Pharmacological Research 125 (2017) 122–131

Contents lists available at ScienceDirect

Pharmacological Research

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

Original article

Alisporivir rescues defective mitochondrial respiration in Duchenne muscular dystrophy

Marco Schiavone [a,1], Alessandra Zulian [a,1], Sara Menazza [a], Valeria Petronilli [a], Francesco Argenton [b], Luciano Merlini [c], Patrizia Sabatelli [d,e], Paolo Bernardi [a,*]

[a] Department of Biomedical Sciences and CNR Neuroscience Institute, University of Padova, Padova, Italy
[b] Department of Biology, University of Padova, Padova, Italy
[c] Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
[d] Institute of Molecular Genetics, CNR, Bologna, Italy
[e] SC Laboratory of Musculoskeletal Cell Biology, IOR-IRCCS, Bologna, Italy

ARTICLE INFO

Article history:
Received 26 July 2017
Received in revised form 5 September 2017
Accepted 5 September 2017
Available online 9 September 2017

Keywords:
Duchenne muscular dystrophy
Mitochondria
Respiration
Cyclophilin
Permeability transition

ABSTRACT

Duchenne muscular dystrophy (DMD) is a severe muscle disease of known etiology without effective, or generally applicable therapy. Mitochondria are affected by the disease in animal models but whether mitochondrial dysfunction is part of the pathogenesis in patients remains unclear. We show that primary cultures obtained from muscle biopsies of DMD patients display a decrease of the respiratory reserve, a consequence of inappropriate opening of the permeability transition pore (PTP). Treatment with the cyclophilin inhibitor alisporivir – a cyclosporin A derivative that desensitizes the PTP but does not inhibit calcineurin – largely restored the maximal respiratory capacity without affecting basal oxygen consumption in cells from patients, thus reinstating a normal respiratory reserve. Treatment with alisporivir, but not with cyclosporin A, led to a substantial recovery of respiratory function matching improved muscle ultrastructure and survival of *sapje* zebrafish, a severe model of DMD where muscle defects are close to those of DMD patients. Alisporivir was generally well tolerated in HCV patients and could be used for the treatment of DMD.

© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Duchenne muscular dystrophy (DMD) is a severe disease caused by defects of the gene encoding for dystrophin, a key component of muscle [1,2]. Dystrophin interacts with several partners to form the dystrophin-associated dystroglycan complex [3,4], which provides a link between the cytoskeleton and the sarcolemma [5,6]. This link further extends to the extracellular matrix through the interactions of dystroglycan and sarcoglycan with a variety of proteins including agrin, biglycan and their ligands laminin and collagen VI [4]. Dystrophin thus plays a key role in the stability of the sarcolemma in the face of muscle contraction, and it is not surprising that muscular dystrophies can arise from defects in the genes encoding for each component of this connecting system [4].

Mechanical stress is an inevitable consequence of muscle activity. Lack of dystrophin sensitizes muscle to damage followed by fiber death, inflammation and repair resulting in fibrosis, which eventually predominates over regeneration [4]. Increased permeability of the sarcolemma to Ca^{2+} is an early event that can lead to cell death [7], yet it is obvious that the defect is initially compensated [4]. Understanding the molecular bases of this compensation – and the reasons underlying their failure – can have a profound influence on rational therapeutic approaches downstream of the genetic lesion [4].

Animal models indicate that increased Ca^{2+} flux is a pathogenic event *per se* and that muscle disease occurs irrespective of the primary Ca^{2+} transport pathway that has been affected [7,8]. Early work had shown that total Ca^{2+} is increased in DMD fibers [9–11], and that the increase of cytosolic [Ca^{2+}] leads to protein degradation [12,13]. Deregulation of Ca^{2+} homeostasis does not necessarily mean that resting cytosolic [Ca^{2+}] is stably higher than normal

Abbreviations: alisporivir, *N*-methyl-D-alanine-3-*N*-ethyl-valine-4-cyclosporin; CyP, cyclophilin; Cs, cyclosporin; DMD, Duchenne muscular dystrophy; DMEM, Dulbecco's modified Eagle's medium; dpf, days post fertilization; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenyl hydrazone; hpf, hours post fertilization; MTJ, myotendinous junction; NIM811, *N*-methyl-isoleucine-4-cyclosporin; OCR, oxygen consumption rate; PTP, permeability transition pore; TMRM, tetramethylrhodamine methyl ester.

* Corresponding author at: Department of Biomedical Sciences, University of Padova, Via Ugo Bassi 58/B, I-35131 Padova, Italy.
E-mail address: bernardi@bio.unipd.it (P. Bernardi).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.phrs.2017.09.001
1043-6618/© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

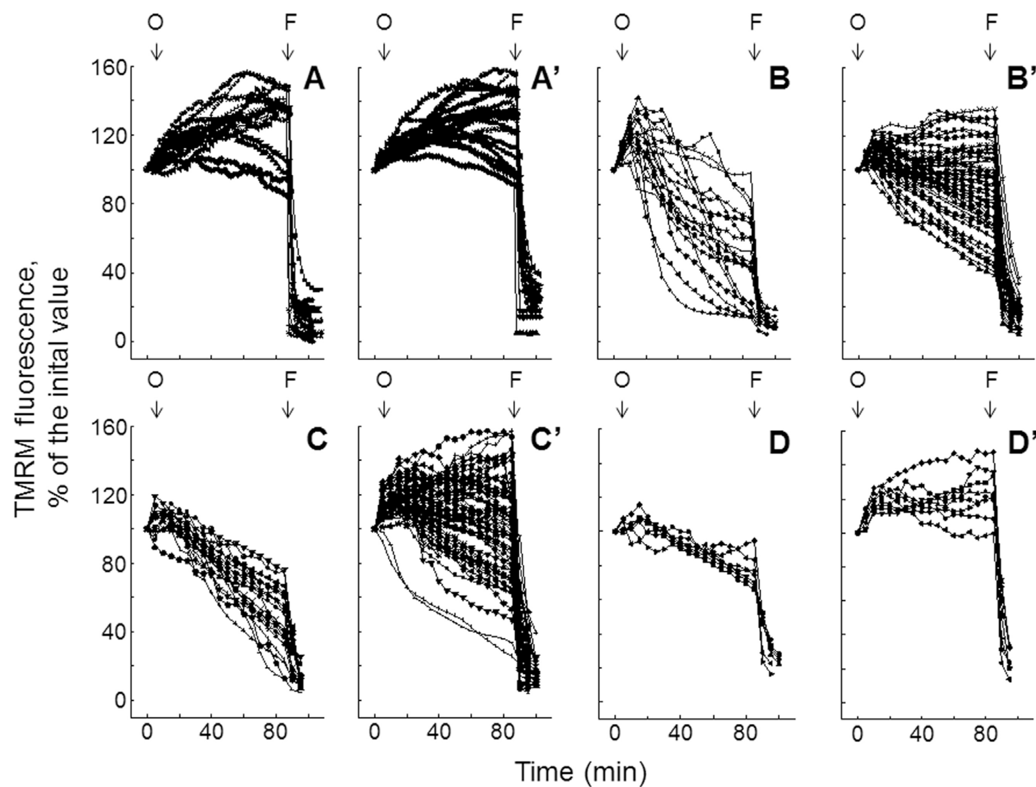

Fig. 1. Effect of alisporivir on oligomycin-induced mitochondrial depolarization in primary muscle cell cultures. Mitochondrial membrane potential was monitored based on TMRM fluorescence changes in primary cultures established from muscle biopsies of one healthy donor (**A, A'**) or of DMD patient 1 (**B, B'**), 2 (**C,C'**) and 3 (**D, D'**) and incubated in serum free-DMEM supplemented with 10 nM TMRM. Fluorescence was monitored over mitochondria-rich regions and acquired every 2 min for healthy donors and every 5 min for DMD patients. Where indicated by arrows, 6 μM oligomycin (O) and 4 μM FCCP (F) were added in the absence (**A–D**) or presence (**A'–D'**) of 1.6 μM alisporivir. Each line reports fluorescence of one individual cell.

[14,15]. It appears likely that increased Ca^{2+} flux at the sarcolemma [16,17] is initially compensated by intracellular organelles, and that a stable cytosolic $[Ca^{2+}]$ rise may be a late event that triggers hypercontracture and activation of proteolytic enzymes, setting the point of no return and fiber death as first proposed 40 years ago [18]. This Ca^{2+}-dependent mitochondrial dysfunction may be due to opening of the mitochondrial permeability transition pore (PTP) [8].

The PTP is an inner membrane channel implicated in a variety of degenerative diseases. Its opening requires matrix Ca^{2+} – a key permissive factor – and is favored by oxidants, while it is counteracted by reducing agents, adenine nucleotides and Mg^{2+} [8]. The PTP appears to originate from a conformational change of the F_1F_O ATP synthase through a Ca^{2+}-dependent mechanism that is the matter of active investigation [19]. A key regulator of the PTP in vertebrates is matrix cyclophilin (CyP) D, whose inhibition is the basis for the PTP desensitizing effects of cyclosporin (Cs) A and of its non-immunosuppressive derivatives *N*-methyl-isoleucine-4-cyclosporin (NIM811) [20] and *N*-methyl-D-alanine-3-*N*-ethyl-valine-4-cyclosporin (alisporivir or Debio025, formerly Unil025) [21]. A role of the PTP in the pathogenesis of muscular dystrophy has been documented particularly for collagen VI diseases. Treatment with CsA and alisporivir was extremely effective in the *Col6a1*$^{-/-}$ myopathic mouse lacking collagen VI [22,23] and in cultured cells form patients [23,24], and NIM811 was superior to CsA in a severe zebrafish model of the disease [25,26]. Is PTP-dependent mitochondrial dysfunction also relevant to DMD? Treatment with alisporivir had beneficial effects in the *mdx* mouse [27,28], where it was more active than prednisone [29]. The limit of these studies is that the *mdx* mouse has a very mild disease and it remains unclear whether CyP inhibitors have therapeutic potential in DMD patients. Here we report (i) the presence of a PTP-dependent mitochondrial defect and (ii) the therapeutic effect of alisporivir in both muscle-derived primary cell cultures from DMD patients and in the *sapje* zebrafish, a severe model of DMD [30].

2. Materials and methods

2.1. Muscle cell cultures

Muscle biopsies were obtained from healthy donors and DMD patients. DMD patient 1 had a deletion of exons 48–54 (out of frame deletion), DMD patient 2 a c5551 C > T stop mutation in exon 39, and DMD patient 3 a deletion of exons 48–50 (out of frame deletion). Cultures were prepared by enzymatic and mechanical treatment of muscle biopsies and by plating in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum, penicillin, streptomycin and amphotericin B (Sigma) as previously described [31] and stored in liquid nitrogen. Cells were expanded and used within the 7th passage.

2.2. Measurement of mitochondrial membrane potential

Mitochondrial membrane potential was measured based on the accumulation of tetramethylrhodamine methyl ester (TMRM). Cells were seeded onto 24 mm diameter round glass coverslips and grown for two days in DMEM supplemented with 20% fetal calf serum. To normalize the loading conditions, in all experiments with TMRM the medium was supplemented with 1.6 μM CsH, which inhibits the multi-drug resistance pump but not the PTP [32]. Cells were rinsed once and then incubated in serum-free DMEM supplemented with 1.6 μM CsH and loaded with 10 nM TMRM for 30 min. At the end of each experiment, mito-

Download English Version:

https://daneshyari.com/en/article/5557187

Download Persian Version:

https://daneshyari.com/article/5557187

Daneshyari.com