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Histological assessment of SJL/J mice treated with the antioxidants Coenzyme Q10 and Resveratrol

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Abstract

The muscular dystrophies (MDs) are genetic disorders of muscle degeneration due to mutations in genes that encode a wide variety of proteins. Dysferlinopathy are characterized by the absence of dysferlin in skeletal muscle and an autosomal recessive mode of inheritance. Both histological and ultrastructural pathology have been well established in dysferlinopathy patients and dysferlin-deficient animal models. To our knowledge the effect of antioxidant supplementation on this level has not been described previously. This article therefore focuses on the histopathology to reveal the effect of antioxidant supplementation. The study aimed to determine, at cellular level, the histopathological changes in the SJL/J mouse model following a 90 day trial with antioxidant supplementation. Markedly reduced inflammatory insult in the more affected quadriceps muscles of animals treated with high doses of CoQ10 and a combination of resveratrol/CoQ10 was observed. The outcome provides evidence that high doses of antioxidant supplementation resulted in decreased dystrophic markers and enhanced tissue integrity at cellular level.

Key words: dysferlinopathy, antioxidant supplementation, coenzyme Q10, resveratrol, histopathology

1. Introduction

Skeletal muscle is a highly specialized tissue with primary function being the generation of physical force. It is therefore more susceptible to plasma membrane damage and requires more efficient membrane repair machinery than perhaps any other tissue (Bansal and Campbell, 2004). The degeneration of skeletal muscle is the most common pathological feature of the muscular dystrophies (MD) (Bansal and Campbell, 2004). Muscles affected by MD characterized by dysferlin deficiency (dysferlinopathy) have been shown to be defective in repairing injuries to the plasma membrane (Bansal *et al.*, 2003). Patients affected by dysferlinopathy display muscle weakness in muscles of the shoulder and pelvic girdles and deterioration progresses with age. Dysferlinopathy is an autosomal recessive inherited disorder but may also occur sporadically. The age of onset of this form of MD is usually around the end of the second decade of life, but may present at any age from as early as two years (Paradas *et al.*, 2009) to as late as the seventies (Klinge *et al.*, 2007).

Skeletal muscle of the SJL mouse strain has been shown to have an increased regenerative capacity (Grounds and McGeachie, 1989; Mitchell *et al.*, 1992) and to demonstrate a spontaneous occurrence of what has previously been designated 'an inflammatory myopathy accompanied by loss of strength' (Hohlfeld *et al.*, 1988; Weller *et al.*, 1997). The spontaneous myopathy is characterized by a progressive loss of muscle mass and strength corresponding with an increase in muscle pathology. Pathological changes include muscle fibers with central nuclei, size variation, splitting, inflammatory infiltrate, necrosis, and eventual replacement of muscle fiber with fat (Weller *et al.*, 1997; Bittner *et al.*, 1999; Suzuki *et al.*, 2005). These pathological alterations are consistent with those found in the human form of muscular dystrophy described in literature (Selcen *et al.*, 2001; Fanin and Angelini 2002; Prella *et al.*, 2003; Cenacchi *et al.*, 2005).

Histopathological examinations of muscles in SJL/J mice of different ages and different sources (SJL/J, SJL/Olac) disclosed features compatible with a progressive muscular dystrophy, including degenerative and regenerative changes of muscle fibers, together with a progressive fibrosis. These changes were found to primarily affect the proximal muscle groups, whereas the distal muscles remained less affected (Bittner *et al.*, 1999). Dysferlinopathy includes Miyoshi myopathy, a distal muscle disorder preferentially affecting the gastrocnemius muscle, and Limb Girdle muscular dystrophy Type 2B, characterized by proximal weakness (quadriceps muscle) at onset. Although the initial presentation may be different, the distinction between distal and proximal onset is very difficult following years of

disease progression (Zatz et al., 2003). A novel spontaneous mutation in the dysferlin gene in the SJL/J mice strain were found to result in a reduction of dysferlin to approximately 15% of levels detected in dysferlin-competent animals and human samples (Bittner *et al.*, 1999). The SJL/J mouse is not only representing the human form of the disease on a histological level, but also on a molecular level. Consequently this model is regarded ideal for research in the field of dysferlinopathy.

Light microscopic investigation of the early pathological abnormalities in non-necrotic muscle fibers of dysferlin-deficient patients revealed that muscle specimens displayed abnormal variation of fiber size, fiber splitting, an increased number of internalized nuclei, scattered necrotic and regenerating fibers, and increased endomysial and perimysial connective tissue (Selcen *et al.*, 2001). Other abnormalities consisted of small, irregularly circumscribed decreases of oxidative enzyme activity, few lobulated fibers, rare ring fibers, and sparse perivascular mononuclear cells (Selcen *et al.*, 2001). Abnormal distribution of the muscle fiber size in muscle cross-section is considered a hallmark of the pathological changes in dystrophic muscle (Briguet *et al.*, 2004).

It has previously been reported that oxidative stress is primarily involved in the pathomechanistic muscle deterioration in the mdx mouse model for dystrophin-deficient MD (Disatnik et al., 1998). This is consistent with the hypothesis of Murphy and Kehrer (1989), based on the similarities between the pathology in the dystrophies and the pathology of muscle exposed to oxidative stress in vitamin E deficiency. Lipid peroxidation is a common index of free radical mediated injury (Halliwell and Gutteridge, 1989). Previous measurements of lipid peroxidation in dystrophin-deficient muscles have indicated elevated levels in both humans and mice (Kar and Pearson, 1979; Jackson et al., 1984; Mechler et al., 1984 (DATE); Ragusa et al., 1997). A number of studies reported the reduction of oxidative stress by various mechanistic approaches, like antioxidant supplementation, led to a reduction in the adverse events in different pathologies (Grounds and Torrisi, 2004; Buck and Chojkier, 1996; Aragno et al., 2002; Buettler et al., 2002; Kaczor et al., 2007).

The effect of the antioxidant CoQ10 on cells and tissues has been proposed to be wide-ranging with the capacity to modulate diverse tissue activities and disease processes via small intrinsic cell metabolic perturbations (Linnane *et al.*, 2002). In reduced form, CoQ10 [known as ubiquinol (CoQH₂)], is an effective antioxidant and inhibits lipid peroxidation (Bentinger *et al.*, 2007). This antioxidant is our only endogenously synthesized lipid soluble antioxidant, and is mainly present in the activated (reduced) form. Resveratrol has been reported to be along with flavonoids, at least partially, responsible for the health benefits of

red wine (Frankel *et al.*, 1993; Soleas *et al.*, 1997). It has previously been reported that resveratrol is a rather weak antioxidant (Hu *et al.*, 2007), and that its antioxidant effects may be due to its direct interaction with biomolecules that confer cellular stress resistance (Robb *et al.*, 2008).

The study of the form of structures with specific focus on the anomalies thereof in the SJL/J mouse, defines the purpose of the current paper. The cellular alterations, as observed with a light microscope, associated with defective membrane repair and the effect of antioxidant supplementation, are investigated. In the present study the distribution of skeletal muscle lesions in the SJL/J mouse and the severity thereof were examined by histopathological examination of untreated 14 and 27 week-old mice, compared with 27 week-old counterparts supplemented with the antioxidants Resveratrol and CoQ10, separately and in combination, for 90 days.

2. Materials and Methods

2.1 Animals and animal care

Sixty female mice with a mean weight of ~ 20 g were imported from the Jackson Laboratory, Bar Harbor, USA for the present study. An import permit was obtained from the Department of Veterinary Services, Ministry of Agriculture. Sample size was determined following convention in ANOVA studies that error degrees of freedom be at least 30. Ten of these animals were of the SWR/J strain and served as the negative control in the study, while the other 50 animals were SJL/J mice. Animals were housed at the laboratory animal facility of the University of Pretoria's Biomedical Research Centre (UPBRC) at Onderstepoort (Pretoria, South Africa). The animals were allowed free access to JL Rat and Mouse 6F-IRRAD food (PMI Nutrition International, LLC) and water *ad libitum*. At the age of 14 weeks and 15 weeks for SJL/J and SWR/J mice, respectively, the animals entered a 90 day experimental study. On this day, six SJL/J animals were terminated for comparison of age-related changes with muscle tissue of 27 week-old SJL/J mice.

When a decrease and slowing in the mobility of the animals was observed, as a result of disease progression, it was decided to supplement the animals' diet by addition of Nestlé Cerelac sterile baby cereal on the floor of each cage, from day 50 until termination. The food supplementation did not contain any of the two products tested in the present study and were not expected to influence the results. Oral dosing was performed for all substances in all groups, using a 1000µl syringe with a mouse oral gavage needle nr. 20g. All animals

were weighed on day one, and thereafter twice weekly. Animals were divided into groups and caged communally in group order (Table 1).

2.2 Termination and tissue preparation

Termination took place on day 91. Animals were anaesthetized by isoflurane inhalation, and terminated by cardiac puncture. Quadriceps muscle samples were collected for histological investigation at termination. A small piece ($\pm 2 \times 3$ mm) of muscle tissue was dissected from the belly area of the quadriceps muscles. Tissue samples were immediately fixed in 2.5% formaldehyde in 0.075 M phosphate buffer, pH 7.4 at room temperature overnight, then rinsed thrice, 10 min each, in 0.075 M phosphate buffer. Serial dehydration was done in ethanol, followed by three changes of absolute ethanol. Tissue was infiltrated with LR White Resin (SPI Supplies, West Chester, PA). Serial sections of between 0.5 μm to 1.5 μm were cut with a Reichert-Jung Ultra Cut E ultramicrotome. Gill's Haematoxylin (Solulab, Johannesburg, South Africa), a general nuclear stain, together with 0.2% Toluidine Blue O were utilized to stain the tissue sections.

Viewing and image capturing were done with a Nikon Optiphot transmitted light microscope, equipped with a Nikon DXM 1200F digital camera. Slides were investigated at 20x, 40x and 100x objective lens magnification. Images were captured at 40x and 100x objective lens magnification for histological analyses. Images were enhanced but not altered with Adobe Photoshop 7.0. Severity of skeletal muscle lesions such as degeneration/necrosis, central nuclei, cellular infiltration by mononuclear cells, and adipose tissue infiltration were evaluated histopathologically and scored by the method of Kobayashi and co-workers, slightly adapted. In short, histological lesions were assessed and scored as, none (0, not present), minimal (\pm , localized lesions), mild (+, scattered lesions), and moderate (+ +, multifocal lesions) (Kobayashi *et al.*, 2009). A summary of the histological lesion assessment is presented in Table 2.

3. Results

3.1 Histological findings

3.1.1 Negative control group

Quadriceps muscle specimens analyzed from all the 28 week-old SWR/J mice that represented the negative control group (Figure 1), displayed normal variation of fiber size. Neither inflammatory infiltrate nor active necrotic processes could be detected. A nerve bundle (indicated by the black rectangle) was observed. Axons (Figure 1, a) and blood

vessels (Figure 1, b) were present in this section. No occurrence of fiber splitting was detected while only $\approx 1.4\%$ of fibers evaluated, displayed nuclei in the central position. Myonuclei (Figure 1, mn) were situated mostly in the peripheral position with a distribution of one to about five peripherally located nuclei per fiber.

3.1.2 Age control group

Mild (+) focal perimysial and endomysial inflammatory changes, (marked by asterisks in Figure 2A), were observed in the age control group (Figure 2). Mononuclear cells (mc) were present between fibers (Figure 2A, mc). Mononuclear cells in this group were observed in smaller quantities, compared to that seen in the 27 week-old SJL/J groups treated with placebo. Early stages of an ongoing necrotic process (Figure 2B, black rectangle) in muscle fibers with the presence of mononucleated cells were observed. Mostly single fiber involvement was seen in necrotic events. Invasion of fibers by mononucleated phagocytic cells (Figure 2B, mc) are characteristic of initial phases of the process, and these invading cells are presumably macrophages. Endomysial infiltrate (between the fibers) was seen to a lesser extent (\pm) and was mostly noted around intact cells (Figure 2A). Inflammatory infiltrate were found to be present to a lesser extent (\pm). 'Moth-eaten' appearance of fibers (Figure 2B, asterisks) was noted, although the overall appearance of the muscle fibers was good. Carpenter and Karpati, 1984, reported that many fibers in biopsies from limb girdle dystrophy patients to show moth-eaten appearance in their centers and to have prominent mitochondria along their periphery. Moth-eaten fibers are common in denervating conditions, though they may represent reinnervated fibers (Carpenter and Karpati, 1984).

Nuclei were mostly peripherally located and found in relative large numbers that vary among individuals. A cluster of structures surrounded by a multiple layered sheath was identified as smooth muscle (Figure 2C, sm) (Carpenter and Karpati, 1984) in one sample in this group. Carpenter and Karpati recorded a case in 1984 where there was marked independent proliferation of smooth muscle cells, accompanied by collagen in the endomysium of a limb girdle myopathy sample. Occasional (\pm) fiber splitting was present in this group. In a number of transverse sectioned samples, but not in all, numerous small circular objects identified as capillaries were found to surround the muscle fibers (Figure 2D). The lumens were mostly filled with erythrocytes, resulting in the dark appearance.

3.1.3 Positive control group

The quadriceps muscle specimens of 27 week-old SJL/J mice treated with placebo displayed abnormal variation of fiber size (Figure 3). Mild (+) to moderate (+ +) perimysial inflammatory changes with endomysial involvement became predominant in this group.

Active myopathic changes were emphasized by the following findings; fiber splitting (fs) was prevalent (+) (Figure 3A) and its appearance was distinct in areas with more inflammatory infiltrate. Numerous necrotic fibers and fiber remnants (+ +) resultant of the ongoing necrotic processes ranging from mild (+) to moderate (+ +) were observed (Figure 3B, asterisk). These fibers ranged from disrupted cells invaded by macrophage activity, to 'ghost' cells (Figure 3C, asterisk), which is marked by a 'see-through' appearance of cell remnants representing the initial shape and size of the dead cell. Numerous mononucleated cells (+ +) were predominant in areas with inflammatory infiltrate.

The appearance of moth-eaten cells (Figure 3C) was frequent (+ +). Inflammatory infiltrate were present, ranging from mild dystrophic changes (+), characterized by numerous small diameter fibers, intercepted by the occasional necrotic fibers, to moderate ongoing dystrophic processes (+ +). In only one out of nine animals, no inflammatory infiltrate (0) could be detected in quadriceps muscle specimens examined, while seven out of nine showed mild (+) to moderate (+ +) dystrophic lesions and one animal showed lesions, more severe than what was classified as moderate in the present study, with up to zero intact cells in one microscopic field. This observation could possibly be explained by the possibility of inter-individual variation amongst animals, where onset of disease might have occurred later in the latter subject.

Ring fibers (Figure 3D, asterisk) were detected in one sample from this group. Ring myofibrils are newly formed structures arising in muscle fibers which were normal, until their growth pattern was modified by the pathological process (Schotland *et al.*, 1966). Fibers affected by this phenomenon are termed ring fibers (Carpenter and Karpati, 1984). In the same sample, fibers of which the fibrils were 'streaming' (Figure 3D) were also seen. This description correlates with what was termed snake coils, by Carpenter and Karpati, 1984. Bethlem and Van Wijngaarden, 1963 suggested that ring myofibrils may also branch from the ring, penetrate the muscle fiber and rejoin the aberrant myofibrils on the opposite side, forming these streaming patterns or snake coils.

Nuclei in the central position of fibers (Figure 3D, arrowheads) were abundant and up to three central nuclei could be detected in a single fiber cross section. Peripheral nuclei were sparsely distributed along less- and unaffected fibers.

The samples analysed from this group served as a baseline/reference for the age-matched experimental groups treated with the different concentrations of different antioxidants throughout the course of the trial. The histological results from 27 week-old, untreated, SJL/J

mice were consistent with previous findings in SJL/J mice at a similar age (Weller *et al.*, 1997; Ho *et al.*, 2002; Suzuki *et al.*, 2005; Nemoto *et al.*, 2007;) and correlated with the histological findings previously described in human dysferlinopathy patients (Gallardo *et al.*, 2001; Selcen *et al.*, 2001; Cenacchi *et al.*, 2005).

3.1.4 Resveratrol group

A distinct variation in fiber size was apparent in the resveratrol group (Figure 4). Fiber splitting (+), was observed as groups of small nested fibers with complementary contours (Figure 4A, black arrows). Carpenter and Karpati, 1984, described a split fiber as one which has a fissure extending from its periphery into its interior, consistent with what was seen on a transverse section in the present study (Figure 3A). Complete separation may result into what appear to be two distinct fibers whose contours are complementary to one another (Carpenter and Karpati, 1984). One or more of these fibers often displayed central nucleation. Mild (+) to moderate (+ +) degeneration and perimysial inflammatory changes with endomysial involvement was present and underlined by the distinct presence of mononucleated cells (+) (Figure 4A, arrowheads).

The presence of necrotic fibers as 'ghost cells' (Figure 4B, asterisk) as well as fiber remnants resultant of the ongoing dystrophic process was observed. Presence of fibers with a moth-eaten appearance (Figure 4B, arrowheads) was moderate (+ +). Fibers of which the fibrils appeared to be 'streaming' (Figure 4B, arrows) were present. Ring fibers (Figure 4C, arrow) were detected in one sample from this group. Intense vacuolation was observed in some fibers (Figure 4D, arrow). The infiltrate in perimysial spaces was mostly dense connective tissue (Figure 4D, asterisk). Fibroblasts (f) (Figure 4D) with fine extending processes were found in conjunction with connective tissue infiltration.

In general, fibers ranged from being intact (no incidence of dystrophic process in a specific microscopic field), healthy appearing cells with peripherally located nuclei to areas with multifocal lesions (+ +). Qualitatively, more intact cells were observed in this group compared to the positive control group.

3.1.5 Low CoQ10 group

A variation in fiber size was prominent in the low CoQ10 group (Figure 5). Groups of small fibers (Figure 5A, arrow) were frequently found. Dense connective tissue (Figure 5A, asterisk) and mononucleated cells (mc) (+ +) were present in perimysial regions.

Inflammatory incidence ranged from no inflammatory infiltrate (0) to mild (+) and moderate (+ +) changes in both perimysial and endomysial regions. Fibers ranged from being intact, healthy appearing cells with peripherally located nuclei to fibers undergoing necrosis. Minimal infiltration of adipose cells (\pm) was observed.

Nuclei, distinctly different in appearance from myonuclei, were found in this group, in intact fibers (Figure 5B, arrowheads). The position of the nuclei fit that of satellite cells. Satellite cells are mononuclear cells found beneath the basal lamina of muscle fibers (Carpenter and Karpati, 1984). These cells are flat and somewhat elongated in the long axis of the muscle fiber. Their nuclei tend to contain darker clumped chromatin than myonuclei. When a muscle becomes necrotic, satellite cells are the source of the myoblasts which regenerate the necrotic segment (Carpenter and Karpati, 1984). From a qualitative perspective; more intact cells were observed in this group compared to the positive control group samples (placebo), but less compared to the resveratrol group.

3.1.6 High CoQ10 group

In samples from the high CoQ10 group (Figure 6), very little (\pm) (Figure 6A) to mild (+) and moderate (+ +) (Figure 6B) degenerative and perimysial inflammatory changes with endomysial involvement were observed. As in the younger 14 week-old age control group, inflammatory changes, although more severe in this group, were found mostly in the perimysial regions where necrotic incidences (Figure 6B) could be detected. Markedly more myonuclei were detected in the peripheral position, and central nucleation was sparsely distributed in less and unaffected areas.

Numerous mononucleated cells (+) were present in infiltrate, indicating the presence of inflammatory incidence. Fiber splitting occurred less frequently (\pm), compared to the resveratrol and low CoQ10 groups, and distinctly less frequently compared to the positive control group. Minimal adipose tissue infiltration (\pm) (Figure 6B) was seen in moderately (+ +) affected areas. Vacuolation were overall minimally (\pm) distributed in moderately (+ +) affected areas, while moth-eaten appearance of cells was observed more frequently (+). No ring fibers were observed in the samples analyzed from this group. Nuclei, presumably belonging to satellite cells, were also observed in this group, in intact fibers (Figure 6 A and C, arrowheads).

3.1.7 Resveratrol/CoQ10 combination group

Samples from the resveratrol/CoQ10 group (Figure 7), showed minimal (\pm) (Figure 7A) to mild (+) (Figure 7B) degeneration and perimysial inflammatory changes with endomysial involvement. As in the high CoQ10 group, inflammatory changes were found mostly in the perimysial regions where necrotic incidences (\pm / +) could be detected extending into endomysial areas. Markedly more myonuclei were detected in the peripheral positions.

Numerous mononucleated cells (mc) (+) (Figure 7B) were present in affected areas. Fiber splitting occurred less frequently (\pm), compared to the positive control, resveratrol and low CoQ10 groups. Minimal adipose tissue infiltration (\pm) was observed. Ghost cells as a result of necrosis were detected. Vacuolation was overall minimally distributed (\pm), while moth-eaten appearance of cells was also markedly reduced compared to the findings in other groups. Ring fibers (Figure 7B, asterisk) were observed in two samples of this group. The rings only affected part of the fibers, and did not form a 'complete ring'. Elongated, thin, and darkly stained nuclei, that presumably belong to satellite cells, were found in this group in intact fibers (Figure 7A, arrowheads), although in smaller numbers than in the high and low CoQ10 groups.

4. Discussion

The selection criteria for an appropriate antioxidant included potent antioxidant potential, evidence of beneficial supplementation, high dose tolerance and a low side effect profile. CoQ10 and resveratrol complied with the criteria. It was decided to test two different concentrations of CoQ10, a relatively low and a relatively high concentration equivalent to human physiological doses. Only one concentration of resveratrol was tested and the antioxidants were also administered in a combination dose to establish whether a combination might have a different effect. For dose translation, a formula based on body surface area normalization (Reagan-Shaw et al., 2008) were utilized to obtain accurate doses expected to provide a reliable indication of antioxidant effectiveness.

Histopathological data from the present study has confirmed that distal muscles are less affected and relatively spared in initial phases of the disease in SJL/J mice. The histological picture of the gastrocnemius muscle samples showed little to no dystrophic changes (data not shown), compared to samples from the quadriceps muscles (Figure 2 to 7). A distinct lower number of nuclei in the central position were observed in gastrocnemius fibers.

The qualitative results of the present study suggest a reduction in necrotic incidence as well as in the amount of inflammatory foci in groups treated with the highest concentrations of antioxidants (Figures 6 and 7). These findings imply that the application of antioxidants

interfere with the ongoing process responsible for initiation of necrosis. It is tempting to speculate that high dose antioxidants are most likely responsible for a reduction in metabolic disturbances, brought about by the continuous inflammatory processes. It can be further suggested that these metabolic disturbances might also allow for accumulation of oxidative stress in the muscle, and that antioxidants scavenges the free radicals. The relief from oxidative stress insult may allow for the sufficient recruitment of anti-inflammatory cells, like neutrophils and macrophages. Thereby providing more effective clearance of necrotic fibers, and thus mediating more effective regeneration.

The supplementation of SJL/J mice with resveratrol alone or low dose CoQ10 was not able to decrease dystrophic alterations at cellular level to the same extent than what was afforded by high dose CoQ10 and resveratrol/CoQ10 in combination. It is therefore possible that supplementation from the age of 14 weeks was too late to result in beneficial effects from these antioxidant doses. This notion further implicates that only high doses of CoQ10 or low doses of resveratrol and CoQ10 in combination will be effective in slowing disease progression in the SJL/J mouse, if supplementation is started after onset of disease.

Conclusion

The underlying biochemical defect of muscular dystrophy, as with nearly all inherited diseases, can only be cured if techniques are developed to repair or replace the defective genetic material (Murphy and Kehrer, 1989). Therefore, if lipid- and water-soluble antioxidants are applied as treatment strategy in the muscular dystrophies, such treatment would only be symptomatic (Murphy and Kehrer, 1989). Although it is unlikely that the supplementation of a dystrophic model with antioxidants will reverse the pathologic remodelling, the present study focused on whether such an approach will provide a relief of the effect of inflammatory insult at the tissue level. Assessment of the histopathology showed, from a qualitative perspective, markedly reduced inflammatory insult in the more affected quadriceps muscles of animals treated with high doses of CoQ10 and a combination of resveratrol/CoQ10. An ongoing inflammatory reaction as the result of attenuated muscle regeneration in dysferlin-deficient muscle was markedly reduced. Collectively the findings of the study allude to the ability of high doses of antioxidant therapy to decrease the ongoing inflammatory process. Although supplementation with antioxidants will not reverse the pathological state in muscular dystrophy, it might serve a purpose in combination with cell-based therapies. From the results of the present study it can be hypothesized that high dose antioxidant supplementation might provide a more favourable

environment at tissue level that might enhance donor cell differentiation and maturation in dysferlin-deficient muscle tissue following cell transplantation.

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Figure Legends - Histological assessment of SJL/J mice treated with the antioxidants
Coenzyme Q10 and Resveratrol

Figure 1: Quadriceps muscle section from the negative control group. An axon (a) and blood vessels (b) was present in a nerve bundle (black rectangle) detected on this section. Myonuclei (mn) was found in a peripheral position. Sections were stained with Toluidine blue O. Scale bar = 50 μ m

Figure 2: Quadriceps muscle sections from the age control group. Perimysial and endomysial inflammatory changes (black asterisks) were observed. (A) Mononuclear cells (mc) were present between fibers. (B) Early stages of an ongoing necrotic process (rectangle) were accompanied by infiltration of mononuclear cells (mc). (C) A cluster of smooth muscle (sm) in muscle tissue. (D) Numerous capillaries (white asterisks) were found to surround fibers. Sections were stained with Toluidine blue O. Scale bar = 50 μ m

Figure 3: Quadriceps muscle sections from the positive control group. (A) Active myopathic changes included fiber splitting (fs), (B) numerous necrotic fibers (asterisks), and (C) ghost cells (asterisk). (D) Ring fibers and (asterisk) were detected. Nuclei in the central position of fibers (arrowheads) were abundant. Sections were stained with Toluidine blue O. Scale bar = 50 μ m

Figure 4: Quadriceps muscle sections from the resveratrol group. (A) Fiber splitting was observed as groups of small nested fibers (arrows) and numerous mononuclear cells (arrow heads) were found in the inflammatory infiltrate. (B) Fibers presented as ghost cells (asterisk), displayed a moth eaten (arrowheads), and a streaming (arrows) appearance. (C) Ring fibers were present (arrow). (D) Intense vacuolation (arrow), dense connective tissue (asterisks) and fibroblasts (f) can be seen in this section. Sections were stained with Toluidine blue O. Scale bar = 50 μ m

Figure 5: Quadriceps muscle sections from the low CoQ10 group. (A) Groups of small fibers (arrow), dense connective tissue (asterisk), and mononucleated cells (mc) were present. (B)

Nuclei distinctly different in appearance (arrowheads) are thought to belong to satellite cells. Sections were stained with Toluidine blue O. Scale bar = 50 μ m

Figure 6: Quadriceps muscle sections from the high CoQ10 group. Very little (A) to mild and moderate (B) degenerative changes were present in this group. Nuclei (A & C, arrowheads), presumably belonging to satellite cells were abundant. Sections were stained with Toluidine blue O. Scale bar = 50 μ m

Figure 7: Quadriceps muscle sections from the resveratrol/CoQ10 group. Minimal (A) to mild (B) degeneration was observed in this group. (A) Nuclei (arrowheads) presumably belonging to satellite cells were abundant. (B) Numerous mononucleated cells (mc) were observed in affected areas in some sections. Ring fibers (asterisk) were present. Sections were stained with Toluidine blue O. Scale bar = 50 μ m

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Tables

Table 1: Animal grouping and treatment doses

Group	Strain	Treatment	Concentration (mg/kg/day)	Age at termination (in weeks)
Negative control	SWR/J	None	Placebo (water)	28
Age control	SJL/J	None	-	14
Positive control	SJL/J	None	Placebo (water)	27
Resveratrol	SJL/J	Resveratrol	60	27
CoQ10(low)	SJL/J	CoQ10	40	27
CoQ10 (high)	SJL/J	CoQ10	120	27
Resveratrol/CoQ10	SJL/J	Resveratrol + CoQ10	60 + 40	27

Table 2: Summary and scoring of incidence of dystrophic processes

Age at termination (weeks)	27	27	27	27	27	14
Group	SJL/J Placebo	SJL/J Resveratrol	SJL/J (low) CoQ10	SJL/J (high) CoQ10	SJL/J Resveratrol + CoQ10	SJL/J No treatment
CHANGES						
Fiber diameter range (μm)	11.82 – 99.01	10.12 – 117.90	12.41 – 117.77	12.06 – 108.06	13.17 – 108.10	15.98 – 112.34
% Central nuclei	35.4	31.6	34.3	22.9	21.0	8.8
Fiber splitting	+	+	±	±	±	±
Necrotic fibers / degeneration	++	+ / ++	+ / ++	+	± / +	±
Inflammatory infiltrate	++	+ / ++	+ / ++	0 / + / ++	+	±
Mononuclear cells (in extracellular spaces)	++	+	++	+	+	±
Ring fibers	Present	Present	Not detected	Not detected	Present	Not detected
Adipose infiltration	± / +	+	±	±	±	±
Vacuoles	+ / ++	+ / ++	+ / ++	±	±	±
Moth-eaten appearance	++	++	++	+	+	±
(0) = none; (±) = minimal; (+) = mild; (+ +) = moderate						













