의학석사 학위논문

Apoptosis in Young and Old Denervated Rat Skeletal Muscle

신경차단된 어린 백서와 노화 백서 골격근의 세포자멸사 연구

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서울대학교 대학원

임상의과학과

이 강 표

Abstract

Introduction: The purpose of this study was to investigate the apoptotic response to different degrees of denervation in two age groups.

Methods: A total of thirty young (3 mos.; n=15) and older (22 mos.; n=15) Sprague-Dawley rats were randomized into three groups: control (C), partial denervation (PD), and complete denervation (CD). The right sciatic nerve was injured in the PD and CD groups. Four weeks after the injury, the muscle wet weight and myosin heavy chain (MHC) isoform composition (via SDS-PAGE) were determined in gastrocnemius (GCM) and soleus (SOL) muscles. Histological appearance and fiber cross-sectional area (FCSA) were studied in GCM. Apoptotic responses in GCM were determined by studying changes in myonuclei and expression of Bcl-2 and BAX. Statistical analysis was done using nonparametric methods and generalized linear model analysis.

Results: No definite interaction between age group and degree of denervation was seen. In general, older animals were heavier ($p<0.001$) but showed non-significant tendency of lower muscle weight to body weight ratio (MBR, $p>0.05$). However, within control rats, we found significant difference of MBR between age groups ($p=0.036$). PD and CD resulted in significant reductions in muscle weight (GCM and SOL) and FCSA (GCM) in young and older rats. Increases in connective tissue were seen after

denervation. Significant changes were seen in the expression of types I (increase, p=0.008) and IIb (decrease, p=0.008) MHC isoforms in young GCM after denervation but no significant changes were seen in older animals. In SOL, CD resulted in significant changes compared to C in type I (decrease, p=0.016) and IIa (increase, p=0.016) MHC isoforms in young rats. In general, there was no difference of apoptotic response between age groups (P>0.05). But, within control group, older rats had significantly more broken myonuclei (p=0.033), higher expression of BAX (p=0.036) and Bcl-2 (p=0.028). A larger number of apoptotic nuclei was seen in PD and CD compared to C in young and older animals (p=0.008). PD and CD resulted in significantly higher expression of BAX and Bcl-2 in both young and older rats (p<0.05) but the BAX/Bcl-2 ratio did not change (p>0.05).

Conclusions: Older age was associated with an increased level of apoptosis but older muscle was not more vulnerable to the effect of denervation on cell death.

Key words: Aging, denervation, muscle atrophy, apoptosis

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INTRODUCTION

Age-related changes in skeletal muscle size and function lead to a loss of independence in activities of daily living, a higher of risk of hospitalization, and increased mortality after falls and fractures (1–3). Some of the cellular mechanisms underlying these changes in muscle include decreased mitochondrial function, impaired satellite cell activity, and increased oxidative stress (4–7). A reductions in muscle mass (sarcopenia) is a combination of fiber atrophy and a decrease in the number of muscle fibers (8–12). One possible mechanism for the loss of muscle fibers in older subjects is apoptosis but its contribution to sarcopenia is still controversial (11,13–15). For example, it has been reported that older muscles have a higher number of apoptotic nuclei but the mitochondrial BAX/Bcl-2 ratio, a marker of apoptotic response, is not different in aging muscles (11,12).

Denervation has been suggested as an important contributor to sarcopenia (7,16–18). Several neuropathic conditions, such as diabetic peripheral neuropathy, cervical and lumbar radiculopathy, and spinal stenosis are frequently seen in older age groups (19,20). Further, older nerves appear to be more vulnerable to injury and may have a lower capacity to regenerate (21). Muscle cell apoptosis is believed to be closely associated with muscle atrophy seen in denervated muscle (22–24). The upregulation of BAX and Bcl-2 or imbalance in the BAX/Bcl-2 ratio has been reported to be one of the determining factors in denervation atrophy (25,26). It is interesting to

consider that apoptotic responses may vary according to the degree of nerve injury, particularly in older subjects.

The purpose of this study was to investigate the differences in apoptosis response in relation to age and in response to different degree of denervation in rat skeletal muscle. Further, we studied the potential interaction between aging and denervation on apoptosis. We hypothesize that: 1) the apoptosis response from aging process is less pronounced than from denervation process; 2) more extensive denervation results in more significant apoptotic responses independent of the age group; 3) the combination of aging and denervation will result in the most extensive apoptotic response compared to either alone.

MATERIALS AND METHODS

Animals and experimental protocol

Fifteen 3 months old and fifteen 22 months old male Sprague-Dawley rats were used. The rats were housed in pathogen-free conditions at ~20℃, were exposed to a reverse light condition of 12:12-h of light/darkness each day, and consumed a regular chow diet and tap water. Older rats were acquired from an animal housing unit (Orient Bio Inc., Seoul) when they were 9 months old and housed in our pre-clinical research unit. A total of five young and five older animals were randomly assigned to one of three groups including control (C), partial denervation (PD), and complete denervation (CD). After 4 weeks all rats were sacrificed using carbon dioxide gas and the gastrocnemius (GCM) and soleus (SOL) muscles were harvested. The study was approved by the Seoul National University Bundang Hospital Institutional Animal Care and Use Committee.

Nerve injury model

Before the experimental nerve injury, the animals were anesthetized with Zoletil (Zolazepam + Tiletamine, 50mg/kg body weight) along with Xylazine (10mg/kg body weight) through intraperitoneal injection. Using sterile technique, a posterior thigh splitting incision was made on the right side to expose the trunk of the sciatic nerve. In the CD group the right sciatic nerve trunk was transected 10 mm distal to the sciatic notch. To ensure permanent denervation, the proximal stump was bent and implanted in the gluteal

muscles, and the distal stump implanted in the popliteal space. In the PD group, the right sciatic nerve trunk 10 mm distal to the sciatic notch was crushed with locking non-serrate hemostatic forceps for 1.5 min. After the nerve injury, the muscle and skin were sutured and the rats were returned to their cage. All animal housing and surgical interventions were performed in accordance with the Canadian Council on Animal Care Guidelines.

Outcomes

Muscle wet weight

Muscle wet weight (MWW) was measured using a laboratory scale, expressed in absolute terms, and as the ratio of muscle weight to body weight (MBR).

Morphometric analysis of GCM

A block of muscle taken from the mid-belly of GCM was fixed in 4% paraformaldehyde. The fixed muscle specimens were washed in water, dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin, and cut into 7-lm-thick sections. Sections were stained with Haematoxylin & Eosin (H&E) and observed under light microscopy. Photographs were taken from five random fields (x200) chosen from the central region of one cross-section of each GCM with the aid of an AxioCam HR digital camera (Carl Zeiss, Göttingen, Germany). Images were digitized into an Axiovision imaging software (Carl Zeiss, Göttingen, Germany) and analyzed with an ImageJ 1.44a software (Wayne Rasband, National Institutes of Health, USA,

http://rsb.knfo.ih.gove/ij). Fiber cross-sectional area (FCSA) was measured in a minimum of 150 fibers per animal.

Myosin heavy chain (MHC) isoform composition in GCM and SOL

MHC isoform composition was analyzed using muscle protein homogenates. The MHC composition was determined using 6% SDS-PAGE. The acrylamide concentration was 4% (v/v) in the stacking gel and 6% in the separating gel, and the gel matrix included 30% glycerol. SDS PAGE was run at a constant voltage of 90V for 30 min and 140V for 5.5 hours (43). Protein homogenate mixtures of GCM and SOL were used as standards. Protein bands in the gels loaded with muscle homogenates were visualized with Coomassie Brilliant Blue. Densitometry was performed using analytic software (Bio-1D Light, VilberLauret, French) to measure relative proportions of MHC isoforms.

In Situ DNA Nick-End Labeling (TUNEL) in GCM

Paraffin-embedded tissues were deparaffinized and proteins in tissue sections were digested by applying proteinase K (20 μg/ml) for 15 min at room temperature. The specimens were then washed with distilled water. Cryostat sections were fixed in 10% neutral buffered formalin for 30 min at room temperature. After washing in phosphate-buffered solution, slides were post-fixed in ethanol/acetic acid (2:1) for 5 min, followed by 2% hydrogen peroxide in phosphate-buffered solution for 5 min to block endogenous peroxidase activity. DNA strand breaks were labeled by

attaching them to digoxygenin-conjugated dUTP in a reaction catalyzed by exogenous terminal deoxynucleotidyl transferase, and then immunodetected by incorporating nucleotide sequences using the ApopTag® *in situ* apoptosis detection kit peroxidase (Oncogene, San Diego, CA), as described by the manufacturer. After counterstaining with methyl-green, positive myonuclei were counted, and expressed as percentages of the total number of nuclei counted in three randomly chosen fields.

Western blot analysis for BAX and Bcl-2 in GCM

The residual GCM tissue of each group was pooled and homogenized using a sonic dimembrator (Fisher Scientific inc., Polytron) in 50 mM KCl containing 10 mM EGTA, 30 μM pepstatin, 12 μM phenylmethylsulfonylfluoride, and 1 mM benzamidine to reduce the effects of endogenous proteases. The homogenates were then centrifuged at 1200 ×*g* for 10 min at 4℃, and the supernatants were collected. Protein concentrations were determined by the Coomassie brilliant blue method using bovine serum albumin as a standard. Aliquots dissolved in sodium dodecyl sulfate (SDS) buffer were analyzed by 12% SDS-PAGE followed by Western blotting (30 μg/lane). The following conditions were used for antibody binding: 5 min exposure with anti-bcl-2 alpha (Neomarkers, Fremont, CA) and anti-BAX (Neomarkers, Fremont, CA) at 1:100 dilution, with anti-mouse conjugated horseradish peroxidase (HRP) (ZYMED, San Francisco, CA) at 1:2000 as a secondary antibody. Anti-α-tubulin antibody (1:1000, Santa Cruz Biotechnology Inc., CA) was included as a reference for the housekeeping

gene, α-tubulin. After applying ECL (enhanced chemiluminescence, Amersham Biosciences, Little Chalfont, UK), blots were illuminated and exposed to x-ray film for 5 min. Immunoblots were densitometrically scanned using a charge-coupled device camera, and the data were quantitatively processed using Bio-ID software (Viber Lourmat, Mame-la-Valle, France). Through the measurement of band density, the expression levels of BAX and Bcl-2 and the ratio of BAX to Bcl-2 were calculated.

Statistical analysis

To evaluate differences among groups and to determine if the effects of aging and degree of denervation on each parameter were significant we used the non-parametric Kruskal-Wallis test. The Mann-Whitney test was applied post hoc with Bonferroni's correction. The interaction between aging and denervation was analyzed using generalized linear model statistics. The goodness of fit was tested with the Wald test. These analyses were performed using the statistical R software application. Statistical significances was accepted at the $p < 0.05$ level.

RESULTS

Two older rats died in the CD group. In general, statistical analyses showed no interaction between aging and denervation for any of the outcome variables. In other words, the changes seen after denervation (see below) were independent of the age group.

Body and muscle weight

The results for the body and muscle wet weights are presented in Table 1. Older rats were heavier than younger rats ($p<0.001$) and denervation did not have an effect on body weight ($p=0.458$). The MBR in older rats were non-significantly lower than young rats in GCM (24.3%, $p=0.128$) and SOL (18.5%, $p=0.299$). However, within control rats, we found significant difference of MBR between age groups ($p=0.036$). In general, MBRs were decreased according to degree of denervation ($p<0.05$), except SOL in older rats ($p=0.055$). Young animals showed good post hoc discrimination of MBR between degree of denervation in GCM ($p=0.008$) and SOL ($p=0.012$), while older animals only had shown that between C and PD in GCM ($p=0.008$).

Morphometry of GCM

No significant difference in FCSA between age groups was seen ($p=0.259$, Table 1 and Figure 1). A statistically significant reduction in FCSA was seen after denervation ($p<0.05$). PD in young rats was associated with a

20.3% reduction in FCSA compared to C (p=0.008) while CD resulted in a 50.2% reduction in FCSA (p=0.008). In older rats FCSA were 38.6% (p=0.008) and 50.0% (p=0.036) smaller in PD and CD, respectively compared to C.

Qualitative analysis of muscle cross-sections stained with H&E (Figure 1) showed increased interfiber connective tissue, and some increase in fiber size variation in older C muscle compared to young C. Denervation in both young and older animals resulted in muscle fiber atrophy, fiber necrosis, and increased collagen content. These changes appear to be more extensive in CD compared to PD.

MHC isoform composition

Densitometric analysis of MHC isoform composition is shown in Figure 2. In GCM, the content of type I was higher (p=0.049) and type IIx lower in young compared to older animals (p=0.018). In young animals, PD resulted in increases in type I (p=0.008) isoform and a corresponding reduction in IIb (p=0.008) compared to C. No changes were seen after CD compared to C in young animals. In older rats, PD and CD showed a non-significant tendency for increases in type I and IIx, and a corresponding reduction in IIb.

In SOL, there was no difference in MHC isoform composition between young and old rats. In young animals, PD did not result in significant changes. However, CD resulted in lower type I compared to C (p=0.016), and higher IIa isoforms compared to C (p=0.016). In older rats, there was

non-significant tendency for increases in type IIx and IIa, and a corresponding reduction in I.

Table 1. Body weight, muscle wet weight, muscle weight-to-bodyweight-ratio, and muscle fiber cross-sectional area in young and old rats

		Young			Older		
		C	PD	CD	C	PD	CD
	BW†	482.00±10.31	428.65±20.83	443.60±20.32	768.64±84.69	716.89±48.46	760.00±1.44
Gastrocnemius	MWW, left†	2.79±0.08	2.60±0.15	2.59±0.16	3.07±0.24	3.26±0.16	3.47±0.00
	MWW, right	2.79±0.08	1.66±0.06*	0.82±0.13*	3.07±0.24	1.54±0.19*	1.49±0.25
	MBR	5.79±0.16	3.90±0.17*	1.81±0.22*	4.13±0.42	2.23±0.36*	1.96±0.33
	FCSA	1686.10±121.75	1343.02±92.65*	839.14±147.95*	2108.98±261.55	1295.22±166.34*	1053.87±83.41
Soleus	MWW, left†	0.18±0.01	0.18±0.01	0.18±0.01	0.23±0.02	0.24±0.03	0.26±0.02
	MWW, right	0.18±0.00	0.10±0.01*	0.08±0.00*	0.23±0.02	0.12±0.02*	0.11±0.01
	MBR	0.38±0.01	0.24±0.01*	0.18±0.01*	0.30±0.03	0.18±0.04	0.15±0.01

Values are means ± SE. Denervation was produced always in the right side. C = control; PD = partial denervation; CD = complete denervation; BW = body weight (g); MWW = muscle wet weight (mg); MBR = muscle weight to body weight ratio (mg/g); FCSA = fiber cross-sectional area; * = significantly different from C in post hoc test within age group ($p<0.05/3$); † = significantly different between young and older rats ($p<0.05$).

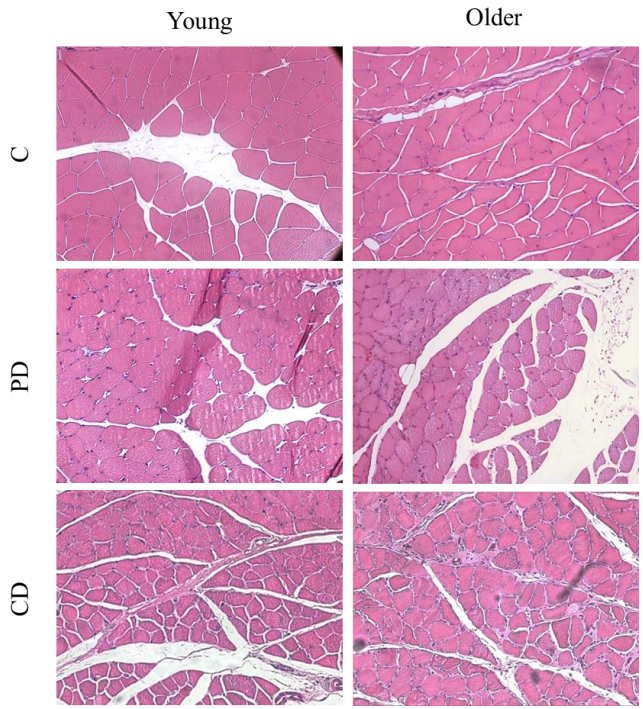

Figure 1. Cross-sections of the gastrocnemius muscle stained with H&E in young and older rats in the three groups; C = control; PD = partially denervation; CD = complete denervation.

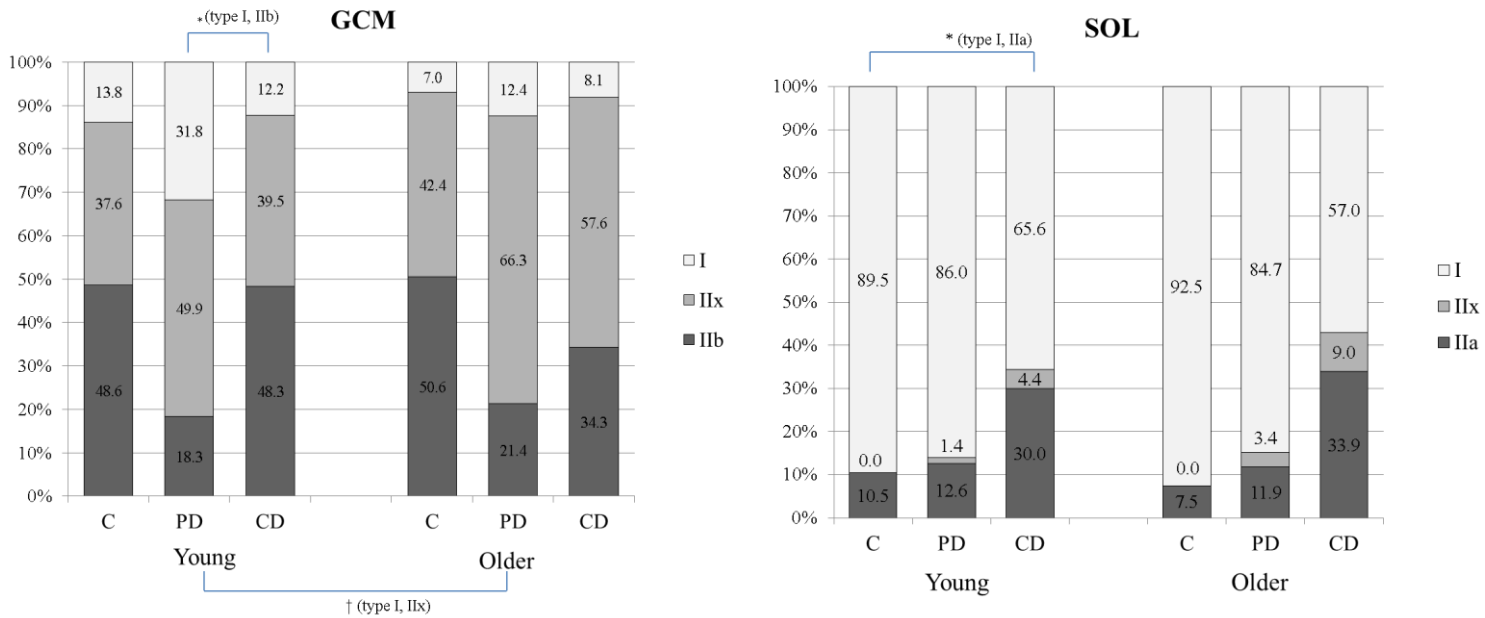

Figure 2. Myosin heavy chain isoform composition (%) in young and old gastrocnemius (GCM-left) and soleus (SOL-right) rat muscles. Composition in each group (C = control; PD = partial denervation; CD = complete denervation) is expressed as a percentage of type I, IIX, and IIB isoforms. * = significantly different with Bonferroni correction ($p<0.05/3$). † = significantly different between young and older rats ($p<0.05$).

Immunohistochemistry: TUNEL assay

No difference (p=0.268) in TUNEL-positive nuclei was seen between young and older GCM (Figure 3). But, within control group, older rats had significantly more broken myonuclei (p=0.033). PD (p=0.012) and CD (p=0.012) showed higher percentage of TUNEL-positive nuclei compared to C in young rats (C vs PD, 1.80 to 9.20%; C vs CD, 1.80 to 13.8%). Similarly, a higher percentage of TUNEL-positive nuclei was seen in PD (C vs PD, 3.8 to 12.6%; p=0.011) and in CD (C vs. CD, 3.8 to 18.7%; p=0.032) in older rats.

The expression of BAX and Bcl-2: Western blot analysis for apoptotic activity

The changes in BAX and Bcl-2 are shown in Figure 4. There was no significant interaction between aging and denervation in both BAX and Bcl-2 results. There were no differences between age groups in BAX (p=0.311) or Bcl-2 (p=0.490). Like the preceding TUNEL assay, within control group, we found significantly higher expression of BAX (p=0.036) and Bcl-2 (p=0.028) in older rats. The expression of BAX showed a statistically significant increase with denervation compared to C ($p<0.001$) and it was higher in CD compared to PD in young (p=0.008) and older animals (p=0.008). The expression of Bcl-2 was also significantly increased with the degree of denervation. In young and older animals, PD (p=0.008) and CD (p=0.008) resulted in significantly higher Bcl-2 expression compared to C. The BAX to Bcl-2 ratio (BBR), a surrogate marker of myonuclear apoptosis in previous studies, did not change with age (p=0.420) or denervation (p=0.237).

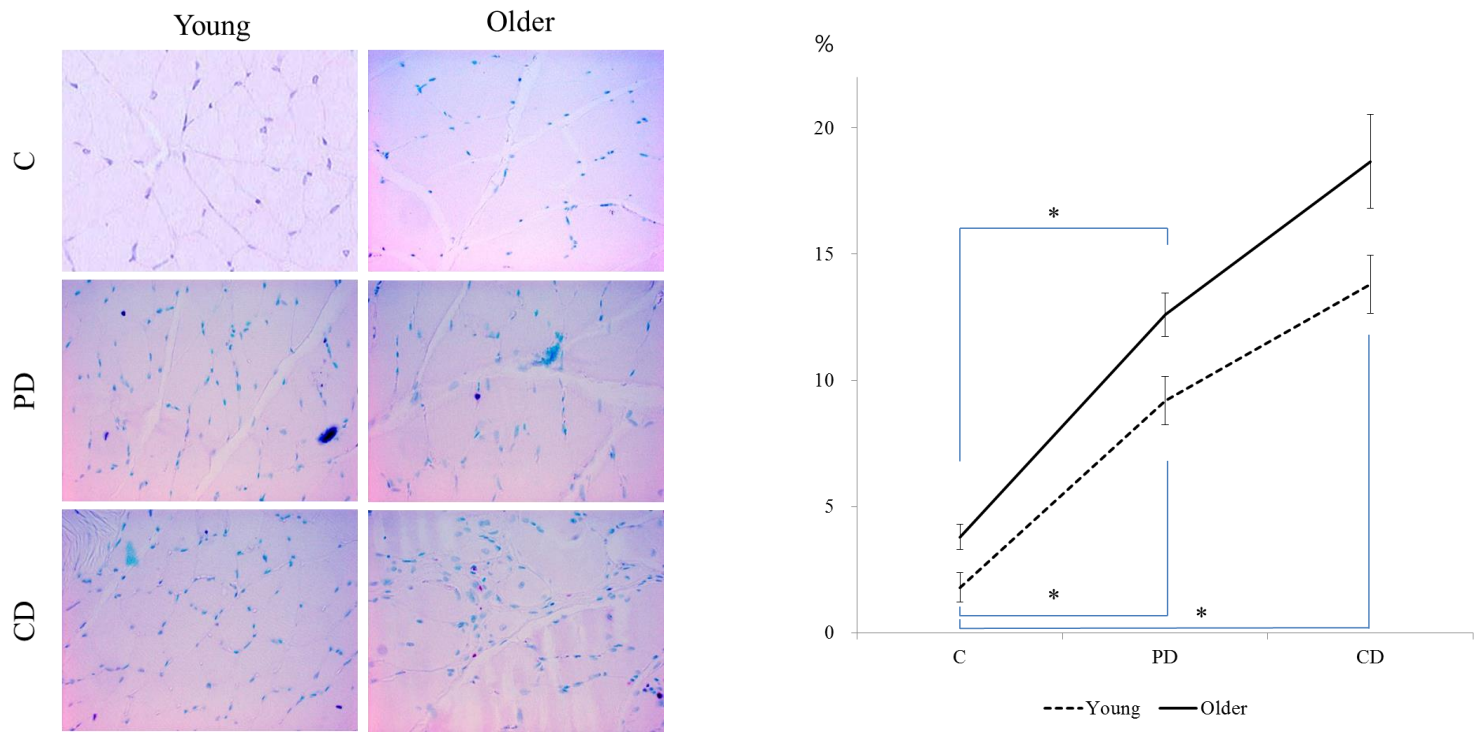

Figure 3. Percentage of TdT-mediated dUTP nick end labeling (TUNEL)-positive nuclei in young and older rat GCM in three groups (C=control; PD=partial denervation; CD=complete denervation). * = significantly different with Bonferroni correction ($p<0.05/3$)

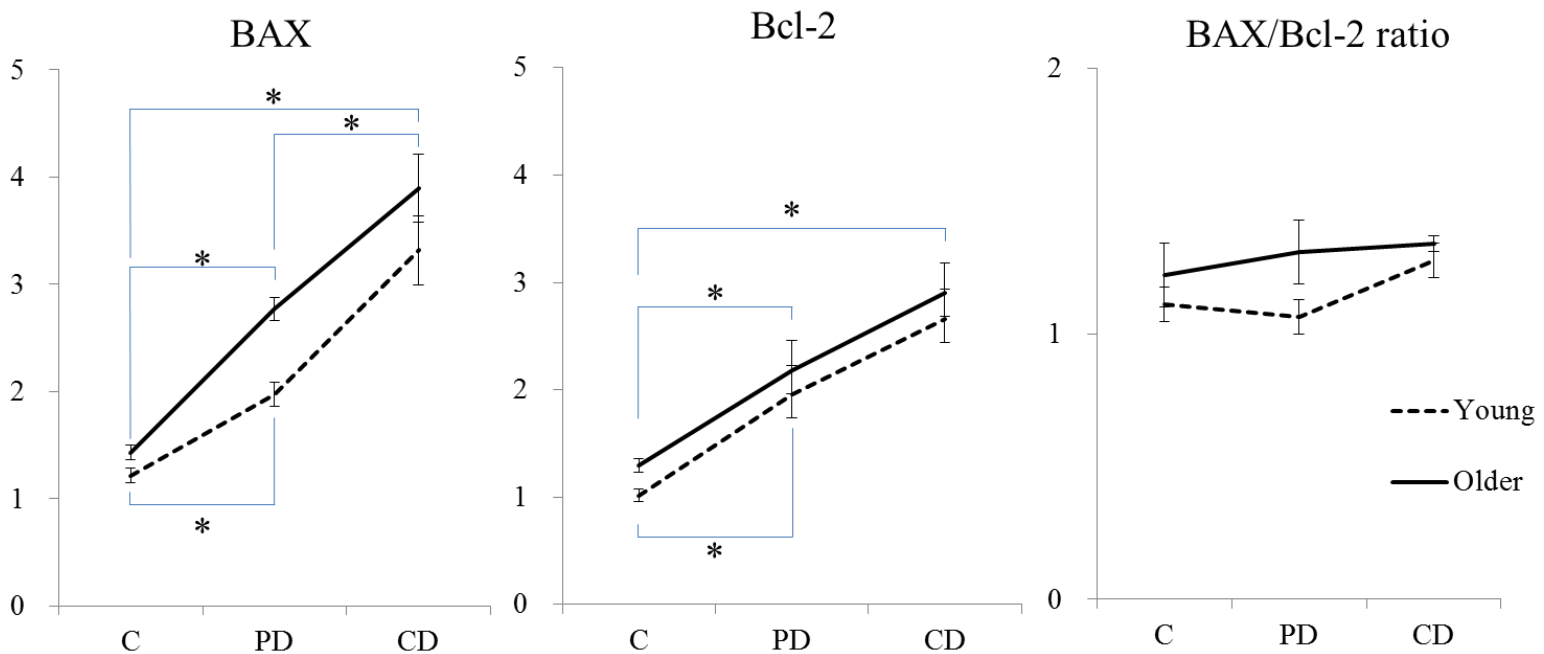

Figure 4. BAX and Bcl-2 expression by Western blot in rat GCM in three groups (C = control; PD = partial denervation; CD = complete denervation) 4 weeks after denervation. * = significantly different with Bonferroni correction ($p<0.05/3$)

DISCUSSION

We investigated changes in skeletal muscle in association with aging and denervation because of the prevalence and clinical importance of these two conditions (27). In this study, older animals were heavier but showed lower MBR. Denervation resulted in reductions in MBR and FCSA in young and older rats. Increases in connective tissue were also seen after denervation. Plastic changes in MHC composition related to aging were found in GCM, but denervation resulted in remodeling of MHC composition in both GCM and SOL (more definite change in SOL). In general, apoptotic responses were increased by aging and degree of denervation, independently. Older rats with complete denervation had most severe apoptotic response. The extent of responses was much bigger from denervation than aging. Furthermore, in this study, no definite interaction between age group and degree of denervation was seen.

Although we could not find thoroughly significant changes of apoptosis from sarcopenia, the trend was definite as has been shown previously for the soleus muscle (14) and gastrocnemius muscle (6,10). The previous studies also reported confusional data among apoptosis products. Some studies showed increased expression of BAX and reduced levels of Bcl-2 (28), but other researchers described up-regulation of both pro- and anti-apoptotic Bcl-2 family proteins with aging (29,30). Our western blot results would favor last studies that reported aging muscles showed increased apoptotic nuclei, but have no change of mitochondrial BAX/Bcl-2 ratio (10,11). As aging

process is long standing and leads relatively small apoptotic response, we thought that the mitochondrial apoptosis with aging was sufficiently compensated by anti-apoptotic mechanism.

Compared to aging effect on apoptosis, this study showed denervation process makes more marked change in apoptotic response. Some studies reported that denervation results in the up-regulation of Bcl-2 family proteins, and increasing BBR (22,31). This study showed significantly increased TUNEL-positive DNA fragmentation as well as up-regulation of Bcl-2 family proteins (BAX, Bcl-2) according to degree of denervation, but the increment of BBR was not statistically confirmed in both young and older rats.

We found the plastic changes from aging and denervation in MHC composition. But the change of MHC isoform composition related to aging process was not accordant with previous studies, which showed fast (type II) to slow fiber type (type I) transition in aging process of rats and human (32). In this study, soleus muscle showed no significant change with aging process. In gastrocnemius muscle, in contrast to previous studies, slow to fast fiber type transition (IIb, no change; IIx, increased; I, decreased) was observed.

The change of MHC isoform composition related to intervention showed decrement of slow fiber type (MHC I) and increment of fast fiber in both soleus and gastrocnemius muscles. The previous studies also revealed the proportion of the MHC I was relatively decreased in both muscle types (33,34).

Actually, we had expected to find some additional effect of aging and denervation beyond simple sum of each independent outcome. But there is

no definite synergistic effect between 2 processes on atrophy. These findings suggest that aged muscle is in apoptosis-favored cellular environment but the apoptotic response to denervation is similar to young muscle.

Compared to previous studies, our experimental model had 2 by 3 groups with 2 factors (aging, denervation). Especially, the intervention for nerve injury performed 2 levels and we could reveal the statistically significant results with serial changes in almost parameters. We also confirmed the possibility of semi-quantitative denervation model of atrophy in rat. Although partial denervation experiment of GCM did not show the satisfactory result in MHC composition, we could observe simultaneous atrophy in 2 muscle types and the difference of MHC composition change.

It is important to recognize the following limitations. Our sample size, 5 rats in each group (total 30 rats) may have been small for the detection of differences between old and younger animals. As 2 older rats died in the CD group, so we thought older group (especially, complete denervation) had weak statistical power. We could not know the reason why the animals were not alive 4 weeks after denervation. Although our study includes various parameters, we evaluated only two apoptotic products in BAX and Bcl-2. Recent studies related to mitochondrial pathways in sarcopenia have included not only intrinsic, caspase-dependent, pathways, but also extrinsic caspase-dependent and independent pathways, environmental dynamics of mitochondria (imbalance in mitochondrial fusion-fission events), and mitochondrial autophagy (35).

CONCLUSION

We found that the level of apoptosis was elevated in older animals and by denervation. Denervation appears to result in larger changes that correlate with the degree of nerve damage. We did not detect synergies between age and degree of denervation. It is reasonable to conclude that older rats have more significant apoptotic changes but no higher vulnerability to further damage from denervation than young.

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국 문 초 록

서론: 본 연구는 어린 백서와 노화 백서의 골격근에서 신경차단 정도에 따른 세포자멸사와 관련된 반응 차이에 대해 살펴보고자 하였다.

방법: Sprague-Dawley종 백서 30마리(월령 3개월 15마리, 22개월 15마리)를 신경차단 정도에 따라 3군으로 무작위 배정하였다: 대조군(C, control), 불완전 신경차단(PD, partial denervation), 완전 신경차단(CD, complete denervation). PD와 CD군에서 신경 손상 조작은 우측 좌골신경(sciatic nerve)에서 시행하였다. 신경차단 4주 후, 비복근(GCM, gastrocnemius)과 가자미근(SOL, soleus)에서 근육의 무게(muscle wet weight)를 측정하였고, SDS-PAGE를 통하여 미오신 중쇄(MHC, myocin heavy chain)의 아이소형(isoform)의 구성비를 구했다. 비복근에서는 추가로 광학현미경을 통하여 조직학적 변화 소견을 관찰하였고, 근섬유의 횡단면(FCSA, fiber cross-sectional area)을 측정하였다. 세포자멸사 반응 역시 비복근에서 얻은 조직을 이용하여 근핵 자체의 변화와 Bcl-2와 BAX 단백의 발현 정도를 통하여 정량화하였다. 통계분석은 비모수적인 방법을 통하여 각 군별 차이를 확인하였고, 일반화 선형 모형을 통하여 신경차단과 월령 간에 상호작용이 있는지 분석하였다.

결과: 신경차단정도와 노화여부 간의 상호작용은 관찰되지 않았다. 노화 백서가 전체 몸무게는 더 무거웠으나($p<0.001$), 근육 대 몸무게 비율(MBR, muscle weight to body weight ratio)은 작은 경향을 확인할

수 있었다($p>0.05$). 하지만 신경차단을 시행하지 않은 군(C) 안에서 비교해 보면, 비복근 대 몸무게 비율이 노화백서에서 유의미하게 작은 것을 확인할 수 있었다($p=0.036$). 두 월령군 모두에서 대조군(C)과 비교하여 신경차단(PD와 CD)을 하였을 때 근육의 무게와 근섬유의 횡단면이 감소한 소견을 보였으며, 결합조직의 증가도 확인되었다. 어린 백서에서는 비복근의 경우 부분신경차단 및 완전신경차단 모두에서 미오신 중쇄 I형은 증가($p=0.008$)와 IIb형은 감소($p=0.008$) 소견이 관찰되었으나, 가자미근의 경우 완전신경차단에서만 I형의 감소($p=0.016$), IIa형의 증가($p=0.016$)가 확인되었다. 하지만 노화 백서에서는 이런 미오신 중쇄의 유의미한 변화는 관찰되지 않았다. 전체적으로 노화 백서와 어린 백서 사이에 세포자멸반응의 차이는 없었다($P>0.05$). 하지만, 신경차단을 시행하지 않은 군(C) 안에서 비교해 보면, 노화백서에서 세포자멸반응이 증가를 확인할 수 있었다($p<0.05$). 부분 및 완전 신경차단은 모두 어린 백서뿐만 아니라 노화 백서에서도 세포자멸반응에 의해 파괴된 근핵의 수는 유의미하게 증가시켰으며($p<0.05$), Bcl-2와 BAX 단백 발현의 증가($p<0.05$) 역시 확인할 수 있었다. 하지만, BAX/Bcl-2 비율은 변화하지 않았다.

결론: 노화된 근육에서는 세포자멸반응이 증가되어 있으나, 노화된 근육이라도 신경차단에 의한 세포사멸에 더 취약하지는 않았다.

주요어: 노화, 탈신경, 근위축, 세포자멸사

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