Original Article

Recovery of skeletal muscle function following injury is not augmented by acute resveratrol supplementation

Russell G Rogers, Cory W Baumann, Jeffrey S Otis

Department of Kinesiology and Health, Muscle Biology Laboratory, Georgia State University, Atlanta, GA 30302, USA

Abstract

Background and Aim: Skeletal muscle function is significantly reduced following traumatic muscle injury and complete recovery can take several weeks. Pharmacological agents like nonsteroidal anti-inflammatory drugs have been used to minimize pain and inflammation associated with muscle injury. However, their safety and efficacy to improve muscle repair have been challenged. To that end, safer and more effective treatment options remain to be identified. Accordingly, resveratrol use has numerous health benefits in clinical population. Further, resveratrol has been shown to promote myoblast differentiation *in vitro*, which suggests it could be used to improve skeletal muscle regeneration *in vivo*.

Methods: Experimental mice received resveratrol supplementation (0.05% wt/wt) 4 weeks prior to injury, which persisted throughout recovery. Muscle injury was induced by injecting a diluted barium chloride solution into the tibialis anterior (TA). Isometric tetanic and twitch torque of the anterior crural muscles were measured prior to the injury and 7, 14, or 21 days postinjury.

Results: Resveratrol supplementation had no effect on preinjury isometric tetanic and twitch torque of the anterior crural muscles. Seven days after injury, tetanic torque production was reduced by 80% compared to preinjury values. Functional and structural recovery steadily improved at 14 days and was completed 21 days after injury. However, resveratrol treatment had no beneficial effects on isometric torque or regenerated fiber area.

Conclusion: Acute, low-dose resveratrol supplementation did not improve functional or structural recovery of regenerating mouse TA after injury. Further research is likely required that explores alternate resveratrol dosing strategies and their effects on skeletal muscle repair processes.

Key words: Fiber cross-sectional area, isometric torque, resveratrol, skeletal muscle regeneration

Received: 11th January, 2015; ***Revised:*** 9th February, 2015; ***Accepted:*** 23rd February, 2015

INTRODUCTION

Intense physical activity or unaccustomed exercise often results in contraction-induced muscle injury, force decrements, and muscle soreness.[1-3] Recently, our lab has shown that repeated eccentric contractions of the anterior crural muscles resulted in substantial loss of isometric tetanic torque immediately (i.e., 0 day) after the injury.[2] Further, restoration of tetanic torque took over 2 weeks to return to preinjury levels. Not surprisingly, the initial mode and severity of injury often plays a significant role in the duration of the degenerative and regenerative phases of skeletal muscle repair. For example, Warren *et al*. (2007) demonstrated freeze injury to the tibialis anterior (TA) resulted in a greater loss of maximal isometric torque immediately after the injury and took longer to fully recover (over 4 weeks) when compared to contraction-induced injury. Further, freeze injury caused a greater degree of damage, increased mononuclear cell infiltration, and increased number of regenerating fibers. Similarly, we have shown that injection of barium chloride ($BaCl_2$) into the mouse TA resulted in extensive damage.[4] However, the impact of $BaCl_2$-induced injury to the TA on tetanic torque production of the anterior compartment musculature remains unknown.

Models of muscle injury such as repeated eccentric contractions, cold probe application, or myotoxin injection provide critical insight into skeletal muscle regenerative processes.[5] Complete recovery may be long and research has been focused on accelerating skeletal

Access this article online	
Quick Response Code:	Website: www.ijcep.org
	DOI: 10.4103/2348-8093.155515

Address for correspondence: Mr. Russell G. Rogers, Department of Kinesiology and Health, Georgia State University, P. O. Box 3975, Atlanta, GA 30302, USA. E-mail: rrogers11@gsu.edu

muscle repair with nutraceutical and pharmacological agents.[6] For example, antioxidants like Vitamin E have been hypothesized to minimize oxidant damage associated with the early inflammatory response after injury.[7] However, evidence supporting a beneficial role for this antioxidant in muscle repair is unsubstantiated. To that end, clinicians have recommended nonsteroidal anti-inflammatory drugs (NSAIDs) to minimize pain and inflammation early after injury. Further, NSAIDs have been shown to reduce swelling and delay the inflammatory process in injured skeletal muscle,[8,9] which may provide a beneficial role in the very early stages of muscle repair. For example, treatment with the NSAID flurbiprofen improved recovery 3 and 7 days after repeated eccentric contractions.[10] However, a reduction in torque production at 28 days after injury was observed suggesting treatment resulted in delayed muscle regeneration. Therefore, continued research to identify a safe, effective treatment option to improve muscle repair following injury is warranted.

Resveratrol (3, 5, 4′-trihydroxystilbene) is a natural phenol found in high concentrations in the skin of red grapes and its use is becoming increasingly common for chronic diseases such as cancer,[11] cardiovascular disease,[12] and diabetes.[13] Resveratrol supplementation also lowered mortality rates and extended life expectancies in mice fed a high-fat diet.[14] Resveratrol was also shown to improve exercise endurance, oxidative metabolism, mitochondrial biogenesis, and caloric expenditure.[15] Importantly, resveratrol also increased C2C12 myoblast differentiation in culture,[16,17] which may suggest resveratrol could be used to improve skeletal muscle regeneration *in vivo*. However, the potential benefits of the extract in a model of muscle injury have not been explored. Accordingly, the purpose of this preliminary study was: (1) to determine the effects of $BaCl_2$ injection on isometric tetanic and twitch torque production during the recovery process and (2) to identify the impact of resveratrol supplementation during the recovery process.

MATERIALS AND METHODS

Animals and resveratrol supplementation

Male C57BL/6 mice (~25–30 g) were purchased from Jackson Laboratories (Bar Harbor, ME), housed in groups of 5, supplied food and water *ad libitum*, and maintained in a room at 20–22°C with a 12 h photoperiod. Experimental mice were provided standard rodent chow supplemented with 0.05% (wt/wt) resveratrol (ChromaDex, Irvine, CA) for 30 days prior to injury, which persisted throughout recovery. Control mice were fed only standard rodent chow (Purina Diet 5001, Indianapolis, IN). All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee.

Barium chloride-induced skeletal muscle injury

A 50-μL volume of a 1.2% $BaCl_2$ solution (diluted in sterile saline) was injected into the left mouse TA muscles using a 28 G needle as previously described.[4,18] The right, contralateral TA muscles served as uninjured controls in both treated and untreated groups. Briefly, the needle was inserted at the origin of the TA, extended through the mid-belly of the muscle to a region just superior to the distal tendon. Next, the diluted $BaCl_2$ solution was continuously injected into the TA as the syringe was removed. Complete serial sections of the injured TA confirmed that the mid-belly section was affected by the myotoxin. Injured and uninjured contralateral control muscles were harvested *postmortem* at 2, 7, 14, and 21 days following injury and processed for histological analysis.

In vivo analysis of skeletal muscle function

Torque – frequency relationships of the left anterior crural muscles (TA, extensor digitorum longus, and extensor hallucis longus) were determined as previously described.[2] Briefly, mice were anesthetized with isoflurane (1.5% isoflurane and 400 mL O_2/min) and placed on a temperature controlled platform to maintain core body temperature between 35°C and 37°C. The left knee was clamped and the left foot was secured to an aluminum "shoe" attached to the shaft of an Aurora Scientific 300B servomotor (Aurora, ON, Canada). Sterilized needles were inserted through the skin for stimulation of the left common peroneal nerve. Stimulation voltage and needle electrode placement were optimized with 5–15 isometric contractions (200 ms train of 0.1 ms pulses at 300 Hz). Following optimization, isometric torque was quantified as a function of stimulation frequency (20–300 Hz) before injury and at 7, 14, or 21 days postinjury. Mice were then euthanized with an overdose of isoflurane followed by cervical dislocation.

Histological assessment of regeneration in injured tibialis anterior muscles

Injured and uninjured TA muscles were removed, embedded in tissue freezing medium, and immediately frozen in 2-methylbutane cooled in liquid nitrogen. To ensure that we were assessing the most damaged portion of the TA, muscles were cut in serial sections at 10 μm beginning at the mid-belly. Sections were then processed for hematoxylin and eosin staining, dehydrated, mounted, and visualized at ×10 with a VanGaurd light microscope as previously described.[18] Cross-sectional areas of approximately 200 uninjured or regenerating fibers (i.e. centrally nucleated fibers) were calculated using ImageJ software (NIH, Bethesda, MD).

Statistical analysis of data

Data are presented as means ± standard error of the mean. Comparisons between groups were done using an

independent *t*-test and a two-way factorial (group × time) ANOVA with a Bonferroni *post-hoc* analysis. All statistical tests were performed with SigmaPlot (Systat Software Inc., San Jose, CA). Significance was accepted at $P \leq 0.05$.

RESULTS

Isometric tetanic and twitch torque of the anterior crural muscles were not different between untreated and resveratrol treated mice prior to the injury ($P = 0.074$). Two days after the injury, aberrant cellular architecture, mononuclear cell infiltration, and edema were clearly evident in both treated and untreated mice [Figure 1a]. At this time point, the severity of the injury prevented us from measuring tetanic or twitch torque.

Seven days following the injury, small centrally nucleated fibers (i.e., regenerating fibers) were present [Figure 1a]. In parallel, tetanic torque and twitch torque from untreated mice were reduced by 80.08% ± 1.20% and 81.41% ± 3.21% [Figure 2a and b]. However, treated mice showed no statistical improvement in force generation nor regenerated fiber cross-sectional area.

Fourteen days following the injury, regenerating fibers continue to hypertrophy with concomitant attenuation of the strength deficit [Figure 1a and b]. Specifically, tetanic torque was reduced 22.69% ± 3.27% while twitch torque returned to baseline (−5.33% ± 13.88%) when compared to preinjury values in untreated mice [Figure 2a and b]. However, resveratrol treatment did not improve these functional deficits nor regenerated fiber cross-sectional area.

Twenty-one days after the injury, cross-sectional area of regenerated fibers was larger than uninjured fibers [Figure 1a and b]. Further, tetanic and twitch torque were similar to preinjury values [Figure 2a and b]. Again, resveratrol treatment had no effect on fiber cross-sectional area or these functional markers.

DISCUSSION

In this study, we attempted to accelerate the recovery of skeletal muscle function following injury with resveratrol. Previous research has shown that resveratrol induced satellite cell differentiation *in vitro*,[16,17] which might suggest that the extract could improve skeletal muscle regeneration when delivered orally as well. Yet, acute low-dose resveratrol supplementation had no effect on recovery of skeletal muscle function and structure following myotoxin-induced injury.

One possible explanation why resveratrol failed to improve regeneration may lie in the severity of the injury. For example, our lab routinely quantifies tetanic and twitch torque in less-severe models of skeletal muscle injury (i.e., repeated eccentric contractions);[2] however, we were unable to quantify these force outputs during the early stages of muscle repair in the current injury model. Thus, it is possible that the extensive necrosis from $BaCl_2$-induced injury may have maximally activated the endogenous repair machinery and treatment with resveratrol was unable to further enhance these processes (e.g., satellite cell activation and proliferation, differentiation and fusion, and re-growth). To that end, supplementation with resveratrol in a less severe model

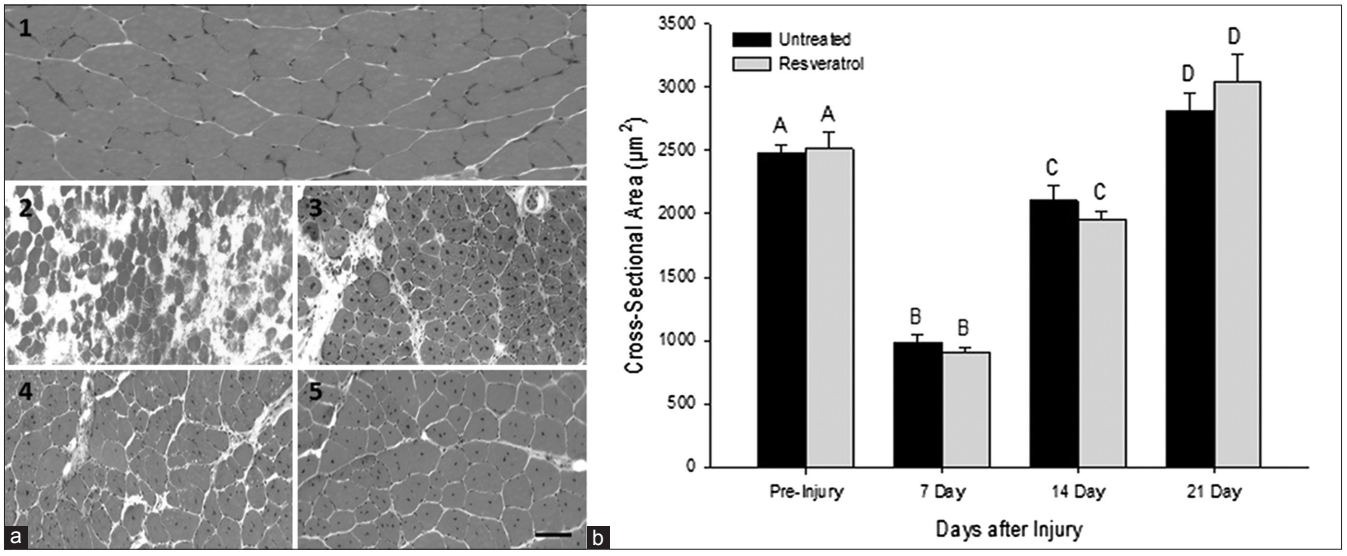

Figure 1: (a) Histological representation of injured muscle during regeneration. (1) Uninjured mouse tibialis anterior muscle, (2) 2 days, (3) 7 days, (4) 14 days, and (5) 21 days after barium chloride-induced injury. Images shown at ×10 magnification and bar in panel 5 = 100 μm. (b) Bar graph representing cross-sectional area of regenerating fibers. Bars with the same letter are not significantly different (accepted at $P \leq 0.05$). Values are reported as means ± standard error of the mean

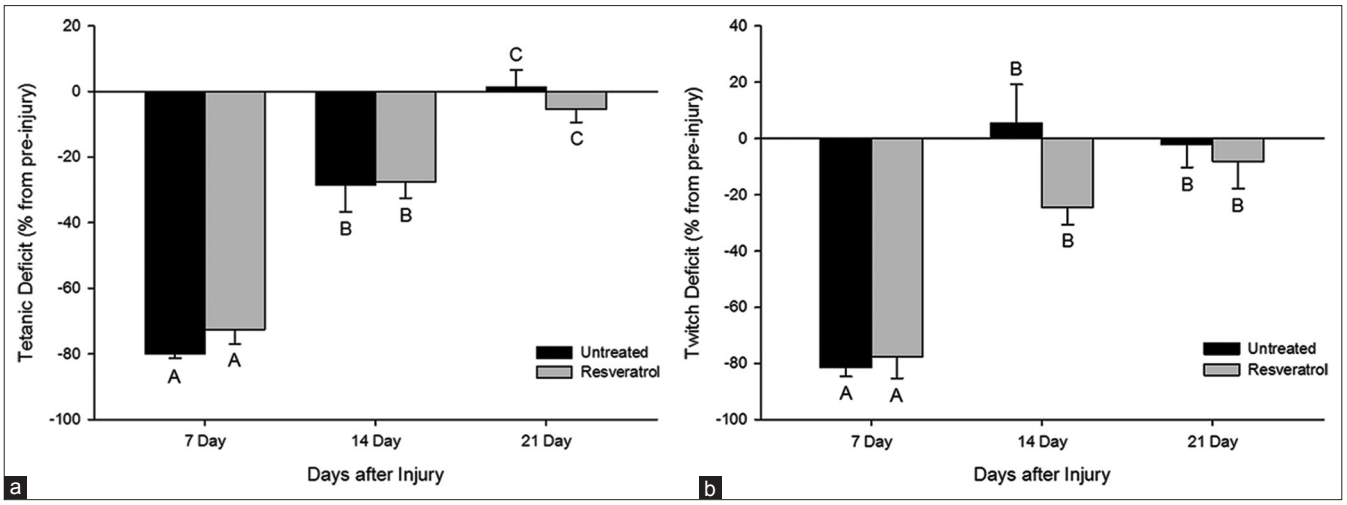

Figure 2: (a) Bar graph representing tetanic torque deficit. (b) Bar graph representing twitch torque deficit. Bars with the same letter are not significantly different (accepted at P≤0.05). Values are reported as means ± standard error of the mean (SEM)

of injury may be required to adequately study its effects on recovery of skeletal muscle function.

Eccentric contraction-induced injury has been shown to damage approximately 5% of the myofibers[19] and likely does not require maximal activity of satellite cells. Corona and Ingalls[20] demonstrated L-NAME administration, a compound known to block nitric oxide synthase, exacerbated strength deficits immediately after eccentric contractions suggesting nitric oxide partially protected skeletal muscle from contraction-induced injury. Moreover, resveratrol has been shown to provide cardioprotection via a nitric oxide mechanism and L-NAME administration was shown to attenuate the protection.[21] Alternatively, the dose of resveratrol and duration of treatment used in this study may have not been sufficient to promote these repair processes *in vivo*. For example, pre-conditioning with resveratrol for a longer duration may provide partial protection from injury and enhance the recovery of muscle function.

CONCLUSION

Acute, low-dose resveratrol supplementation did not improve functional or structural recovery of regenerating mouse TA after injury. Future research is likely required that explores differential dosing strategies in a physiological injury model. Together, this may provide a therapeutic approach to attenuate the loss of skeletal muscle function and pain associated with intense physical activity or unaccustomed exercise.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (NIAAA: K01 017190).

REFERENCES

1. Warren GL, Summan M, Gao X, Chapman R, Hulderman T, Simeonova PP. Mechanisms of skeletal muscle injury and repair revealed by gene expression studies in mouse models. J Physiol 2007;582:825-41.
2. Baumann CW, Rogers RG, Gahlot N, Ingalls CP. Eccentric contractions disrupt FKBP12 content in mouse skeletal muscle. Physiol Rep 2014;2:1-12.
3. Proske U, Morgan DL. Muscle damage from eccentric exercise: Mechanism, mechanical signs, adaptation and clinical applications. J Physiol 2001;537:333-45.
4. Otis JS, Niccoli S, Hawdon N, Sarvas JL, Frye MA, Chicco AJ, *et al.* Pro-inflammatory mediation of myoblast proliferation. PLoS One 2014;9:e92363.
5. Huard J, Li Y, Fu FH. Muscle injuries and repair: Current trends in research. J Bone Joint Surg Am 2002;84-A: 822-32.
6. Prisk V, Huard J. Muscle injuries and repair: The role of prostaglandins and inflammation. Histol Histopathol 2003;18:1243-56.
7. Beaton LJ, Allan DA, Tarnopolsky MA, Tiidus PM, Phillips SM. Contraction-induced muscle damage is unaffected by vitamin E supplementation. Med Sci Sports Exerc 2002;34:798-805.
8. Almekinders LC, Gilbert JA. Healing of experimental muscle strains and the effects of nonsteroidal antiinflammatory medication. Am J Sports Med 1986;14:303-8.
9. Trappe TA, Fluckey JD, White F, Lambert CP, Evans WJ. Skeletal muscle PGF (2)(alpha) and PGE (2) in response to eccentric resistance exercise: Influence of ibuprofen acetaminophen. J Clin Endocrinol Metab 2001;86:5067-70.
10. Mishra DK, Fridén J, Schmitz MC, Lieber RL. Anti-inflammatory medication after muscle injury. A treatment resulting in short-term improvement but subsequent loss of muscle function. J Bone Joint Surg Am 1995;77:1510-9.
11. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, *et al.* Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997;275:218-20.
12. Szmitko PE, Verma S. Cardiology patient pages. Red wine and your heart. Circulation 2005;111:e10-1.
13. Su HC, Hung LM, Chen JK. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in

streptozotocin-induced diabetic rats. Am J Physiol Endocrinol Metab 2006;290:E1339-46.
14. Baur JA, Ungvari Z, Minor RK, Le Couteur DG, de Cabo R. Are sirtuins viable targets for improving healthspan and lifespan? Nat Rev Drug Discov 2012;11:443-61.
15. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, *et al.* Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006;127:1109-22.
16. Montesano A, Luzi L, Senesi P, Mazzocchi N, Terruzzi I. Resveratrol promotes myogenesis and hypertrophy in murine myoblasts. J Transl Med 2013;11:310.
17. Kaminski J, Lançon A, Aires V, Limagne E, Tili E, Michaille JJ, *et al.* Resveratrol initiates differentiation of mouse skeletal muscle-derived C2C12 myoblasts. Biochem Pharmacol 2012;84:1251-9.
18. Dekeyser GJ, Clary CR, Otis JS. Chronic alcohol ingestion delays skeletal muscle regeneration following injury. Regen Med Res 2013;1:2.
19. Lowe DA, Warren GL, Ingalls CP, Boorstein DB, Armstrong RB. Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. J Appl Physiol (1985) 1995;79:1260-70.
20. Corona BT, Ingalls CP. Immediate force loss after eccentric contractions is increased with L-NAME administration, a nitric oxide synthase inhibitor. Muscle Nerve 2013;47:271-3.
21. Bradamante S, Barenghi L, Piccinini F, Bertelli AA, De Jonge R, Beemster P, *et al.* Resveratrol provides late-phase cardioprotection by means of a nitric oxide- and adenosine-mediated mechanism. Eur J Pharmacol 2003;465:115-23.

How to cite this article: Rogers RG, Baumann CW, Otis JS. Recovery of skeletal muscle function following injury is not augmented by acute resveratrol supplementation. Int J Clin Exp Physiol 2015;2:29-33.

Source of Support: This work was supported by the National Institutes of Health (NIAAA: K01 017190), **Conflict of Interest:** Nil.