ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)
Biopolymers and Cell. 2016. Vol. 32. N 2. P 111–117
doi: http://dx.doi.org/10.7124/bc.000913

UDC 577

Uncoupling of oxidative phosphorylation and antioxidants affect fusion of primary human myoblasts *in vitro*

V. V. Zakharova[1,2,3], C. Dib[3], Y. Bou Saada[3], Y. S. Vassetzky[1,3], I. I. Galkin[1], B. V. Chernyak[1], E. N. Popova[1]

[1] A. N. Belozersky Institute of Physico-Chemical Biology, M. V. Lomonosov Moscow State University Vorobyevy gory, house 1, building 40, Moscow, Russian Federation, 119992

[2] Faculty of Bioengineering and Bioinformatics, M. V. Lomonosov Moscow State University Vorobyevy gory, house 1, building 40, Moscow, Russian Federation, 119992

[3] CNRS UMR 8126, Universit Paris-Sud 11, Institut Gustave Roussy 114, rue Edouard Vaillant, Villejuif, France, 94805
k_popova_ch@mail.ru

Reactive oxygen species are at the origin of muscular fatigue and atrophy. They are also linked to muscular dystrophies, a group of human genetic diseases. Several studies point to the benefits of application of antioxidants and uncouplers of oxidative phosphorylation to improve the functional activity of normal and pathological muscles. Other studies point to potential dangers of these compounds. **Aim.** To study the effect of mitochondria-targeted antioxidants and uncouplers of oxidative phosphorylation on muscle differentiation. **Methods.** Muscle differentiation was induced by serum starvation and montored by troponin T stainig. **Results.** the mitochondria-targeted uncoupler of oxidative phosphorylation C_{12}TPP, but not the mitochondria-targeted antioxidant SkQ1, inhibit fusion of primary myoblasts upon their differentiation, but do not affect the synthesis of troponin T, a protein marker of muscle differentiation. **Conclusion.** The effect of C_{12}TPP could be at least partially mediated by inhibition of reactive oxygen species (ROS) production since antioxidant N-acetylcysteine at high doses also inhibited differentiation of myoblasts.

K e y w o r d s: skeletal muscle, muscle differentiation, mitochondria-targeted antioxidants, uncouplers of oxidative phosphorylation

Introduction

Oxidative stress generated in human muscles increases fatigue and may even contribute to muscular atrophy (reviewed in [1]). In skeletal muscles, oxidative stress is produced in mitochondria and cytosol and expresses itself in a high level of reactive oxygen species (ROS), mostly originating from mitochondria (mtROS; reviewed in [2,3]). Oxidative stress, ROS and mtROS are linked to the pathological conditions such as muscular dystrophies, a group of heterogeneous human genetic diseases mostly affecting skeletal muscles. Indeed, oxidative stress was shown to play a role in the pathogenesis of Duchenne muscular dystrophy (DMD) [4], facioscapulo-humeral dystrophy (FSHD) [5–7] and some other muscular dystrophies (reviewed in [8]).

During muscle repair and regeneration, satellite-derived $CD56^+$ myoblasts differentiate into myotubes. This process may be inhibited by high ROS levels, e.g. due to glutathione depletion [9]. The level of ROS may be reduced by both specific enzymes and low-molecular weight compounds, called antioxidants. ROS play an important role in

various cell processes such as proliferation and differentiation, in defense against infections. ROS may activate redox-sensitive transcription factors and cascades, in a number of cellular signaling systems (reviewed in [2]). At the same time, high ROS levels are detrimental for cells. Antioxidants are widely used as dietary supplements in treating muscular fatigue and atrophy [1], and they are currently studied as potential treatment of muscular dystrophies (Passerieux *et al.*, 2015; Turki *et al.*, 2012; Whitehead *et al.*, 2010) (reviewed in [8]). For example, N-acetylcysteine (NAC), a classical antioxidant was tested in the DMD murine model where it decreased inflammation and myonecrosis (de Senzi Moraes Pinto *et al.*, 2013). At the same time, it appears that ROS are required for muscle differentiation [11, 12]; therefore a complete ROS inhibition may be deleterious for successful muscle regeneration.

In the skeletal muscle, most of the ROS production occurs in mitochondria [13], e.g. during contraction [14], or in response to the external signals such as cytokines [15]. Thus, it would be tempting to use a class of antioxidants specifically targeted to mitochondria to improve the specificity and the impact of antioxidant action. The mitochondria-targeted antioxidants are a class of molecules combining lipophilic cations capable of penetrating through the mitochondrial membranes using the energy of the transmembrane potential (reviewed in [16]) with an antioxidant part. These molecules were successfully tested for their capacity to protect muscles from senescence [17] and immobilization-induced activation of muscle proteolysis [18]. Mitochondria-targeted peptide SS-31which protected mitochondria against an oxidative damage was shown to improve the skeletal muscle performance in aged mice [19]. At the same time, like general antioxidants, a negative effect of mitochondria-targeted compounds was observed: high doses of mitochondria-targeted antioxidants MitoQ and MitoTEMPOL (100 nM and 100 mM, respectively) were shown to repress the myotubes fusion in H9c2 transformed rat cardiac myoblasts [12].

Another class of mitochondria-targeted molecules includes uncouplers of oxidative phosphorylation. At low doses these molecules reduce the mtROS production by mildly decreasing the mitochondrial transmembrane potential without repressing ATP synthesis [20]. For example, 2,4-dinitrophenol (DNP) has multiple tissue-protective effects in different animal and *in vitro* models due to the suppression of oxidative stress [21]. Unfortunately, DNP has some toxic effects and a narrow window of therapeutic activity. The other possible therapeutic strategy exploiting the effect of mild uncoupling is based on the expression of the uncoupling protein specific for brown fat (UCP1); however, the overexpression of UCP1 in muscles led to a significant delay in the re-establishment of neuro-muscular junctions after injury exacerbated the pathology in amyotrophic lateral sclerosis animal model [22].

Recently, a new class of uncouplers, lipophilic cations, has been described. One of the representatives of this new generation of uncouplers, a lipophilic membrane-penetrating cation dodecyltriphenylphosphonium (C_{12}TPP), was found to uncouple oxidative phosphorylation *via* the stimulation of transmembrane cycling of endogenous free fatty acids [23]. Later the uncoupling effect of other lipophilic cations was described and some of them (derivatives of Rhodamine19) catalyzed the transmembrane transport of protons in the absence of free fatty acids due to the protonation/deprotonation reactions of the cation [24]. These compounds have a wider effective concentration range because of their self-limiting mechanism of uncoupling and their therapeutic effects were demonstrated in the animal models of ischemic brain [25,26] and kidney [25] injury. The effect of lipophilic cations has not been tested on muscles yet.

Here we analyzed the effect of different concentrations of the C_{12}TPP lipophilic cation on the differentiation of human $CD56^+$ primary myoblasts *in vitro* and compared it to the antioxidants: mitochondria-targeted antioxidant SkQ1 and NAC (a general antioxidant).

Materials and Methods

Cell culture conditions and transfection. Primary myoblasts (a kind gift of Prof. Dalila Laoudj-Chenivesse) were isolated from skeletal muscle biopsies, purified using CD56/NCAM magnetic beads (Miltenyui Biotec) and cultured as described elsewhere [27].

Myogenic differentiation and myotubes quantification were carried out essentially as described in [28] Primary myoblasts were seeded at a low density and cultured in a proliferation medium, 10 ml of which supplemented or not with the indicated concentrations of antioxidants or uncouplers. 48 h later the cells were collected, washed and seeded in the 35 mm collagen-coated petri dishes at 80–100 % confluence. Myogenic differentiation was induced in the differentiation medium, supplemented or not with the indicated concentrations of antioxidants or uncouplers. The cells were kept 6 days under differentiation conditions and were then fixed and immuno-stained with anti-Troponin T primary antibody, as described below.

Analysis of myotube fusion. Fusion was analyzed after 6 days of differentiation using the Myotube Fusion Index (MFI) determined by dividing the number of nuclei in multi-nucleated myotubes by the total number of nuclei in a given microscopic field. Three fields per culture were counted using the ImageJ software.

Immunofluorescence staining and quantification. Differentiated myotubes were stained with mouse monoclonal anti-Troponin T (Sigma # T6277, 1:50) and observed under a fluorescent microscope as described elsewhere [28]. To create a full image of the specimen, the images of adjacent fields were stitched together using Cartograph software (Microvision).

Results and Discussion

Satellite-derived myoblasts from skeletal muscles differentiate into myotubes. This process is essential for the muscle repair and regeneration. The conflicting data exist about a role of oxidative stress in this process. ROS produced by NADPH oxidase are considered to be an essential signaling components for the embryonic cardiomyogenesis [29]. Scavenging of mtROS using mitochondria-targeted antioxidants (MitoQ and MitoTEMPOL) and mitochondria-targeted catalase prevents the murine the cardiac myoblasts differentiation (Lee *et al.*, 2011). On the other hand, a severe oxidative stress induced by glutathione depletion inhibits the differentiation of murine skeletal muscle cells [9]. Most of these studies were carried on the immortalized animal cells. We decided to test the effect of cationic mitochondria-targeted uncoupler of oxidative phosphorylation C_{12}TPP on the differentiation of primary human $CD56^+$ myoblasts and compare it to the effects of mitochondria-targeted and general antioxidants. The cells were isolated from the skeletal muscle biopsies of two different individuals and induced to differentiate *in vitro* using serum starvation as described elsewhere [30]. Different concentrations of C_{12}TPP, mitochondria-targeted antioxidant SkQ1 or NAC, were added to myoblasts 48h prior to the onset of differentiation, then the same amount of the tested compound was added to the differentiation medium. The results of the differentiation were assayed 6 days after its onset.

The control non-treated cells produced the branched myotubes with bright troponin T staining (Figure 1A). Similar results were obtained with the cells, treated with 2–200 nM SkQ1, 2–20 nM C_{12}TPP and 1 mM NAC (Figure 1). In contrast, the myoblasts pre-treated with 200 nM C_{12}TPP and 5 mM NAC did not form prolonged myotubes, although the individual cells had a prolonged shape and demonstrated troponin T staining (Figure 1C, G).

Next we have determined the myotube fusion index (MFI) for all experimental conditions. This parameter was obtained by dividing the number of nuclei in multi-nucleated myotubes, containing more than 3 nuclei by the total number of nuclei. The MFI was 72 % for the control cells. In cells treated with 1 mM NAC and 200 nM SkQ1, the MFI slightly increased, although this was not statistically significant, in the cells treated with 5 mM NAC, the MFI was significantly lower than in the control cells,

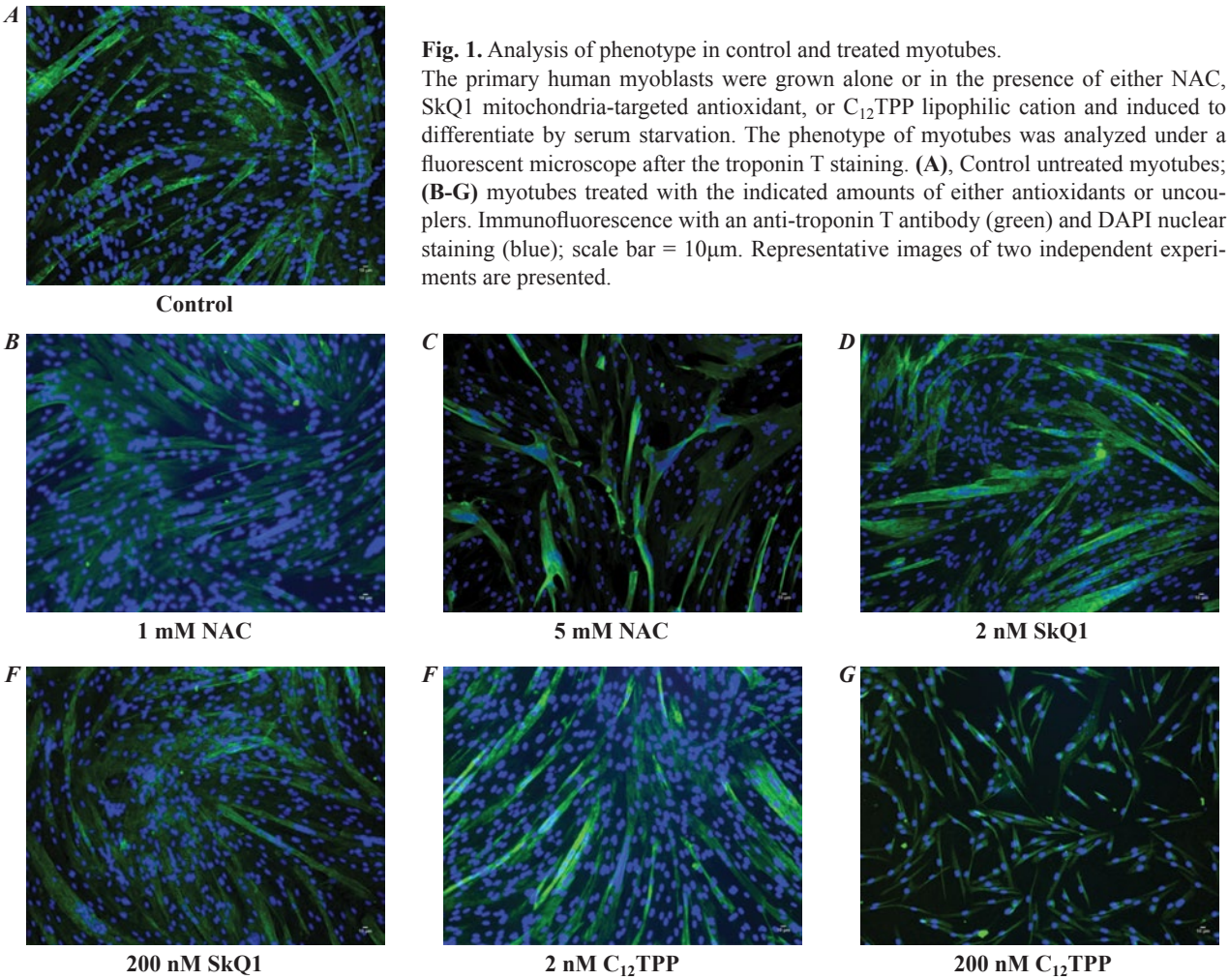

Fig. 1. Analysis of phenotype in control and treated myotubes.
The primary human myoblasts were grown alone or in the presence of either NAC, SkQ1 mitochondria-targeted antioxidant, or C_{12}TPP lipophilic cation and induced to differentiate by serum starvation. The phenotype of myotubes was analyzed under a fluorescent microscope after the troponin T staining. **(A)**, Control untreated myotubes; **(B-G)** myotubes treated with the indicated amounts of either antioxidants or uncouplers. Immunofluorescence with an anti-troponin T antibody (green) and DAPI nuclear staining (blue); scale bar = 10μm. Representative images of two independent experiments are presented.

21±10 %, and 200 nM and C_{12}TPP completely abolished cell fusion (Figure 2).

Thus, we have shown that C_{12}TPP lipophilic cation interferes with the human muscle differentiation by inhibiting the fusion of myoblasts into polynucleated myotubes at a concentration of 200 nM. The differentiation process is not completely inhibited, as the cells acquire an elongated shape (Figure 1G) and express troponin T, a marker of the muscular differentiation. Previously, the high doses of mitochondria-targeted antioxidants MitoQ and MitoTEMPOL (100 nM and 100 mM, respectively) were shown to repress the myotubes fusion in the H9c2 transformed rat cardiac myoblasts cell line [12]. We have next compared the effect of C_{12}TPP with that of NAC, the classical antioxidant and SkQ1, the mitochondria-targeted antioxidant whose lipophilic part is identical to that of C_{12}TPP [31].

The treatment of myoblasts with 5 mM NAC significantly inhibited the myoblast fusion while a lower dose of NAC (1 mM) did not affect the MFI. This raises the question of the safety of this compound, as the opposite effects can be obtained in a very small range of concentrations. Comparing the effects of C_{12}TPP and SkQ1, one can notice that their effects are similar at the concentration of 2–20 nM, but opposite at 200 nM. This may be due to the fact that the uncoupling effect of SkQ1 is significantly less pro-

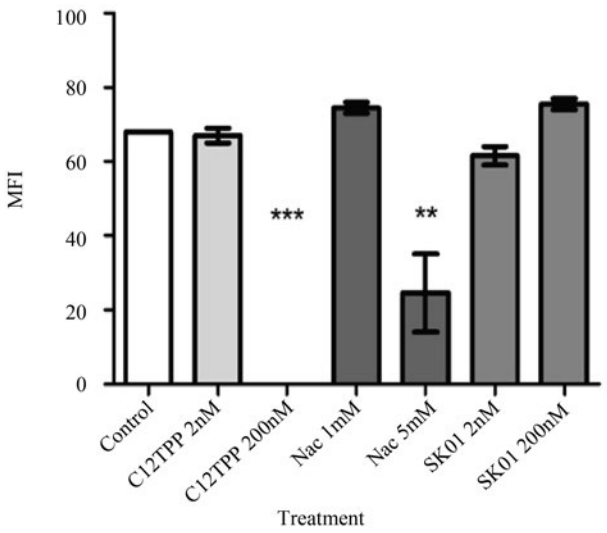

Fig. 2. Effect of antioxidants and uncouplers on the myoblast fusion index. MFI calculated by dividing the number of nuclei in multi-nucleated myotubes by the total number of nuclei in a given microscopic field. A minimum of 1000 nuclei per condition was scored in two independent experiments; **, $p<0.01$; ***, $p<0.001$.

nounced than that of C_{12}TPP, and its mitochondria-targeted antioxidant effect is not detrimental to the cell differentiation. Moreover, the mild uncoupling effect of C_{12}TPP may be also linked to the activation of AMP-activated protein kinase (AMPK) [32], an enzyme known to inhibit the differentiation of C2C12 myoblasts [33].

Another conclusion from this study concerns SkQ1. Its analog MitoQ was shown to repress differentiation of murine cardiac myoblasts cell lines at a concentration of 100 nM [12]. In our hands, a similar dose of SkQ1 did not affect the myoblast fusion, suggesting that SkQ1 has a better range of effective and safe concentrations as compared to MitoQ, although we cannot completely exclude that the differentiation of human myoblasts does not depend on mtROS as dramatically as in case of the murine cardiac myoblasts.

Acknowledgements

The primary human myoblasts were a kind gift of Prof. Dalila Laoudj-Chenivesse (Montpellier). This work was supported by the Russian Scientific Foundation grant #14-24-00107 (SkQ1 studies) and the Russian Foundation for Basic Research (16-04-01074A and 16-54-16015). VZ was supported by the André Mazon fellowship from the French Embassy in Russia.

Funding

This work was supported by the Russian Scientific Foundation (14-24-00107) and the Russian Foundation for Basic Research (16-04-01074A and 16-54-16015).

REFERENCES

1. *Powers SK, Smuder AJ, Judge AR.* Oxidative stress and disuse muscle atrophy: cause or consequence? *Curr Opin Clin Nutr Metab Care.* 2012;**15**(3):240–5.
2. *Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J.* Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007;**39**(1):44–84.
3. *Murphy MP.* How mitochondria produce reactive oxygen species. *Biochem J.* 2009;**417**(1):1–13.
4. *Messina S, Altavilla D, Aguennouz M, Seminara P, Minutoli L, Monici MC, Bitto A, Mazzeo A, Marini H, Squadrito F, Vita G.* Lipid peroxidation inhibition blunts nuclear factor-kappaB activation, reduces skeletal muscle degeneration, and enhances muscle function in mdx mice. *Am J Pathol.* 2006;**168**(3):918–26.
5. *Bosnakovski D, Xu Z, Gang EJ, Galindo CL, Liu M, Simsek T, Garner HR, Agha-Mohammadi S, Tassin A, Coppée F, Belayew A, Perlingeiro RR, Kyba M.* An isogenetic myoblast expression screen identifies DUX4-mediated FSHD-associated molecular pathologies. *EMBO J.* 2008;**27**(20):2766–79.
6. *Turki A, Hayot M, Carnac G, Pillard F, Passerieux E, Bommart S, Raynaud de Mauverger E, Hugon G, Pincemail J, Pietri S, Lambert K, Belayew A, Vassetzky Y, Juntas Morales R, Mercier J, Laoudj-Chenivesse D.* Functional muscle impairment in facioscapulohumeral muscular dystrophy is correlated with oxidative stress and mitochondrial dysfunction. *Free Radic Biol Med.* 2012;**53**(5):1068–79.
7. *Passerieux E, Hayot M, Jaussent A, Carnac G, Gouzi F, Pillard F, Picot MC, Böcker K, Hugon G, Pincemail J, Defraigne JO, Verrips T, Mercier J, Laoudj-Chenivesse D.* Effects of vitamin C, vitamin E, zinc gluconate, and selenomethionine supplementation on muscle function and oxidative stress biomarkers in patients with facioscapulohumeral dystrophy: a double-blind randomized controlled clinical trial. *Free Radic Biol Med.* 2015;**81**:158–69.

8. *Canton M, Menazza S, Di Lisa F.* Oxidative stress in muscular dystrophy: from generic evidence to specific sources and targets. *J Muscle Res Cell Motil.* 2014;**35**(1):23–36.
9. *Ardite E, Barbera JA, Roca J, Fernández-Checa JC.* Glutathione depletion impairs myogenic differentiation of murine skeletal muscle C2C12 cells through sustained NF-kappaB activation. *Am J Pathol.* 2004;**165**(3):719–28.
10. *Whitehead NP, Yeung EW, Froehner SC, Allen DG.* Skeletal muscle NADPH oxidase is increased and triggers stretch-induced damage in the mdx mouse. *PLoS One.* 2010;**5**(12): e15354.
11. *Lee KH, Kim DG, Shin NY, Song WK, Kwon H, Chung CH, Kang MS.* NF-kappaB-dependent expression of nitric oxide synthase is required for membrane fusion of chick embryonic myoblasts. *Biochem J.* 1997;**324** (Pt 1):237–42.
12. *Lee S, Tak E, Lee J, Rashid MA, Murphy MP, Ha J, Kim SS.* Mitochondrial H2O2 generated from electron transport chain complex I stimulates muscle differentiation. *Cell Res.* 2011;**21**(5):817–34.
13. *Koren A, Sauber C, Sentjurc M, Schara M.* Free radicals in tetanic activity of isolated skeletal muscle. *Comp Biochem Physiol B.* 1983;**74**(3):633–5.
14. *Jackson MJ, Pye D, Palomero J.* The production of reactive oxygen and nitrogen species by skeletal muscle. *J Appl Physiol (1985).* 2007;**102**(4):1664–70.
15. *Langen RC, Van Der Velden JL, Schols AM, Kelders MC, Wouters EF, Janssen-Heininger YM.* Tumor necrosis factor-alpha inhibits myogenic differentiation through MyoD protein destabilization. *FASEB J.* 2004;**18**(2):227–37.
16. *Sheu SS, Nauduri D, Anders MW.* Targeting antioxidants to mitochondria: a new therapeutic direction. *Biochim Biophys Acta.* 2006;**1762**(2):256–65.
17. *Mistry Y, Poolman T, Williams B, Herbert KE.* A role for mitochondrial oxidants in stress-induced premature senescence of human vascular smooth muscle cells. *Redox Biol.* 2013;**1**:411–7.
18. *Talbert EE, Smuder AJ, Min K, Kwon OS, Szeto HH, Powers SK.* Immobilization-induced activation of key proteolytic systems in skeletal muscles is prevented by a mitochondria-targeted antioxidant. *J Appl Physiol (1985).* 2013; **115**(4):529–38.
19. *Siegel MP, Kruse SE, Percival JM, Goh J, White CC, Hopkins HC, Kavanagh TJ, Szeto HH, Rabinovitch PS, Marcinek DJ.* Mitochondrial-targeted peptide rapidly improves mitochondrial energetics and skeletal muscle performance in aged mice. *Aging Cell.* 2013;**12**(5):763–71.
20. *Cunha FM, Caldeira da Silva CC, Cerqueira FM, Kowaltowski AJ.* Mild mitochondrial uncoupling as a therapeutic strategy. *Curr Drug Targets.* 2011;**12**(6):783–9.
21. *Caldeira da Silva CC, Cerqueira FM, Barbosa LF, Medeiros MH, Kowaltowski AJ.* Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance and longevity. *Aging Cell.* 2008;**7**(4):552–60.
22. *Dupuis L, Gonzalez de Aguilar JL, Echaniz-Laguna A, Eschbach J, Rene F, Oudart H, Halter B, Huze C, Schaeffer L, Bouillaud F, Loeffler JP.* Muscle mitochondrial uncoupling dismantles neuromuscular junction and triggers distal degeneration of motor neurons. *PLoS One.* 2009;**4**(4):e5390.
23. *Severin FF, Severina II, Antonenko YN, Rokitskaya TI, Cherepanov DA, Mokhova EN, Vyssokikh MY, Pustovidko AV, Markova OV, Yaguzhinsky LS, Korshunova GA, Sumbatyan NV, Skulachev MV, Skulachev VP.* Penetrating cation/fatty acid anion pair as a mitochondria-targeted protonophore. *Proc Natl Acad Sci U S A.* 2010;**107**(2):663–8.
24. *Antonenko YN, Avetisyan AV, Cherepanov DA, Knorre DA, Korshunova GA, Markova OV, Ojovan SM, Perevoshchikova IV, Pustovidko AV, Rokitskaya TI, Severina II, Simonyan RA, Smirnova EA, Sobko AA, Sumbatyan NV, Severin FF, Skulachev VP.* Derivatives of rhodamine 19 as mild mitochondria-targeted cationic uncouplers. *J Biol Chem.* 2011;**286**(20):17831–40.
25. *Plotnikov EY, Silachev DN, Jankauskas SS, Rokitskaya TI, Chupyrkina AA, Pevzner IB, Zorova LD, Isaev NK, Antonenko YN, Skulachev VP, Zorov DB.* Mild uncoupling of respiration and phosphorylation as a mechanism providing nephro- and neuroprotective effects of penetrating cations of the SkQ family. *Biochemistry (Mosc).* 2012;**77**(9):1029–37.
26. *Khailova LS, Silachev DN, Rokitskaya TI, Avetisyan AV, Lyamsaev KG, Severina II, Il'yasova TM, Gulyaev MV, Dedukhova VI, Trendeleva TA, Plotnikov EY, Zvyagilskaya RA, Chernyak BV, Zorov DB, Antonenko YN, Skulachev VP.* A short-chain alkyl derivative of Rhodamine 19 acts as a mild uncoupler of mitochondria and a neuroprotector. *Biochim Biophys Acta.* 2014;**1837**(10):1739–47.
27. *Barro M, Carnac G, Flavier S, Mercier J, Vassetzky Y, Laoudj-Chenivesse D.* Myoblasts from affected and non-affected FSHD muscles exhibit morphological differentiation defects. *J Cell Mol Med.* 2010;**14**(1–2):275–89.
28. *Dib C, Bou Saada Y, Dmitriev P, Richon C, Dessen P, Laoudj-Chenivesse D, Carnac G, Lipinski M, Vassetzky YS.* Correction of the FSHD myoblast differentiation defect by fusion with healthy myoblasts. *J Cell Physiol.* 2016;**231**(1): 62–71.
29. *Buggisch M, Ateghang B, Ruhe C, Strobel C, Lange S, Wartenberg M, Sauer H.* Stimulation of ES-cell-derived cardiomyogenesis and neonatal cardiac cell proliferation by reactive oxygen species and NADPH oxidase. *J Cell Sci.* 2007;**120**(Pt 5):885–94.
30. *Dmitriev P, Barat A, Polesskaya A, O'Connell MJ, Robert T, Dessen P, Walsh TA, Lazar V, Turki A, Carnac G, Laoudj-Chenivesse D, Lipinski M, Vassetzky YS.* Simultaneous miRNA and mRNA transcriptome profiling of human myoblasts reveals a novel set of myogenic differentiation-associated miRNAs and their target genes. *BMC Genomics.* 2013;**14**:265.
31. *Antonenko YN, Avetisyan AV, Bakeeva LE, Chernyak BV, Chertkov VA, Domnina LV, Ivanova OY, Izyumov DS,*

Khailova LS, Klishin SS, Korshunova GA, Lyamzaev KG, Muntyan MS, Nepryakhina OK, Pashkovskaya AA, Pletjushkina OY, Pustovidko AV, Roginsky VA, Rokitskaya TI, Ruuge EK, Saprunova VB, Severina II, Simonyan RA, Skulachev IV, Skulachev MV, Sumbatyan NV, Sviryaeva IV, Tashlitsky VN, Vassiliev JM, Vyssokikh MY, Yaguzhinsky LS, Zamyatnin AA Jr, Skulachev VP. Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 1. Cationic plastoquinone derivatives: synthesis and in vitro studies. *Biochemistry (Mosc)*. 2008 Dec;**73**(12):1273–87.

32. *Apostolova N, Victor VM.* Molecular strategies for targeting antioxidants to mitochondria: therapeutic implications. *Antioxid Redox Signal.* 2015;**22**(8):686–729.
33. *Williamson DL, Butler DC, Alway SE.* AMPK inhibits myoblast differentiation through a PGC-1alpha-dependent mechanism. *Am J Physiol Endocrinol Metab.* 2009;**297**(2): E304–14.

Роз'єднання окисного фосфорилювання і антиоксидантів впливають на злиття первинних людських міобластів in vitro

В. В. Захарова, К. Діб, Я. Б. Саада, Е. С. Васецкий, І. І. Галкін, Б. В. Черняк, Є. М. Попова

Активні форми кисню (АФК) можуть викликати м'язову втому і атрофію м'язів. АФК також пов'язані з м'язовими дистрофії. Безліч досліджень вказує на позитивний вплив антиоксидантів і разобщітелей окисного фосфорилювання на функціональну активність м'язів в нормі та патології. **Мета.** Вивчити вплив мітохондріальної-спрямованих антиоксидантів і разобщітелей окисного фосфорилювання на диференціювання первинних міобластів людини. **Методи.** М'язове діфеернціювання викликали інкубацією міобластів в середовищі з низькою концентрацію сироватки. Міотрубочкі детектували антитілами до тропоніну Т. **Результати.** мітохондріальної-спрямований разобщитель окисного фосфорилювання C_{12}TPP, але не мітохондріальної-спрямований антиоксидант SkQ1, пригнічує злиття міобластів при диференціюванні, при цьому не впливаючи на експресію тропонина Т, білкового маркера м'язової диференціювання. **Висновки.** Вплив C_{12}TPP може бути частково викликано пригніченням АФК, так як високі концентрації класичного антиоксиданту N-ацетилцистеїну також інгібували диференціювання міобластів людини.

Ключові слова: скелетні м'язи, м'язове диференціювання, мітохондріально-спрямовані антиоксиданти, роз'єднувачі окисного фосфорилювання.

Разобщение окислительного фосфорилирования и антиоксидантов влияют на слияние первичных человеческих миобластов в пробирке

В. В. Захарова, К. Диб, Я. Б. Саада, Е. С. Васецкий, И. И. Галкин, Б. В. Черняк, Е. Н. Попова

Активные формы кислорода (АФК) могут вызывать мышечную усталость и атрофию мышц. АФК также связаны с мышечными дистрофиями. Множество исследований указывает на положительное влияние антиоксидантов и разобщителей окислительного фосфорилирования на функциональную активность мышц в норме и патологии. **Цель.** Изучить влияние митохондриально-направленных антиоксидантов и разобщителей окислительного фосфорилирования на дифференцировку первичных миобластов человека. **Методы:** Мышечную дифеернцировку вызывали инкубацией миобластов в среде, содержащей низкую концентрацию сыворотки. Миотрубочки окрашивакли антителми к Тропонину Т. **Результаты.** митохондриально-направленный разобщитель окислительного фосфорилирования C_{12}TPP, но не митохондриально-направленный антиоксидант SkQ1, ингибирует слияние миобластов при дифференцировке, при этом не влияя на экспрессию тропонина Т, белкового маркера мышечной дифференцировки. **Выводы:** Влияние C_{12}TPP может быть частично вызвано ингибированием АФК, так как высокие концентрации классического антиоксиданта N-ацетилцистеина также ингибировали дифференцировку миобластов человека.

Ключевые слова: Скелетные мышцы, мышечная дифференцировка, митохондриально-направленные антиоксиданты, разобщители окислительного фосфорилирования.

Received 14.02.2016