JSES Open Access 2 (2018) 91–96

Contents lists available at ScienceDirect

JSES Open Access

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

Antioxidant treatment with vitamin C attenuated rotator cuff degeneration caused by oxidative stress in *Sod1*-deficient mice

Daichi Morikawa, MD, PhD [a,b], Hidetoshi Nojiri, MD, PhD [a,*], Yoshiaki Itoigawa, MD, PhD [a], Yusuke Ozawa [b], Kazuo Kaneko, MD, PhD [a], Takahiko Shimizu, PhD [b]

[a] *Department of Orthopaedics, Juntendo University Graduate School of Medicine, Tokyo, Japan*
[b] *Department of Advanced Aging Medicine, Chiba University Graduate School of Medicine, Chiba, Japan*

ARTICLE INFO

Keywords:
Oxidative stress
Rotator cuff degeneration
Enthesis
Vitamin C administration
Sod1-deficient mice
Antioxidant

Level of evidence: Basic Science Study, Histology, Animal Model

Background: Rotator cuff degeneration is 1 of several factors that lead to rotator cuff tears; however, the mechanism of this degeneration remains unclear. We previously reported that deficiency of an antioxidant enzyme, superoxide dismutase 1 (*Sod1*), in mice induced degeneration in supraspinatus tendon entheses, a model that replicates human rotator cuff degeneration. In this study, we analyzed possible effects of vitamin C (VC), a major antioxidant, on the degenerative changes of supraspinatus entheses in *Sod1*$^{-/-}$ mice.

Methods: We administered VC or vehicle, distilled water, for 8 weeks to *Sod1*$^{-/-}$ and wild-type male mice beginning at 12 weeks of age (n = 5-8 per group). When mice were 20 weeks of age, we sectioned rotator cuff tissue samples and performed hematoxylin-eosin and toluidine blue staining for quantitative histologic evaluation.

Results: VC administration, compared with vehicle administration, attenuated the histologic changes, including a misaligned 4-layered structure, fragmented tidemark, and toluidine blue staining, in the supraspinatus entheses of *Sod1*$^{-/-}$ mice. In the quantitative histologic evaluation, all parameters were significantly decreased in *Sod1*$^{-/-}$ mice compared with wild-type mice, except for the number of nonchondrocytes.

Conclusion: We demonstrated that an antioxidant treatment, VC administration, attenuated the rotator cuff degeneration, similar to that observed in humans, that is caused by oxidative stress in *Sod1*$^{-/-}$ mice. VC effects included improvements in quantitative histologic parameters and other histologic changes. These results suggest that VC treatment can prevent oxidative stress–induced degeneration of the rotator cuff.

Rotator cuff tears are the most common tendon injury in orthopedic patients and are associated with shoulder pain and dysfunction.[28] The factors contributing to the tears fall under 2 main categories: extrinsic factors (including shoulder overuse, presence of spurs, and acromion morphology)[13,19] and intrinsic factors (including aging, inflammation, oxidative stress, and hypovascularity).[35,40] Degeneration of the rotator cuff entheses is an intrinsic factor and consists of pathologic changes of cuff insertion, such as thinning and disorientation of collagen fibers and loss of cellularity, vascularity, and fibrocartilage mass at the site of insertion.[6,19] Previous studies reported that degeneration of the rotator cuff was associated with reduction of its tensile strength,[30,31] but the precise mechanisms of age-related rotator cuff degeneration remain unclear.

We previously investigated the contribution of oxidative stress to rotator cuff degeneration. Oxidative stress results from an imbalance between oxidation caused by reactive oxygen species (ROS) and reduction catalyzed by antioxidant systems. We analyzed the supraspinatus entheses of mice deficient in superoxide dismutase 1 (*Sod1*), an important antioxidant enzyme.[17,22] The *Sod1*$^{-/-}$ mice showed rotator cuff degeneration with histologic changes that were similar to those observed in humans. These included a misaligned 4-layered structure and fragmented tidemark as well as altered tissue elasticity in the supraspinatus enthesis.[15]

In this study, we administrated vitamin C (VC), one of the primary antioxidants obtained through foods and supplementation, to *Sod1*-deficient mice to examine effects of VC antioxidant treatment on rotator cuff degeneration and to confirm the link between this degeneration and oxidative stress.

* Corresponding author: Hidetoshi Nojiri, MD, PhD, Department of Orthopaedics, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.
E-mail address: hnojiri@juntendo.ac.jp (H. Nojiri)

https://doi.org/10.1016/j.jses.2017.11.003

Methods

Animals

Sod1-deficient mice (*Sod1*$^{-/-}$) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The *Sod1*$^{-/-}$ mice were backcrossed with C57BL/6NCrSlc mice (Nilson SLC, Shizuoka, Japan) 5-6 times. The mice were maintained and studied according to protocols approved by the Animal Care Committees of the authors' institutions based on Guidelines for Proper Conduct of Animal Experiments.

Oral administration of the antioxidant vitamin C

To analyze effectiveness of antioxidant therapy against the degenerative changes of supraspinatus entheses in *Sod1*$^{-/-}$ mice, we administrated the antioxidant VC or vehicle orally to *Sod1*$^{-/-}$ and wild-type (WT) male mice. VC (sodium L-ascorbate; Sigma-Aldrich Chemicals, St. Louis, MO, USA) was dissolved in water at 1% (w/v). Oral administration began at the age of 12 weeks and continued for 8 weeks. The method of dosing was to provide the mice, ad libitum, with drinking water containing VC. The VC solution was replenished with fresh solution twice per week.

Tissue preparation

The *Sod1*$^{-/-}$ and WT mice were sacrificed with proper euthanasia procedures at 20 weeks of age (n = 5-8 per group). The complexes of the supraspinatus and infraspinatus muscles, tendons, and humeral head were removed together, fixed in 4% paraformaldehyde at room temperature overnight, decalcified with 10% ethylenediaminetetraacetic acid in 10 mM of phosphate buffer (pH 7.4) for 1 week, and then embedded in paraffin blocks. The paraffin blocks were cut on a standardized frontal plane and stained with hematoxylin-eosin and toluidine blue (TB).

Histologic analysis of the supraspinatus enthesis

We analyzed sections under an optical microscope to assess overall histologic structure and microstructure of the supraspinatus entheses. This analysis included examination of the 4-layered structure, including the tendon, nonmineralized and mineralized fibrocartilage, and bone, and the tidemark, which forms a boundary between 2 fibrocartilages.

Histologic analysis of collagen fibers in the entheses

To analyze collagen fiber structure in the entheses, we observed the sections under a polarizing microscope. This analysis was based on the principle that polarizing light directed at spatially oriented collagen fibers in tissue sections is diffracted and shines brightly against a dark background.[4,11] The slides were rotated for 360° on the microscope tray to select the position showing maximum brightness.[5,11] In the intact enthesis, collagen fibrils are spatially aligned, conferring a high tensile strength to fibrocartilage.[11]

Histologic evaluation of supraspinatus entheses

Quantitative histologic measurements were performed as described previously.[11,15] Parameters analyzed were the number of chondrocytes, number of nonchondrocytes, percentage of aligned chondrocytes, spatial arrangement of collagen fibers, and area of metachromasia.

Number of chondrocytes

At the enthesis, the number of chondrocytes was counted in a standardized rectangle field on hematoxylin-eosin–stained section. Cells displaying 3 or 4 of the following were defined as chondrocytes: large nucleus, basophilic and shrunken cytoplasm, lacuna around the cytoplasm, and halo around the lacuna.

Number of nonchondrocytes

Non–chondrocytic cells were counted in the same fields as the chondrocytes. Non–chondrocytic cells indicated mesenchymal cells, fibroblasts, endothelial cells, or adipocytes.[11]

Percentage of aligned chondrocytes

In the same rectangular field used for the number of chondrocytes, the number of chondrocytes forming rows was counted. A row was defined as 3 or more chondrocytes aligned longitudinally. The number of chondrocytes aligned in rows divided by the total number of chondrocytes provided the percentage of chondrocytes aligned in rows. In a normal mature enthesis, chondrocytes are aligned in rows in nonmineralized and mineralized fibrocartilage.[11]

Area of metachromasia

Fibrocartilage binds basic blue dyes, such as TB, changing its color to reddish blue, a property known as metachromasia. Intensity of metachromasia staining with TB indicated proteoglycan content. The area of intense metachromasia was quantified using the image analysis software. On the TB-stained slides, a standardized field starting at the bone-tendon junction was captured. Intense metachromatic areas within the standardized field were measured automatically and interpreted as fibrocartilage.

Statistical analysis

Statistical analyses were performed using analysis of variance followed by Tukey test. All data are expressed as means ± standard deviation. $P \leq .05$ was considered statistically significant.

Results

Vitamin C administration attenuated histologic changes of the supraspinatus entheses in Sod1$^{-/-}$ mice

According to histologic analyses under the optical microscope, the WT mice had a well-organized 4-layered structure (tendon proper, nonmineralized fibrocartilage, mineralized fibrocartilage, and bone) and a tidemark with a boundary between nonmineralized fibrocartilage and mineralized fibrocartilage (Fig. 1, *A*). In contrast, *Sod1*$^{-/-}$ mice had a misaligned 4-layered structure and a fragmented tidemark in the entheses (Fig. 1, *B*, *arrowheads*). VC administration (Fig. 1, *D*), compared with vehicle administration (Fig. 1, *B*), attenuated these histologic changes, improving the misaligned 4-layered structure and fragmented tidemark in *Sod1*$^{-/-}$ mice. According to TB staining, the WT mice had an area of reddish blue staining, known as metachromasia, in the supraspinatus entheses (Fig. 2, *A*). The *Sod1*$^{-/-}$ mice (Fig. 2, *B*) had weaker staining than the WT mice (Fig. 2, *A*). VC administration increased TB staining in the supraspinatus entheses of the *Sod1*$^{-/-}$ mice (Fig. 2, *D*).

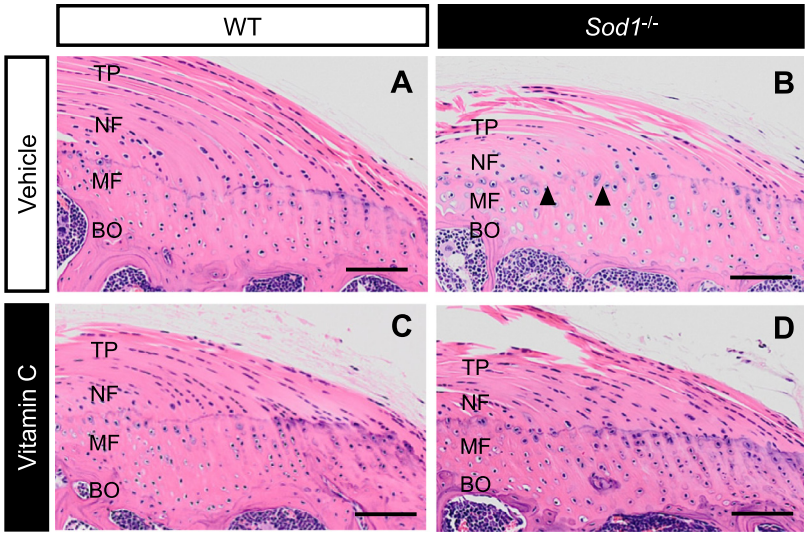

Figure 1 Vitamin C (VC) administration attenuated histologic changes of the supraspinatus enthesis in *Sod1*$^{-/-}$ mice. Hematoxylin-eosin staining of the supraspinatus enthesis (original magnification ×100). (**A**) Wild-type (*WT*) mice with vehicle administration. (**B**) *Sod1*$^{-/-}$ mice with vehicle administration. (**C**) WT mice with VC administration. (**D**) *Sod1*$^{-/-}$ mice with VC administration. The WT mice exhibited a well-organized 4-layered structure (*TP*, tendon proper; *NF*, nonmineralized fibrocartilage; *MF*, mineralized fibrocartilage; and *BO*, bone) and tidemark, which forms a boundary between the NF and MF (**A**). In contrast, the *Sod1*$^{-/-}$ mice exhibited a misaligned 4-layered structure and fragmented tidemark (*arrowheads*) in the enthesis (**B**). In *Sod1*$^{-/-}$ mice, VC administration attenuated the histologic changes (ie, the misaligned 4-layered structure and fragmented tidemark) compared with vehicle administration (**B** and **D**). The *scale bars* indicate 100 μm.

Vitamin C administration attenuated deterioration of spatially aligned collagen fibers of the supraspinatus entheses in Sod1$^{-/-}$ *mice*

We next evaluated the alignment of collagen fibers in the supraspinatus entheses using polarizing microscopy. The WT mice exhibited brightly diffracted light at the entheses along the tendon (Fig. 3, *A*). In contrast, the *Sod1*$^{-/-}$ mice had markedly less brightly diffracted light in the entheses compared with that observed in WT mice (Fig. 3, *A* and *B*). Furthermore, *Sod1*$^{-/-}$ mice treated with VC (Fig. 3, *D*) showed an increase in brightly diffracted light in the enthesis compared with those receiving vehicle (Fig. 3, *B*). These results indicated that VC administration attenuated deterioration of spatially aligned collagen fibers in the supraspinatus entheses of the *Sod1*$^{-/-}$ mice.

Quantitative histologic changes of the supraspinatus entheses

To quantify the histologic changes, we measured 5 parameters: the number of chondrocytes (Fig. 4, *A*) and nonchondrocytes (Fig. 4, *B*), percentage of aligned chondrocytes (Fig. 4, *C*), area of diffracted polarized light (Fig. 4, *D*), and area of metachromasia (Fig. 4, *E*). With vehicle administration, *Sod1*$^{-/-}$ mice showed significant reduction of these quantitative histologic measurements compared with WT mice, with the exception of the number of nonchondrocytes

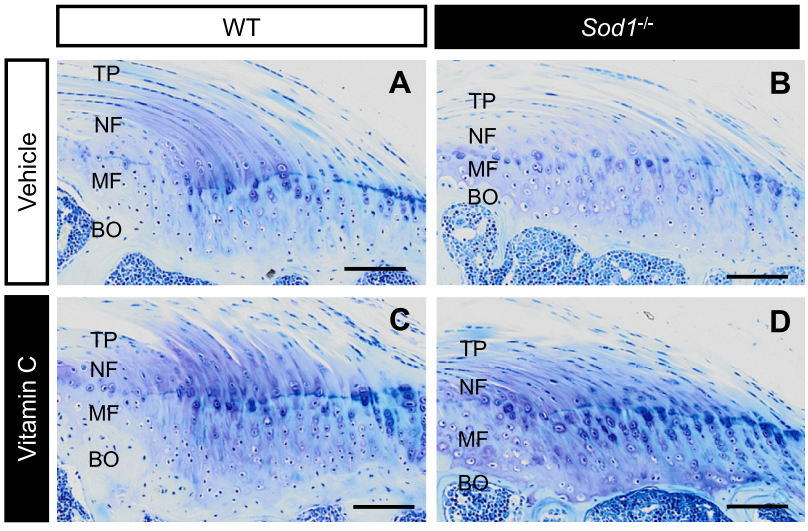

Figure 2 Vitamin C (VC) administration increased toluidine blue (TB) staining of the supraspinatus enthesis in *Sod1*$^{-/-}$ mice. TB staining of the supraspinatus enthesis (original magnification ×100). (**A**) Wild-type (*WT*) mice with vehicle administration. (**B**) *Sod1*$^{-/-}$ mice with vehicle administration. (**C**) WT mice with VC administration. (**D**) *Sod1*$^{-/-}$ mice with VC administration. In WT mice, there was an area of reddish blue staining, known as metachromasia, in the supraspinatus enthesis. In contrast, *Sod1*$^{-/-}$ mice had much weaker TB staining than WT mice. VC administration increased the staining of TB in the supraspinatus enthesis of the *Sod1*$^{-/-}$ mice. *TP*, tendon proper; *NF*, nonmineralized fibrocartilage; *MF*, mineralized fibrocartilage; *BO*, bone. The *scale bars* indicate 100 μm.

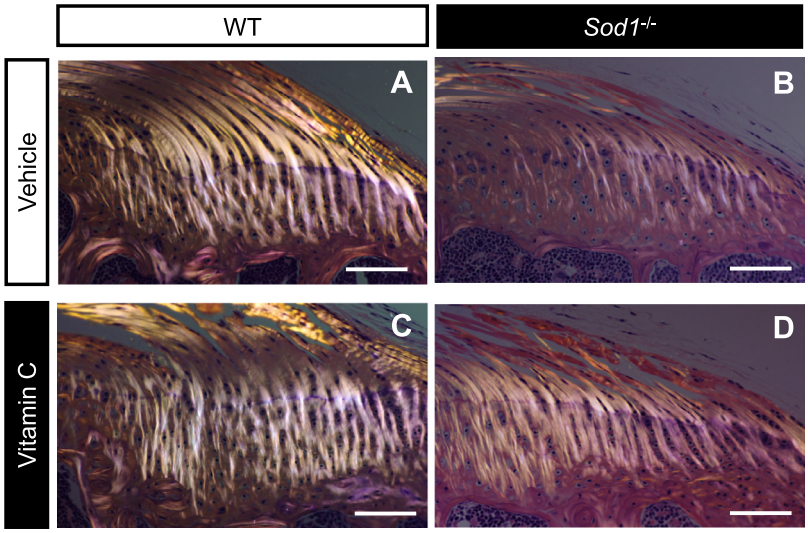

Figure 3 Vitamin C (VC) administration attenuated deterioration of spatially aligned collagen fibers of the supraspinatus enthesis in *Sod1*$^{-/-}$ mice. Polarizing microscopic images of the supraspinatus enthesis in wild-type (*WT*) mice with vehicle administration (**A**), *Sod1*$^{-/-}$ mice with vehicle administration (**B**), WT mice with VC administration (**C**), and *Sod1*$^{-/-}$ mice with VC administration (**D**). The *Sod1*$^{-/-}$ mice displayed a decrease in brightly diffracted light in the enthesis compared with that observed in the WT mice (**A** and **B**). With VC administration, the brightly diffracted light was increased in the enthesis of *Sod1*$^{-/-}$ mice (**B** and **D**). The *scale bars* indicate 50 μm.

(Fig. 4, *A-E*). However, in *Sod1*$^{-/-}$ mice receiving VC, these parameters were improved (Fig. 4, *A* and *C-E*). In WT mice, no difference in any parameter was observed between VC and vehicle administration (Fig. 4).

Discussion

In this study, we showed that an antioxidant treatment, VC administration, attenuated the quantitative histologic measurements and other histologic changes caused by oxidative stress in *Sod1*$^{-/-}$ mice (Figs. 1-4). These histologic changes were similar to those observed in humans with rotator cuff degeneration. VC is a simple, safe, and inexpensive treatment believed to be beneficial for many conditions including cancer, the common cold, and smoking-related problems.[8,14,23,29,34,41] VC is not commonly used to address musculoskeletal problems but has been recommended to prevent occurrence of complex regional pain syndrome after extremity surgery and injury, although the mechanism of this effect is not known.[3,27,32] Several animal studies have demonstrated the benefits of VC for musculoskeletal conditions. Passage et al reported that high doses of VC ameliorated the phenotype of a mouse model for Charcot-Marie-Tooth, the most common hereditary peripheral neuropathy.[26] Omeroğlu et al showed that local injection of a high dose of VC (150 mg/d) accelerated healing of the Achilles tendon in rats.[24] Hung et al reported that local VC injection at a low concentration (5 mg/mL) reduced the extent of adhesion in healing tendons better than a high concentration (50 mg/mL) in a chicken model.[7] However, effects of VC on rotator cuff tissue have not been examined. In 2 reports testing antioxidant therapies, for tendon tissue, hydrogen peroxide (H_2O_2) exposure increased ROS generation and death of tenofibroblasts, and antioxidants, anthocyanins, or cyanidin treatments decreased ROS and increased the viability of tenofibroblasts from supraspinatus tendon tissues of rats and humans.[10,25] In our study, oral administration of VC was effective against rotator cuff degeneration induced by endogenous oxidative stress caused by *Sod1*-deficiency. To our knowledge, this is the first in vivo study showing effectiveness of an orally administered antioxidant against oxidative stress in rotator cuff tissue. Based on our findings, it is possible that oral antioxidant treatment would be useful for human rotator cuff degeneration.

VC has two major biologic functions: as a scavenger of ROS, such as O_2^- and H_2O_2, and as a cofactor for collagen synthesis.[20,36] Our data showed that VC administration attenuated the histologic changes of supraspinatus entheses (Figs. 1-3), improving quantitative histologic parameters in *Sod1*$^{-/-}$ mice (Fig. 4). Yet, in the WT mice, VC administration for 8 weeks did not affect the histologic findings (Figs. 1 and 2), including the quantitative histology in supraspinatus entheses (Fig. 3). These results indicated that the protective effects of VC in *Sod1*$^{-/-}$ mice were caused by its ROS scavenger activity rather than its actions as a cofactor for collagen synthesis. In 1 animal study, VC accelerated tendon healing, restoring normal structure.[24] Other antioxidant treatments were reported to improve total collagen levels and collagen orientation as well as to increase strength during Achilles tendon healing.[1,2] Moreover, previous data from our group showed that VC accelerated the healing and outgrowth of *Sod1*$^{-/-}$ fibroblasts and a VC derivative increased cell viability during oxidative stress in vitro.[33,38] Together, these findings suggest that redox balance regulation, especially through VC treatment, prevented the degeneration of supraspinatus entheses in *Sod1*$^{-/-}$ mice.

In this study, VC administration improved the 4 histologic parameters of entheses, such as number of chondrocyte, chondrocytes aligned in rows, area of metachromasia, and diffracted polarized light, in *Sod1*$^{-/-}$ mice. In the intact enthesis, chondrocytes are aligned in a row and maintain the integrity of fibrocartilaginous matrix, which was indicated by metachromasia. As well as these, collagen fibrils are spatially aligned in the intact enthesis, conferring a high tensile strength to fibrocartilage.[11] These indicated that histologic findings with VC treatment are more similar to those of intact enthesis compared with those with vehicle administration in *Sod1*$^{-/-}$ mice.

Limitations

There were several limitations in this study. First, it is difficult to measure oxidative stress or to analyze oxidative stress–related genes in mouse rotator cuff tissues because the supraspinatus tendon is very small, with a tendon width of approximately 1 mm. Therefore, we did not confirm that the VC treatment decreased oxidative stress in supraspinatus tendon. However, *Sod1* deficiency causes several age-related changes attributed to oxidative stress in mice,

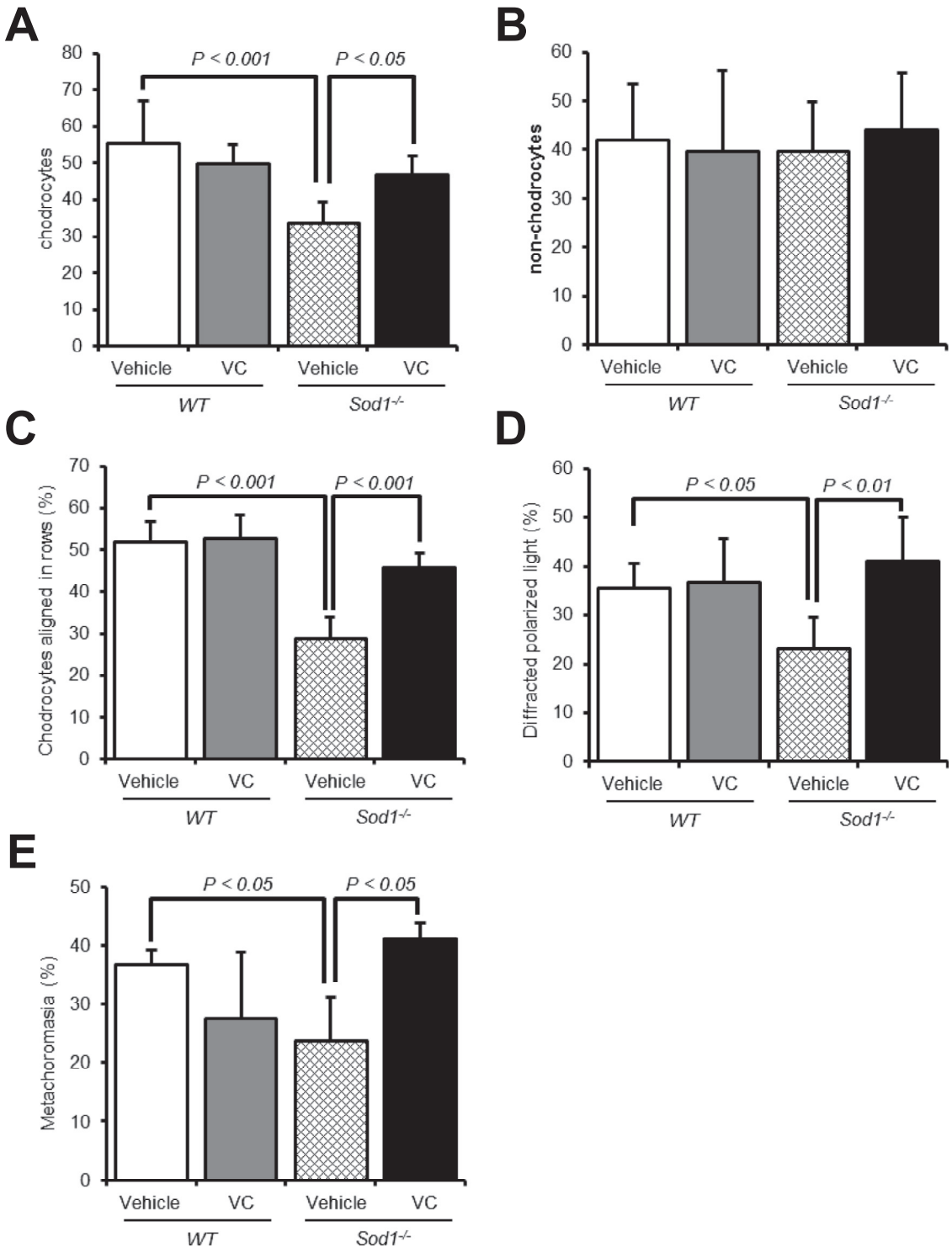

Figure 4 Histologic evaluation of supraspinatus enthesis in wild-type (*WT*) and *Sod1*$^{-/-}$ mice with vehicle and vitamin C (*VC*) administration. Quantitative histology measured 5 parameters: (**A**) the number of chondrocytes, (**B**) the number of nonchondrocytes, (**C**) the percentage of aligned chondrocytes, (**D**) the spatial arrangement of collagen fibers, and (**E**) the area of metachromasia. n = 5-8 per group. The *error bars* indicate standard deviation.

and several studies have shown that VC has antioxidant effects in *Sod1*$^{-/-}$ mice.[9,12,16-18,21,22,37,39] As a second limitation, we could not perform tensile testing because of the small size of the tendons. Third, our study used a relatively limited sample size. Finally, this study has been performed in only 1 protocol regarding VC concentration and duration of oral administration, meaning that we have not checked the dose-dependent or duration-dependent effects of VC administration. Furthermore, the exact concentration and in vivo kinetics of VC in tissues were unclear because these could not be monitored in this examination and depended on the amount of drinking and the timing of measurement. However, we measured total amount of daily drinking of VC and vehicle in WT and *Sod1*$^{-/-}$ mice and found no difference among the 4 groups (data not shown). Further analyses, including studies using human samples, will be

needed to fully clarify the protective role of VC against rotator cuff degeneration.

Conclusion

We have demonstrated that antioxidant treatment, through VC administration, attenuated histologic changes in the supraspinatus entheses induced by *Sod1* deficiency. Our findings suggest that antioxidant treatment may prevent oxidative stress–induced degeneration of the rotator cuff.

Disclaimer

The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

Acknowledgments

The mice were maintained and studied according to protocols approved by the Animal Care Committees of the authors' institutions.

References

1. Akdemir O, Lineaweaver WC, Cavusoglu T, Binboga E, Uyanikgil Y, Zhang F, et al. Effect of taurine on rat Achilles tendon healing. Connect Tissue Res 2015;56:300-6. http://dx.doi.org/10.3109/03008207.2015.1026437
2. Aro AA, Perez MO, Vieira CP, Esquisatto MA, Rodrigues RA, Gomes L, et al. Effect of *Calendula officinalis* cream on Achilles tendon healing. Anat Rec (Hoboken) 2015;298:428-35. http://dx.doi.org/10.1002/ar.23057
3. Cazeneuve JF, Leborgne JM, Kermad K, Hassan Y. Vitamin C and prevention of reflex sympathetic dystrophy following surgical management of distal radius fractures. Acta Orthop Belg 2002;68:481-4.
4. Cummins CA, Murrell GA. Mode of failure for rotator cuff repair with suture anchors identified at revision surgery. J Shoulder Elbow Surg 2003;12:128-33. http://dx.doi.org/10.1067/mse.2003.21
5. Drexler W, Stamper D, Jesser C, Li X, Pitris C, Saunders K, et al. Correlation of collagen organization with polarization sensitive imaging of in vitro cartilage: implications for osteoarthritis. J Rheumatol 2001;28:1311-8.
6. Hashimoto T, Nobuhara K, Hamada T. Pathologic evidence of degeneration as a primary cause of rotator cuff tear. Clin Orthop Relat Res 2003;111-20.
7. Hung LK, Fu SC, Lee YW, Mok TY, Chan KM. Local vitamin-C injection reduced tendon adhesion in a chicken model of flexor digitorum profundus tendon injury. J Bone Joint Surg Am 2013;95:e41. http://dx.doi.org/10.2106/JBJS.K.00988
8. Ichim TE, Minev B, Braciak T, Luna B, Hunninghake R, Mikirova NA, et al. Intravenous ascorbic acid to prevent and treat cancer-associated sepsis? J Transl Med 2011;9:25. http://dx.doi.org/10.1186/1479-5876-9-25
9. Imamura Y, Noda S, Hashizume K, Shinoda K, Yamaguchi M, Uchiyama S, et al. Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. Proc Natl Acad Sci U S A 2006;103:11282-7. http://dx.doi.org/10.1073/pnas.0602131103
10. Kim RJ, Hah YS, Sung CM, Kang JR, Park HB. Do antioxidants inhibit oxidative-stress-induced autophagy of tenofibroblasts? J Orthop Res 2014;32:937-43. http://dx.doi.org/10.1002/jor.22608
11. Koike Y, Trudel G, Uhthoff HK. Formation of a new enthesis after attachment of the supraspinatus tendon: a quantitative histologic study in rabbits. J Orthop Res 2005;23:1433-40. http://dx.doi.org/10.1016/j.orthres.2005.02.015.1100230628
12. Kojima T, Wakamatsu TH, Dogru M, Ogawa Y, Igarashi A, Ibrahim OM, et al. Age-related dysfunction of the lacrimal gland and oxidative stress: evidence from the Cu,Zn-superoxide dismutase-1 (Sod1) knockout mice. Am J Pathol 2012;180:1879-96. http://dx.doi.org/10.1016/j.ajpath.2012.01.019
13. Maffulli N, Longo UG, Berton A, Loppini M, Denaro V. Biological factors in the pathogenesis of rotator cuff tears. Sports Med Arthrosc 2011;19:194-201. http://dx.doi.org/10.1097/JSA.0b013e3182250cad
14. Maggini S, Beveridge S, Suter M. A combination of high-dose vitamin C plus zinc for the common cold. J Int Med Res 2012;40:28-42. http://dx.doi.org/10.1177/147323001204000104
15. Morikawa D, Itoigawa Y, Nojiri H, Sano H, Itoi E, Saijo Y, et al. Contribution of oxidative stress to the degeneration of rotator cuff entheses. J Shoulder Elbow Surg 2014;23:628-35. http://dx.doi.org/10.1016/j.jse.2014.01.041
16. Morikawa D, Nojiri H, Saita Y, Kobayashi K, Watanabe K, Ozawa Y, et al. Cytoplasmic reactive oxygen species and SOD1 regulate bone mass during mechanical unloading. J Bone Miner Res 2013;28:2368-80. http://dx.doi.org/10.1002/jbmr.1981
17. Murakami K, Inagaki J, Saito M, Ikeda Y, Tsuda C, Noda Y, et al. Skin atrophy in cytoplasmic SOD-deficient mice and its complete recovery using a vitamin C derivative. Biochem Biophys Res Commun 2009;382:457-61. http://dx.doi.org/10.1016/j.bbrc.2009.03.053
18. Murakami K, Murata N, Noda Y, Tahara S, Kaneko T, Kinoshita N, et al. SOD1 (copper/zinc superoxide dismutase) deficiency drives amyloid β protein oligomerization and memory loss in mouse model of Alzheimer disease. J Biol Chem 2011;286:44557-68. http://dx.doi.org/10.1074/jbc.M111.279208
19. Nho SJ, Yadav H, Shindle MK, Macgillivray JD. Rotator cuff degeneration: etiology and pathogenesis. Am J Sports Med 2008;36:987-93. http://dx.doi.org/10.1177/0363546508317344
20. Nishikimi M. Oxidation of ascorbic acid with superoxide anion generated by the xanthine-xanthine oxidase system. Biochem Biophys Res Commun 1975;63:463-8.
21. Noda Y, Ota K, Shirasawa T, Shimizu T. Copper/zinc superoxide dismutase insufficiency impairs progesterone secretion and fertility in female mice. Biol Reprod 2012;86:1-8. http://dx.doi.org/10.1095/biolreprod.111.092999
22. Nojiri H, Saita Y, Morikawa D, Kobayashi K, Tsuda C, Miyazaki T, et al. Cytoplasmic superoxide causes bone fragility owing to low-turnover osteoporosis and impaired collagen cross-linking. J Bone Miner Res 2011;26:2682-94. http://dx.doi.org/10.1002/jbmr.489
23. Ohno S, Ohno Y, Suzuki N, Soma G, Inoue M. High-dose vitamin C (ascorbic acid) therapy in the treatment of patients with advanced cancer. Anticancer Res 2009;29:809-15.
24. Omeroğlu S, Peker T, Türközkan N, Omeroğlu H. High-dose vitamin C supplementation accelerates the Achilles tendon healing in healthy rats. Arch Orthop Trauma Surg 2009;129:281-6. http://dx.doi.org/10.1007/s00402-008-0603-0
25. Park HB, Hah YS, Yang JW, Nam JB, Cho SH, Jeong ST. Antiapoptotic effects of anthocyanins on rotator cuff tenofibroblasts. J Orthop Res 2010;28:1162-9. http://dx.doi.org/10.1002/jor.21097
26. Passage E, Norreel JC, Noack-Fraissignes P, Sanguedolce V, Pizant J, Thirion X, et al. Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. Nat Med 2004;10:396-401. http://dx.doi.org/10.1038/nm1023
27. Perez RS, Zollinger PE, Dijkstra PU, Thomassen-Hilgersom IL, Zuurmond WW, Rosenbrand KC, et al. Evidence based guidelines for complex regional pain syndrome type 1. BMC Neurol 2010;10:20. http://dx.doi.org/10.1186/1471-2377-10-20
28. Rees JD, Wilson AM, Wolman RL. Current concepts in the management of tendon disorders. Rheumatology (Oxford) 2006;45:508-21. http://dx.doi.org/10.1093/rheumatology/kel046
29. Riordan HD, Casciari JJ, González MJ, Riordan NH, Miranda-Massari JR, Taylor P, et al. A pilot clinical study of continuous intravenous ascorbate in terminal cancer patients. P R Health Sci J 2005;24:269-76.
30. Sano H, Ishii H, Yeadon A, Backman DS, Brunet JA, Uhthoff HK. Degeneration at the insertion weakens the tensile strength of the supraspinatus tendon: a comparative mechanical and histologic study of the bone-tendon complex. J Orthop Res 1997;15:719-26.
31. Sano H, Uhthoff HK, Backman DS, Brunet JA, Trudel G, Pham B, et al. Structural disorders at the insertion of the supraspinatus tendon. Relation to tensile strength. J Bone Joint Surg Br 1998;80:720-5.
32. Shibuya N, Humphers JM, Agarwal MR, Jupiter DC. Efficacy and safety of high-dose vitamin C on complex regional pain syndrome in extremity trauma and surgery—systematic review and meta-analysis. J Foot Ankle Surg 2013;52:62-6. http://dx.doi.org/10.1053/j.jfas.2012.08.003
33. Shibuya S, Ozawa Y, Toda T, Watanabe K, Tometsuka C, Ogura T, et al. Collagen peptide and vitamin C additively attenuate age-related skin atrophy in Sod1-deficient mice. Biosci Biotechnol Biochem 2014;78:1212-20. http://dx.doi.org/10.1080/09168451.2014.915728
34. Suh SY, Bae WK, Ahn HY, Choi SE, Jung GC, Yeom CH. Intravenous vitamin C administration reduces fatigue in office workers: a double-blind randomized controlled trial. Nutr J 2012;11:7. http://dx.doi.org/10.1186/1475-2891-11-7
35. Tempelhof S, Rupp S, Seil R. Age-related prevalence of rotator cuff tears in asymptomatic shoulders. J Shoulder Elbow Surg 1999;8:296-9.
36. Traber MG, Stevens JF. Vitamins C and E: beneficial effects from a mechanistic perspective. Free Radic Biol Med 2011;51:1000-13. http://dx.doi.org/10.1016/j.freeradbiomed.2011.05.017
37. Uchiyama S, Shimizu T, Shirasawa T. CuZn-SOD deficiency causes ApoB degradation and induces hepatic lipid accumulation by impaired lipoprotein secretion in mice. J Biol Chem 2006;281:31713-9. http://dx.doi.org/10.1074/jbc.M603422200
38. Watanabe K, Shibuya S, Koyama H, Ozawa Y, Toda T, Yokote K, et al. Sod1 loss induces intrinsic superoxide accumulation leading to p53-mediated growth arrest and apoptosis. Int J Mol Sci 2013;14:10998-1010. http://dx.doi.org/10.3390/ijms140610998
39. Watanabe K, Shibuya S, Ozawa Y, Nojiri H, Izuo N, Yokote K, et al. Superoxide dismutase 1 loss disturbs intracellular redox signaling, resulting in global age-related pathological changes. Biomed Res Int 2014;2014:140165. http://dx.doi.org/10.1155/2014/140165
40. Yamamoto A, Takagishi K, Osawa T, Yanagawa T, Nakajima D, Shitara H, et al. Prevalence and risk factors of a rotator cuff tear in the general population. J Shoulder Elbow Surg 2010;19:116-20. http://dx.doi.org/10.1016/j.jse.2009.04.006
41. Zhang J, Ying X, Lu Q, Kallner A, Xiu RJ, Henriksson P, et al. A single high dose of vitamin C counteracts the acute negative effect on microcirculation induced by smoking a cigarette. Microvasc Res 1999;58:305-11.