Acta Biochimica Polonica

Vol. 52 No. 2/2005, 449–452

on-line at: www.actabp.pl

The involvement of oxidative stress in determining the severity and progress of pathological processes in dystrophin-deficient muscles[✪]

Irena Niebrój-Dobosz[✉] and Irena Hausmanowa-Petrusewicz

Neuromuscular Unit, Institute of Experimental and Clinical Medicine, Polish Academy of Sciences, Warszawa, Poland; ✉e-mail: neurmyol@cmdik.pan.pl

Received: 06 January, 2005; revised: 06 May, 2005; accepted: 20 May, 2005
available on-line: 25 May, 2005

In both forms of muscular dystrophy, the severe Duchenne's muscular dystrophy (DMD) with lifespan shortened to about 20 years and the milder Becker dystrophy (BDM) with normal lifespan, the gene defect is located at chromosome locus Xp21. The location is the same in the experimental model of DMD in the mdx mice. As the result of the gene defect a protein called dystrophin is either not synthesized, or is produced in traces. Although the structure of this protein is rather well established there are still many controversies about the dystrophin function. The most accepted suggestion supposes that it stabilizes sarcolemma in the course of the contraction-relaxation cycle. Solving the problem of dystrophin function is a prerequisite for introduction of an effective therapy. Among the different factors which might be responsible for the appearance and progress of dystrophic changes in muscles there is an excessive action of oxidative stress. In this review data indicating the influence of oxidative stress on the severity of the pathologic processes in dystrophy are discussed. Several pieces of data indicating the action of oxidative damage to different macromolecules in DMD/BDM are presented. Special attention is devoted to the degree of oxidative damage to muscle proteins, the activity of neuronal nitric oxide synthase (nNOS) and their involvement in defining the severity of the dystrophic processes. It is indicated that the severity of the morbid process is related to the degree of oxidative damage to muscle proteins and the decrease of the nNOS activity in muscles. Estimation of the degree of the destructive action of oxidative stress in muscular dystrophy may be a useful marker facilitating introduction of an effective antioxidant therapy and regulation of nNOS activity.

Keywords: Duchenne's muscular dystrophy, oxidative stress, oxidative damage to macromolecules

Duchenne's dystrophy (DMD) is a severe genetically determined disease. The patients are immobilized at the age of about 10 yrs and their lifespan is shortened to about 20 yrs. Becker dystrophy (BDM) is a milder form of dystrophy. The first clinical symptoms appear at the age of about 10 years, the patients are usually not immobilized and the lifespan is nearly normal. The mdx mice, an experimental model of DMD, is clinically almost asymptomatic. The gene is located at chromosome locus Xp21. Its product called dystrophin is located at the cytoplasmic face of the sarcolemma (Zubrzycka-Gaarn *et al.*, 1988). In DMD, BDM and the mdx mice dystrophin is either absent, or appears in traces. The structure of this protein has been extensively studied for the last 15 years (Fig. 1). It is known already that several proteins, localized mainly in the membrane, collaborate with dystrophin. Special attention should be focused on neuronal nitric synthase (nNOS), which is attached either to syntrophin-α, or directly to dystrophin and might be responsible, at least partly, for the severe and widespread pathologic changes in DMD muscles.

Dystrophin deficiency has been related to the degeneration and insufficient regeneration of muscle fibers and extensive proliferation of connective tissue, mainly in limb and trunk muscles. Some muscles such as the extraocular and laryngeal muscles, despite being dystrophin-deficient, are spared. In the mdx mice the limb musculature undergoes widespread degeneration, which is followed by an effective regenerative response. In contrast, the mdx mouse diaphragm more closely mimics the process that characterizes DMD. The lack of clinical symptoms from skeletal muscles in mdx mice, the differential response of mdx skeletal muscles and the

[✪]Paper dedicated to the memory of Professor Witold Drabikowski and Professor Gabriela Sarzała-Drabikowska.

Abbreviations: BMD, Becker dystrophy; DMD, Duchenne's dystrophy; LGMD, Limb-girdle dystrophy; nNOS, neuronal nitric oxide synthase; NO, nitric oxide.

mdx diaphragm and also sparing of some muscles in DMD/BDM is among the most fundamental questions which remain to be answered. The answer can provide important clues regarding the disease etiology and also therapeutic measures.

The function of dystrophin in the muscle is still a mystery. It has been suggested that:

1) dystrophin supports sarcolemma against mechanical stress and stabilizes it in course of the contraction–relaxation cycle (Koenig *et al.*, 1988),

2) dystrophin takes part in the regulation of intracellular calcium and the further cascade of calcium-related events (Franco & Lansman, 1990),

3) dystrophin works in force and signal transduction (Gee *et al.*, 1998),

4) dystrophin influences the aggregation of neurotransmitter receptors (Kong & Anderson, 1999),

5) dystrophin prevents excessive generation of reactive oxygen free radical species (Brown, 1995).

All these mechanisms are in no way exclusive and may interact with one another to a significant degree.

OXIDATIVE STRESS IN DYSTROPHINOPATHIES

Recent studies strongly support the notion of the importance of oxidative stress in dystrophinopathies. Free radical injury to sarcolemma may contribute to changes of its integrity (Murphy & Kehrer, 1989). There is increasing evidence that the degenerative processes in dystrophic muscles may be due to oxidative stress. An imbalance of free radicals synthesis and the antioxidant capacity may contribute to the necrotic process (Mendell *et al.*, 1971). The significance and precise extent of the oxidative stress contribution is, however, poorly understood.

Free radicals are produced in the course of different physiological processes. Their action should be limited by several defense mechanisms (Woodford & Whitehead, 1998). Free radicals are known to be responsible for chemical and molecular damage of DNA, nucleotides, proteins, lipids, carbohydrates and cell membrane structure (Slater, 1984).

The increased action of oxidative stress in Duchenne's dystrophy is indicated by:

1) increased excretion of 8-hydroxy-2'-deoxyguanosine indicating oxidative damage of DNA (Rodriquez & Tarnopolsky, 2003),

2) changes in proteins (Hunter *et al.*, 1986, Haycock *et al.*, 1996; 1998; Niebroj-Dobosz *et al.*, 2002), enhanced lipid peroxidation (Kar & Pearson, 1979; Matkovics *et al.*, 1982; Hunter *et al.*, 1986; Ragusa *et al.*, 1997) and induction of antioxidant enzymes (Kar & Pearson, 1979; Matkovics *et al.*, 1982; Mechler *et al.*, 1984),

3) similarities between changes in Duchenn's dystrophy and such conditions as ischemia, exhaustive exercise, and vitamin E deficiency (Mendell *et al.*, 1971; Irintchev & Wernig, 1987; Murphy & Kehrer, 1989),

4) increased sensitivity of dystrophin-deficient cells to injury from oxidative stress (Degl'Innocenti *et al.*, 1999).

5) lipofuscin accumulation in dystrophic muscle (Nakae *et al.*, 2004)

OXIDATIVE STATUS OF MUSCLE PROTEINS IN DYSTROPHINOPATHIES

In addressing the notion that the severity of muscle changes is mediated by reactive free radical species (ROS) it is of value to determine the formation of carbonyl derivatives of amino-acid residues

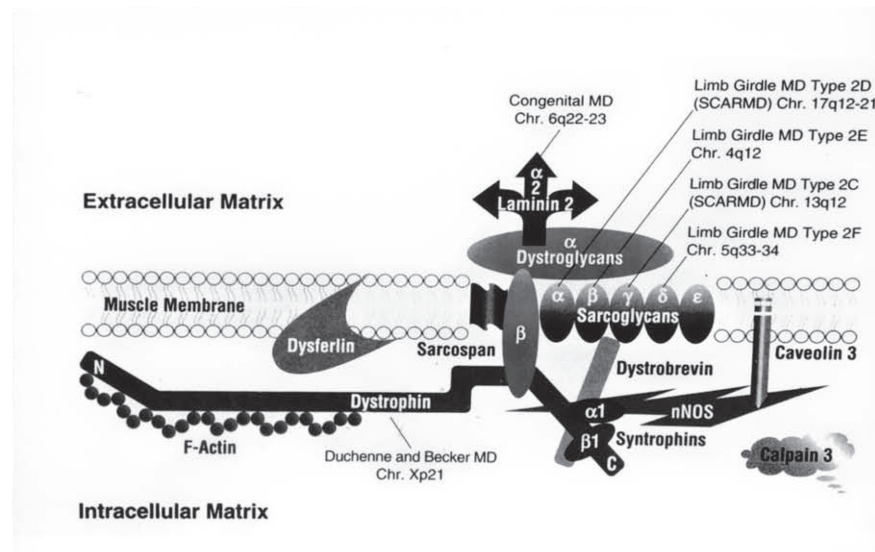

Figure 1. Dystrophin and dystrophin-associated proteins (based mainly on scheme presented in Novocastra Lab. Ltd. Catalogue, 2003/04, p. 123).

in proteins. Their content indicates the proteins oxidative status. Their quantification by testing the reaction of these groups with carbonyl-specific reagents is considered to be the most sensitive and reliable method for determining the oxidative modification to proteins and the free radical-induced protein damage (Levine *et al.*, 1994). Proteins of different structure, function or intracellular localization exhibit various susceptibility to oxidative damage (Haycock *et al.*, 1996).

In nearly all DMD cases increased protein carbonyl values are found (Haycock *et al.*, 1996; Niebroj-Dobosz *et al.*, 2002). In BMD and LGMD this is a rare finding. In mdx mice the carbonyl level is normal both in the hind limb and in the diaphragm muscles (Niebroj-Dobosz *et al.*, 2002). The most heavily oxidized proteins in DMD appear to be actin (43 kDa), desmin (57 kDa) and an unidentified 34 kDa protein, and less oxidized proteins appear at 125 and 83 kDa (Niebroj-Dobosz *et al.*, 2002). In BDM generally the same pattern is present, although it is less expressed. In some LGMD cases increased oxidation of proteins between 83 and 34 kDa is also observed. In a previously published paper (Haycock *et al.*, 1998) the only oxidized protein in DMD and BDM was a 125 kDa protein. Although in the mdx mice the carbonyl content both in the hind limb muscle and the diaphragm is normal, an 83 kDa protein is more heavily oxidized. This protein has been identified as α-actinin (Niebroj-Dobosz *et al.*, 2002).

THE CONTRIBUTION OF NEURONAL NITRIC OXIDE SYNTHASE (nNOS) TO OXIDATIVE DAMAGE OF PROTEINS AND ENHANCEMENT OF DEGENERATIVE PROCESSES OF MUSCLE FIBRES

nNOS is a member of the dystrophin-glycoprotein complex and is implicated in several vital functions including regulation of the homeostasis of reactive free radical species including NO which protects the muscles against oxidative injury and may function as an antioxidant and takes part in mediating the signaling function of dystrophin and related proteins (Wink *et al.*, 1993; 1995). Measurements of nNOS/NO may be helpful in answering the question whether there is a correlation between its activity and the severity of the dystrophic process.

The protective action of nNOS is reduced in dystrophinopathies (Brenman *et al.*, 1996; Chang *et al.*, 1996; Bredt, 1999; Niebroj-Dobosz *et al.*, 2002). In Duchenne's dystrophy nNOS (Niebroj-Dobosz *et al.*, 2001) appears to be either drastically reduced or even absent. In very advanced stages of this disease only endomysial tissue reacts with nNOS antibodies. In Becker dystrophy (Niebroj-Dobosz *et al.*, 2002) nNOS is either decreased or normal, in limb-girdle dystrophy staining of nNOS appears in the cytoplasm. In mdx mouse muscles nNOS reactivity is observed on the surface of the fiber and from day 30 of life clusters of nNOS are present (Niebroj-Dobosz *et al.*, 2001). When immuno-detected in Western blotting, nNOS appears either reduced or absent in DMD, decreased or normal in BMD and LGMD, and normal or slightly decreased in mdx mice (Niebroj-Dobosz *et al.*, 2001)

Altered activity of nNOS, apart from its influence on the homeostasis of ROS, is implicated also in the abnormalities in blood flow during exercise in dystrophic patients. Contraction of dystrophic muscles may not properly stimulate a level of NO production (Gucuyener *et al.*, 2000; Kasai *et al.*, 2004) sufficient for relieving vasoconstriction. This results in local muscle ischemia because of inadequate blood flow to the actively contracting muscles. The sustained vascular constriction in Duchenne dystrophy may explain the focal necroses in dystrophin-deficient muscles. At least part of muscle degeneration in DMD may result from the reduced production of nNOS/NO as it may lead to impaired regulation of vasocontrictor response (Crosbie, 2001).

The oxidative damage of macromolecules possibly appears as a result of insufficient antioxidant defense and abnormal nNOS/NO, which correlates with the severity of the dystrophic process.

STRATEGY OF MUSCULAR DYSTROPHY TREATMENT BASED ON PREVENTION OF OXIDATIVE STRESS ACTION AND IMPROVEMENT OF nNOS ACTIVITY

No effective treatment of muscular dystrophies is known yet. Prevention of ROS damage by providing different antioxidants could have positive clinical effect. Administration of a set of naturally occurring antioxidants and manipulation of the nNOS activity and NO level by exercise. (Tidball *et al.*, 1998) plus physiotherapy could improve the quality of life and decrease the severity of the disease.

CONCLUSIONS

1. In dystrophinopathies myofibers are subject to intra-/extracellular oxidative stress. The oxidized macromolecules are more susceptible to degradation.

2. Oxidative stress is a potential pathogenetic factor which may determine the severity of pathological changes in dystrophic muscle.

3. Oxidative stress in dystrophinopathies interacts with sarcolemmal weakening, inappropriate calcium influx, aberrant cell signaling and recurrent muscle ischemia.

4. In dystrophinopathies oxidative stress markers, such as 8-hydroxy-2′-deoxyguanosine, may be used in clinical trials.

5. Lowering the oxidative stress by increasing the antioxidant capacity through application of antioxidants and corrections of the nNOS activity/NO level by daily long-term physiotherapy and exercise, opens a new strategy of treatment which may modify the disease process and slow down its progress.

REFERENCES

Bredt DS (1999) Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Rad Res* **31:** 577–596.

Brenman JE, Chao DS, Xia H, Aldape K, Bredt DS (1995) Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. *Cell* **82:** 743–752.

Brown RH (1995) Free radicals, programmed death and muscular dystrophy. *Curr Opin Neurol* **8:** 373–378.

Chang WJ, Iannaccone ST, Lau KS, Masters BS, McCabe TJ, McMillan K, Padre RC, Spencer MJ, Tidball JG, Stull JT (1996) Neuronal nitric oxide synthase and dystrophin-deficient muscular dystrophy. *Proc Nat Acad Sci USA* **93:** 9142–9147.

Crosbie RH (2001) NO vascular control in Duchenne muscular dystrophy. *Nat Med* **7:** 27–29.

Degl'Innocenti D, Pieri A, Rosati F, Ramponi G (1999) Oxidative stress and calcium homeostasis in skin fibroblasts. *IUBMB Life* **48:** 391–396.

Franco A, Lansman JB (1990) Calcium entry through stretch-inactivated ion channels in mdx myotubes. *Nature* **334:** 670–673.

Gee SH, Madhavan R, Levinson SR, Caldwell JH, Sealock R, Froehner SC (1998) Interaction of muscle and brain sodium channels with multiple members of the syntrophin family of dystrophin-associated proteins. *J Neurosci* **18:** 128–137.

Gucuyener K, Ergenekon E, Erbas D, Pinarli G, Serdaroglu A (2000) The serum nitric oxide levels in patients with Duchenne muscular dystrophy. *Brain Dev* **22:** 181–183.

Haycock JW, Jones P, Harris JB, Mantle D (1996a) Differential susceptibility of human skeletal muscle proteins to free radical induced oxidative damage: a histochemical, immunocytochemical and electron microscopical study *in vitro. Acta Neuropathol* **92:** 331–340.

Haycock JW, Mac Neil S, Jones PJ, Harris JB, Mantle D (1996b) Oxidative damage to muscle protein in Duchenne muscular dystrophy. *NeuroReport* **8:** 357–361.

Haycock JW, Mac Neil S, Mantle D (1998a) Differential protein oxidation in Duchenne and Becker muscular dystrophy. *NeuroReport* **9:** 2201–2207.

Hunter M, Ian S, Mohamed JB (1986b) Plasma antioxidants and lipid peroxidation products in Duchenne muscular dystrophy. *Clin Chim Acta* **255:** 123–132.

Irintchev A, Wernig A (1987) Muscle damage and repair in voluntarily running mice: strain and muscle differences. *Cell Tissue Res* **249:** 509–521.

Kar NC, Pearson CM (1979) Catalase, superoxide dismutase, glutathione reductase and thiobarbituric acid-reactive products in normal and dystrophic human muscle. *Clin Chim Acta* **94:** 277–280.

Kasai T, Abeyana K, Hashiguchi T, Fukunaga H, Osame M, Maruyama I (2004) Decreased total nitric oxide production in patients with Duchenne muscular dystrophy. *J Biomed Sci* **11:** 534–537.

Koenig M, Monaco AP, Kunkel CM (1988) The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell* **53:** 219–228.

Kong J, Anderson JE (1999) Dystrophin is required for organizing large acetylocholine receptor aggregates. *Brain Res* **839:** 298–304.

Levine RL, Williams JA, Stadman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol* **233:** 346–357.

Matkovics B, Laszlo A, Szabo L (1982) A comparative study of superoxide dismutase, catalase and lipid peroxidation in red blood cells from muscular dystrophy patients and normal controls. *Clin Chim Acta* **118:** 289–292.

Mechler F, Imre S, Dioszeghy P (1984) Lipid peroxidation and superoxide dismutase activity in muscle and erythrocytes in Duchenne muscular dystrophy. *J Neurol Sci* ?: 00–00.

Mendell JR, Engel WK, Derrer EC (1971) Duchenne muscular dystrophy: Functional ischemia reproduces characteristic lesions. *Science* **172:** 1143–1145.

Murphy ME, Kehrer JP (1989) Oxidative stress and muscular dystrophy. *Chem-Biol Interact* **69:** 101–178.

Nakae Y, Stoward PJ, Kashiyama T, Shono M, Akagi A, Matsuzaki T, Nonaka I (2004) Early onset of lipofuscin accumulation in dystrophin-deficient skeletal muscles of DMD patients and mdx mice. *J Mol Histol* **35:** 489–499.

Niebroj-Dobosz I, Fidziańska A, Hausmanowa-Petrusewicz I (2001) Does normal nitric oxide synthase prevent pathologic muscle changes in dystrophin deficiency? *Basic Appl Myol* **11:** 105–110.

Niebroj-Dobosz I, Fidziańska A, Hausmanowa-Petrusewicz I (2002) Oxidative damage to muscle proteins in dystrophinopathies. *Acta Myol* **21:** 12–17.

Ragusa RJ, Chow CK, Porter JD (1997) Oxidative stress as a potential pathogenic mechanism in animal model of Duchenne muscular dystrophy. *Neuromuscul Disord* **7:** 379–380.

Rodriques MCh, Tarnopolsky MA (2003) Patients with dystrophinopathy show evidence of increased oxidative stress. *Free Rad Biol Med* **9:** 1217–1220.

Slater TF (1984) Free-radical mechanisms in tissue injury. *Biochem J* **222:** 1–15.

Tidball JG, Lavergne B, Lau KS, Spencer MJ, Stull JT, Wehling M (1998) Mechanical loading regulates NOS expression and activity in developing and adult skeletal muscle. *Am J Physiol* **82:** C260–266.

Wink DA, Hanbauer I, Krishna MC, DeGraff W, Gamson J, Mitchell JB (1993) Nitric oxide protects against cellular damage and cytotoxity from reactive oxygen species. *Proc Natl Acad Sci USA* **90:** 9813–9817.

Wink DA, Cook JA, Pacelli R, Liebman J, Krishna MC, Mitchell JB (1995) Nitric oxide (NO) protects against cellular damage by reactive oxygen species. *Toxicol Lett* **82–83:** 221–226.

Woodford FP, Whitehead TP (1998) Is measuring serum antioxidant capacity clinically useful? *Ann Clin Biochem* **35:** 48–56.

Zubrzycka-Gaarn EE, Bulman DE, Karpati G, Burghes AH, Belfall B, Klamut HJ, Talbot J, Hodges RS, Ray PN, Worton RG (1988) The Duchenne muscular dystrophy gene product is localized in sarcolemma of human skeletal muscle. *Nature* **333:** 466–469.