities of daily living (ADL). Similarly, results of a meta-analysis of 25 studies suggest that depression increases the risk of mortality,[3] although those studies did not assess ADL in detail. The risk of mortality in Japanese community-dwelling elderly people is reported herein, focusing on DSs and ADLs in a 4-year longitudinal survey.

The study population included 1,818 community-dwelling individuals aged 65 and older in Tosa Town, Japan; 1,600 (88.0%) participants who completed self-reported geriatric questionnaires in 2006 were included in the study. The questionnaires consisted of questions on ADLs and the 15-item Geriatric Depression Scale (GDS-15).[4] For ADL assessment, participants rated their

Journal of Neuroimmunology 245 (2012) 75–78

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Short communication

3,4-Diaminopyridine improves neuromuscular transmission in a MuSK antibody-induced mouse model of myasthenia gravis

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ARTICLE INFO

Article history:
Received 4 November 2011
Received in revised form 15 February 2012
Accepted 18 February 2012

Keywords:
Myasthenia gravis
MuSK
3,4-Diaminopyridine

ABSTRACT

This study investigated the effect of 3,4-diaminopyridine (3,4-DAP), a potent potentiator of transmitter release, on neuromuscular transmission *in vivo* in a mouse model of myasthenia gravis (MG) caused by antibodies against muscle-specific kinase (MuSK; MuSK-MG) and *ex vivo* in diaphragm muscle from these mice. 3,4-DAP significantly improved neuromuscular transmission, predominantly by increasing acetylcholine (ACh) release, supporting presynaptic potentiation as an effective treatment strategy for MuSK-MG patients who have defective transmitter release. In MuSK-MG, we suggest that only low-dose acetylcholinesterase (AChE) inhibitors be used to avoid side effects, and we propose that 3,4-DAP may be effective as a symptomatic therapy.

1. Introduction

Myasthenia gravis (MG) is caused by autoantibodies against postsynaptic membranes at neuromuscular junctions (NMJs), leading to failure of neuromuscular transmission mediated by acetylcholine (ACh) and clinical symptoms of ptosis, fatigue and muscle weakness. While the majority of MG cases (~90%) have antibodies against ACh receptors (AChRs; AChR-MG), MG caused by antibodies against muscle-specific kinase (MuSK; MuSK-MG) is frequently severe and requires emergent and aggressive therapy to manage respiratory distress (Vincent et al., 2008).

The therapeutic protocol for MG includes symptomatic and immunosuppressive treatments. In general, first-line symptomatic treatment is required in most patients until immunosuppressive treatment is effective. The strategy of symptomatic drugs is to improve neuromuscular transmission by increasing presynaptic transmitter release and potentiating postsynaptic effects. Acetylcholinesterase (AChE) inhibitors, which could potentiate postsynaptic effects, are generally effective for most AChR-MG patients. However, MuSK-MG patients are frequently unresponsive to these drugs or develop cholinergic crisis, characterized by increasing muscle weakness that causes dysphagia and respiratory insufficiency (Evoli et al., 2003; Sanders et al., 2003; Hatanaka et al., 2005; Evoli et al., 2008). In addition, MuSK-MG patients receiving AChE inhibitors may show abnormal patterns of repetitive firing to low-frequency motor nerve stimulation via electromyography (EMG). The emergence of repetitive firing indicates an increased sensitivity to ACh (Punga et al., 2006), which may result from the interference of MuSK with accumulation of AChE in synaptic basal lamina of NMJs (Cartaud et al., 2004). Recently, our animal model in which 100% of mice develop experimental autoimmune MG (EAMG) after immunization with MuSK protein reproduced the same EMG patterns showing hypersensitivity to ACh as MG patients. These mice also exhibited decreased levels of AChE and AChE-anchoring protein collagen Q at postsynaptic membranes, revealing the mechanism by which AChE inhibitor treatment exacerbates MuSK-MG symptoms *in vivo* (Mori et al., 2012).

Animal models of EAMG are integral for developing and assessing appropriate medications for patients afflicted with MuSK-MG. The current study focused on improving neuromuscular transmission in MG by increasing transmitter release. Specifically, we determined whether 3,4-diaminopyridine (3,4-DAP), a potent potentiator of transmitter release, could improve neuromuscular transmission *in vivo* in mice with MuSK-EAMG and *ex vivo* in diaphragm muscle from these mice.

2. Materials and methods

2.1. Immunization of mice

All procedures were approved by the Animal Care and Use Committee of Tokyo Metropolitan Geriatric Hospital and Institute of

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doi:10.1016/j.jneuroim.2012.02.010

Gerontology. Female A/WySnJ mice aged 8 weeks or older (The Jackson Laboratory) were anesthetized and injected with 20 μg MuSK emulsified with complete Freund's adjuvant (CFA) on day 0, then boosted with 20 μg MuSK emulsified with incomplete Freund's adjuvant (IFA) on day 14. Recombinant MuSK protein was prepared as described previously (Mori et al., 2012). Control mice were injected with PBS and CFA on day 0, then boosted with PBS and IFA on day 14.

2.2. EMG

Changes in compound muscle action potential (CMAP) were determined as described previously (Mori et al., 2012). Decrement was calculated as the percent amplitude change between the first CMAP and the smallest CMAP that were evoked by a train of 10 impulses. If the amplitude of the first CMAP was also the smallest, the decrement was designated as 0%. 3,4-DAP (Tokyo Kasei) was freshly prepared in PBS and administered at 8 mg/kg, i.p. A typical mouse weighing 20 g received 100 μl of 1.6 mg/ml 3,4-DAP. EMG was performed 20 min later.

2.3. Ex vivo electrophysiology

Membrane potentials and miniature endplate potentials (MEPPs) were recorded using a specimen composed of left phrenic nerve and hemi-diaphragm muscle as described previously (Mori et al., 2012). To measure evoked endplate potentials (EPPs), μ-conotoxin GIIIB (1 μM final concentration, Peptide Institute) was applied to suppress muscle contraction, and the phrenic nerve was stimulated with supramaximal voltage at 0.7 Hz. 3,4-DAP was applied to the specimen-immersed chamber (100 μM final concentration), and synaptic events were recorded 20 min later. Amplitudes of EPPs and MEPPs were standardized to a membrane potential of −75 mV. Quantal content was calculated by using the values of mean MEPP amplitude, mean EPP amplitude and membrane potential in the same muscle fiber in the formula described previously (McLachlan and Martin, 1981). A total of 8–15 NMJs were assessed from each mouse.

2.4. Statistics

Group differences between control and MuSK-injected mice were analyzed by either unpaired *t*-tests or Mann–Whitney *U*-tests. Paired *t*-tests were used to analyze the effects of 3,4-DAP treatment in EMG experiments. One-way ANOVAs were used to assess parameters of synaptic events from *ex vivo* electrophysiology experiments. Statistical significance was set at $P<0.05$.

3. Results

3.1. 3,4-DAP improves neuromuscular transmission

About two weeks after treatment with recombinant MuSK protein (see Materials and methods), all five A/WySnJ mice exhibited MG-like phenotypes, including weight loss and muscle weakness. In addition, while EMG recordings from control gastrocnemius muscle in response to 3-Hz repetitive nerve stimulation showed no abnormal CMAP decrements (defined as >10%) (0.26 ± 0.14%; range 0–0.76%) (Fig. 1A and D), MuSK-injected gastrocnemius muscle showed significant decrements (24.0 ± 2.62%; range 16.6–32.4%) (Fig. 1B and D), indicating neuromuscular transmission failure. Again, the amplitude of the first CMAP in MuSK-injected mice (69.8 ± 7.24 mV) was significantly decreased relative to controls (102.6 ± 3.75 mV) (Fig. 1E). A single injection of 3,4-DAP (8 mg/kg) to MuSK-injected mice reversed the CMAP decrease (3.45 ± 1.51%; range 0.6–8.9%; Fig. 1C and F) and significantly increased the amplitude of the first CMAP from 12.0 mV to a maximum of 34.7 mV (25.4 ± 3.93 mV; Fig. 1G). Similarly, 3,4-DAP also significantly increased the first CMAP amplitude in control mice (15.3 ± 3.45 mV) (data not shown). These results demonstrate that 3,4-DAP improved neuromuscular transmission in MuSK-injected

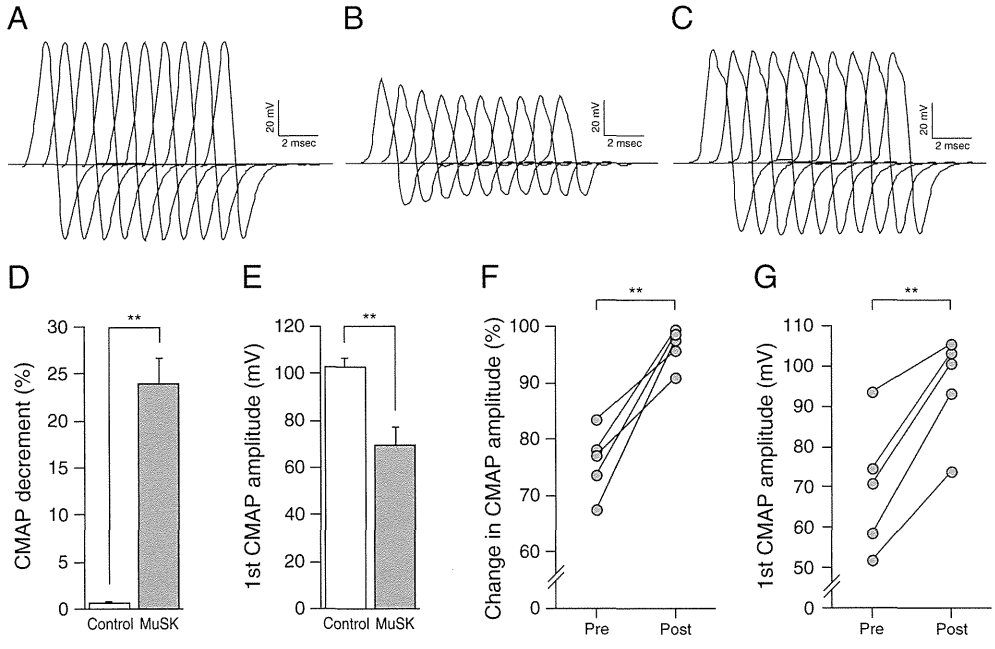

Fig. 1. Effect of 3,4-DAP on CMAP decrement.Representative EMG traces from gastrocnemius muscles of control mice (A) and MuSK-injected mice before (B) and after (C) treatment with 3,4-DAP. MuSK-injected mice exhibited significant CMAP decrement (D) and reduction in the amplitude of the first CMAP (E) (n = 5 mice/group). $^{**}P<0.01$ by Mann–Whitney *U*-test (D) or unpaired *t*-test (E). (F) Changes in CMAP decrement before and after 3,4-DAP administration in MuSK-treated mice. 3,4-DAP significantly improved CMAP decrement (84.1 ± 7.2%; range, 60.7 to 95.4%). (G) Changes in the first CMAP amplitude before and after 3,4-DAP administration in MuSK-treated mice. 3,4-DAP significantly increased CMAP amplitude (39.1 ± 7.5%, range, 12.8 to 59.3%). $^{**}P<0.01$ (paired *t*-test).

Table 1
Effect of 3,4-DAP on synaptic event parameters in control and MuSK-injected diaphragms.

		No. of NMJs (no. of mice)	MEPP amplitude (mV)	EPP amplitude (mV)	EPP area (mV × ms)	Quantal content
Control	Before 3,4-DAP	52 (5)	0.91 ± 0.05	25.5 ± 1.22	139.5 ± 7.72	41.5 ± 1.85
	After 3,4-DAP	69 (5)	1.08 ± 0.04	38.9 ± 1.23	436.7 ± 17.7	70.5 ± 3.03
MuSK-MG	Before 3,4-DAP	35 (4)	0.47 ± 0.03	11.4 ± 1.47	49.7 ± 5.73	30.1 ± 2.83
	After 3,4-DAP	54 (4)	0.64 ± 0.04	26.9 ± 1.73	178.7 ± 0.16	72.4 ± 4.30
*P, Control before 3,4-DAP *vs.* MuSK-MG before 3,4-DAP			<0.0001	<0.0001	<0.0001	<0.001
**P, Control before 3,4-DAP *vs.* Control after 3,4-DAP			<0.01	<0.0001	<0.0001	<0.0001
**P, MuSK-MG before 3,4-DAP *vs.* MuSK-MG after 3,4-DAP			<0.01	<0.0001	<0.0001	<0.0001

Data are means ± SEM. *P determined in Mann–Whitney *U*-test, and **P determined in one-way ANOVA.

mice, supporting its potential to relieve muscle fatigability and increase muscle strength, as previously described (Wirtz et al., 2009).

3.2. 3,4-DAP induces presynaptic potentiation

To examine the mechanisms underlying 3,4-DAP-induced improvement of neuromuscular transmission, we used intracellular recording of muscle fibers from excised hemi-diaphragms. As described previously (Mori et al., 2012), MEPP amplitude, EPP amplitude and mean quantal content (steady state number of quanta released by a single nerve impulse at 0.7 Hz) were significantly decreased in MuSK-injected mice compared to controls (Table 1). These results suggest that both defective transmitter release and attenuated postsynaptic sensitivity contribute to decreased EPP amplitude and CMAP decrement. Application of 3,4-DAP (100 μM) increased EPP amplitude and area by 136% and 259%, respectively (Fig. 2A, B and Table 1) and increased mean quantal content by 141% compared to pre-treatment (Table 1). No significant effect of 3,4-DAP on resting membrane potential was observed (baseline, 64.8 ± 1.78 mV; 3,4-DAP, 61.2 ± 1.61 mV). Similar effects were observed in control diaphragms (Table 1). These results indicate that 3,4-DAP potentiated ACh release from presynaptic membranes of NMJs and increased both the amplitude and duration of EPPs, resulting in reversal of CMAP decrement and increase in CMAP amplitude, as observed in EMG *in vivo*. Unexpectedly, MEPP amplitude, which is not affected by aminopyridines (Thomsen and Wilson, 1983; Giovannini et al., 2002), was increased by 36% by 3,4-DAP. Moderate, but significant, increases in MEPP amplitude were also observed in controls. Thus, increased postsynaptic membrane sensitivity may also contribute to 3,4-DAP-induced improvement in neuromuscular transmission (Table 1).

4. Discussion

Aminopyridines such as 4-AP and 3,4-DAP have been proposed to be potassium channel blockers, but a recent study demonstrated that these compounds stimulate voltage-gated calcium channels (VGCCs) (Wu et al., 2009), prolong the duration of nerve action potentials and increase ACh release from nerve terminals of NMJs (Thomsen and Wilson, 1983). In particular, 3,4-DAP has the advantage of lower brain penetration than 4-AP (Lemeignan et al., 1984) and thus is the drug of choice for neuromuscular disorders such as Lambert–Eaton myasthenic syndrome (LEMS) (Sanders, 2003).

In MuSK-MG, *in vitro* electrophysiology has shown decreased ACh release in NMJs (Burges et al., 1994; Niks et al., 2010). Consistent with these studies, the current study showed decreased ACh release in NMJs of MuSK-MG mice, demonstrating that the presynaptic defect, in addition to the smaller postsynaptic effect (Mori et al., 2012), could contribute to failure of neuromuscular transmission. In AChR-MG, which is caused exclusively by postsynaptic defects, enhanced ACh release has been attributed to compensatory increases in transmitter release due to retrograde signaling from the postsynaptic area to presynaptic terminals (Cull-Candy et al., 1980; Plomp et al., 1995). However, it seems that dysfunction of MuSK is unable to trigger such a mechanism. In this study, 3,4-DAP significantly improved both CMAP decrement and amplitude, mainly via increased ACh release from nerve terminals. Thus, these results suggest that presynaptic potentiation could be an effective strategy to treat MuSK-MG patients who have defective transmitter release. Interestingly, our results also showed that 3,4-DAP increased MEPP amplitude in both control (19%) and MuSK-MG (36%) mice. It has been demonstrated that aminopyridines have no effect on postsynaptic function in normal or LEMS IgG-treated NMJs (Thomsen and Wilson, 1983; Giovannini et al., 2002), whereas other study demonstrated that 4-AP produced a slight, non-significant increase in MEPP amplitude (7–11%) in normal NMJs (Kim et al., 1980). Detailed analysis will be needed to elucidate the effect of 3,4-DAP on the increased sensitivity of postsynaptic membranes in the NMJs of normal and MuSK-MG mice.

Symptomatic treatment for MG includes increasing presynaptic transmitter release and potentiating postsynaptic effects. In fact, administration of aminopyridines such as 3,4-DAP and 4-AP has been shown to improve muscle strength and neuromuscular transmission in AChR-MG patients and might therefore be valuable adjunctive treatments to AChE inhibitors (Lundh et al., 1979, 1985). However, in MuSK-MG, AChE inhibitors cause hypersensitivity of postsynaptic membranes to ACh, increasing the risk for cholinergic crisis and leading to muscle cramps and fasciculations (Punga et al., 2006; Mori et al., 2012). Therefore, in MuSK-MG, we suggest that only low-dose AChE inhibitors should be used to avoid side effects, and we propose that 3,4-DAP may be useful as a symptomatic therapy.

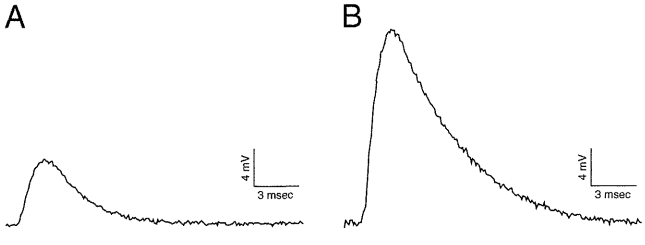

Fig. 2. Effect of 3,4-DAP on EPP.Representative EPP traces at NMJs before (A) and after (B) application of 3,4-DAP to diaphragm from MuSK-treated mice. 3,4-DAP increased both the amplitude and duration of EPPs.

Acknowledgment

Our studies were supported by grants from the Health Science Research Grants for Research on Psychiatric and Neurological Diseases and Mental Health (H19-Psycho-General-19), Comprehensive Research of Aging and Health (H22-Aging-General-002) from the Ministry of Health, Labor, and Welfare, Japan, Grants-in-Aid for Scientific Research on Innovative Area (21200023), Grant-in-Aid for Scientific Research (C) (21591102), Grant-in-aid for Young Scientists (B) (23791009) from the Ministry of Education, Science, and Culture,

Japan, and Intramural Research Grant (22-5) for Neurological and Psychiatric Disorders of National Center of Neurology and Psychiatry.

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基礎老化研究　36(3);47－49,2012

【トピックス】<第35回日本基礎老化学会大会奨励賞受賞>

老齢マウスの筋線維タイプ特異的な筋萎縮の病態解明

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1. はじめに

加齢に伴い骨格筋量は減少する。一般的に、骨格筋量は40歳から徐々に減少し始め、80歳までには30-40%の筋量が失われることが知られている[1]。筋肉量減少に伴う筋萎縮は転倒等によるケガの危険性を増加させ[2]、場合によっては寝たきり状態となり要介護問題へと発展し得るため非常に重要な研究課題である。基礎疾患を伴わない加齢性の筋萎縮（加齢性筋肉減少症：サルコペニア）の原因としては酸化ストレスの増加をはじめ、ミトコンドリア機能の低下など様々な要因が提唱されているが詳細な発症メカニズムは未解明である[3]。

筋は多様な細胞から構成される複雑な組織である。筋を構成する筋線維は、収縮特性・代謝特性・疲労耐性等の違いから遅筋線維と速筋線維に大別することができ、酸化的な代謝特性を持ち持久力を発揮するTypeⅠ筋線維や嫌気的な代謝特性を持ち瞬発力を発揮するTypeⅡb筋線維、そして両方の性質を持つTypeⅡaやⅡx筋線維といった、それぞれ性質が全く異なる筋線維タイプに分類されている(表1)[4]。従って、筋は解剖学的及び機能的にも異なる細胞から構成される集団として捉える必要がある。そして、加齢性の筋萎縮に伴い筋線維タイプが変化することはこれまで多く報告されている[5-7]。

筋線維タイプ	遅筋線維	速筋線維		
	Ⅰ	Ⅱa	Ⅱx	Ⅱb
収縮スピード	遅い	速い（Ⅱb＞Ⅱx＞Ⅱa）		
疲労耐性	高い	高い	やや高い	低い
代謝	酸化系	酸化系/解糖系	解糖系	解糖系
エネルギー効率	優	やや優	劣	劣
解剖学的（色）	赤	赤	白	白
筋線維の大きさ	やや大きい	小さい	中間	大きい

表1 筋線維タイプの特性
骨格筋を構成する筋線維は表に示した性質の違いから、遅筋線維と速筋線維に大別される。
酸化的な代謝特性を持ち、持久力に優れるTypeⅠ,Ⅱa,Ⅱx筋線維や嫌気的な代謝特性を持ち、瞬発力に優れるTypeⅡb,(Ⅱx)筋線維に分類することができ、各筋線維タイプは全く異なる性質を持つ。 このように筋は、多様な細胞から構成される非常に複雑な組織である。

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しかしながら、筋萎縮の機構と筋線維タイプの変化との因果関係については未解明である。そこで我々は、若齢(8ヶ月齢)及び老齢(32ヶ月齢)のC57BL/6NCrマウス(♀)における下肢筋の凍結筋横断切片を作製し、筋萎縮に伴う筋線維タイプレベルの筋機能の変化を、組織化学的・病理学的方法で体系的に解析を行った。本稿では、速筋の長指伸筋（TypeⅡa,x,b 筋線維で構成される)と遅筋のひらめ筋（TypeⅠ,Ⅱa筋線維から構成される)を対象にして、筋線維タイプ単位の筋萎縮とミトコンドリア機能の変化に着目した研究成果について紹介する。

2. 老齢マウスは筋線維タイプ特異的に筋萎縮の様式が異なる

老齢マウスのひらめ筋及び長指伸筋は共に筋重量の減少と、顕著な萎縮を示した。次に、各筋線維タイプ別の筋萎縮を解析するためにATPase(pH4.7)染色と各筋線維タイプに対する免疫染色を行った。その結果、老齢マウスのひらめ筋(TypeⅠ,Ⅱa筋線維から構成される)はTypeⅡa筋線維特異的な筋線維数の減少を示したが、筋線維の面積はTypeⅠ,Ⅱa筋線維共に維持された。一方、老齢マウスの長指伸筋(TypeⅡa,x,b筋線維で構成される)ではTypeⅡb筋線維特異的な筋線維数の減少と筋線維面積の低下を示した。このように、老齢マウスのひらめ筋と長指伸筋は筋線維タイプ特異的に異なる筋萎縮の様式を示すことが明らかとなった(表2)。

	ひらめ筋		長指伸筋		
	TypeⅠ	TypeⅡa	TypeⅡa	TypeⅡx	TypeⅡb
筋線維数	変化なし	減少	変化なし	変化なし	減少
筋線維面積	変化なし	変化なし	変化なし	変化なし	減少
筋線維タイプ群化	群化	変化なし	群化	群化	脱群化
ミトコンドリア活性	低下	変化なし	変化なし	変化なし	変化なし

表2
下肢骨格筋の加齢変化
表に示したように、老齢マウスのひらめ筋と長指伸筋は筋線維タイプ特異的に筋萎縮の様式やミトコンドリア活性の変化が異なることが明らかとなった。

3. 老齢マウス骨格筋における筋線維タイプ群化

これまで、加齢に伴い神経筋接合部の機能・形態が変化し筋線維の脱神経支配が生じて筋の萎縮が進行することが示唆されている[8-10]。そして、老齢マウスや高齢者の萎縮した筋の断面を解析すると随伴して同じ筋線維タイプの群化が報告されている[5,6]。筋線維タイプの