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Sodium phenylbutyrate reverses lysosomal dysfunction and decreases amyloid-β42 in an in vitro-model of inclusion-body myositis

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ABSTRACT

Sporadic inclusion-body myositis (s-IBM) is a severe, progressive muscle disease for which there is no enduring treatment. Pathologically characteristic are vacuolated muscle fibers having: accumulations of multi-protein aggregates, including amyloid-β(Aβ) 42 and its toxic oligomers; increased γ-secretase activity; and impaired autophagy. Cultured human muscle fibers with experimentally-impaired autophagy recapitulate some of the s-IBM muscle abnormalities, including vacuolization and decreased activity of lysosomal enzymes, accompanied by increased Aβ42, Aβ42 oligomers, and increased γ-secretase activity. Sodium phenylbutyrate (NaPB) is an orally bioavailable small molecule approved by the FDA for treatment of urea-cycle disorders. Here we describe that NaPB treatment reverses lysosomal dysfunction in an in vitro model of inclusion-body myositis, involving cultured human muscle fibers. NaPB treatment improved lysosomal activity, decreased Aβ42 and its oligomers, decreased γ-secretase activity, and virtually prevented muscle-fiber vacuolization. Accordingly, NaPB might be considered a potential treatment of s-IBM patients.

Introduction

Sodium phenylbutyrate (NaPB) is an orally bioavailable small molecule approved by the FDA for treatment of urea cycle-disorders (reviewed in Cuadrado-Tejedor et al., 2011; Iannitti and Palmieri, 2011; Papp and Csermely, 2006). In patients with urea-cycle disorders, NaPB is metabolized to phenylacetate, which conjugates with glutamine, and then as phenylacetylglutamine it scavenges ammonia to facilitate its excretion (Cuadrado-Tejedor et al., 2011; Iannitti and Palmieri, 2011; Monteleone et al., 2013). When taken daily and long-term by infants, children and adults, it is usually well tolerated (Iannitti and Palmieri, 2011). NaPB was also reported to be well-tolerated in preliminary trials of patients with amyotrophic lateral sclerosis (ALS) (Cudkowicz et al., 2009).

In addition to is action in urea-cycle disorders, NaPB is a histone-deacetylase (HDAC) inhibitor (reviewed in Cuadrado-Tejedor et al., 2011). NaPB also mimics the function of intracellular molecular chaperones by preventing protein aggregation, oligomerization and misfolding, and by reducing endoplasmic reticulum stress (Burrows et al., 2000; Kubota et al., 2006; Rubenstein et al., 1997).

Experimentally, NaPB was reported to be beneficial in mouse models of various neurodegenerative diseases (Cuadrado-Tejedor et al., 2013; Ono et al., 2009; Ricobaraza et al., 2009, 2011; Roy et al., 2012; Zhou et al., 2011). For example, in transgenic Alzheimer disease (AD) mouse models, NaPB reversed memory deficits, which correlated with a decrease of the intraneuronal amyloid-β (Aβ) and Aβ plaques. In addition, it also decreased tau phosphorylation (Cuadrado-Tejedor et al., 2013; Ricobaraza et al., 2009, 2011). In a mouse model of Parkinson disease (PD), NaPB was reported to decrease activation of NF-κB and to reduce oxidative stress, including reduction of inducible nitric-oxide synthase (iNOS) — these effects correlated with stopping the disease progression in this PD model (Roy et al., 2012). In cell culture and in a PD mouse model, NaPB has upregulated anti-oxidant DJ-1, and exerted a neuroprotective effect (Zhou et al., 2011).

Sporadic inclusion-body myositis (s-IBM) is a common degenerative muscle disease associated with aging. Its progressive course leads to severe weakness and muscle atrophy, and there is no effective treatment currently available (Askanas and Engel, 2011; Dalakas, 2011; Dimachkie and Barohn, 2013; Engel and Askanas, 2006). The s-IBM pathogenesis appears to be multifactorial, but the sequence of events in the pathogenic cascade is not known (reviewed in Askanas and Engel, 2011; Askanas et al., 2012). s-IBM muscle biopsies display both lymphocytic inflammation and muscle-fiber degeneration, but how they relate to each other,

Abbreviations: Aβ42, amyloid beta 42; AD, Alzheimer disease; Baf, bafilomycin A1; CHMFs, cultured human muscle fibers; Chl, chloroquine; HDAC, histone deacetylase; iNOS, inducible nitric oxide synthase; NaPB, sodium phenylbutyrate; PD, Parkinson disease; s-IBM, sporadic inclusion-body myositis.

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and which precedes the other, are debated but not established (Askanas and Engel, 2011; Askanas et al., 2012; Dalakas, 2008). Even though a corticosteroid is sometimes transiently beneficial, in general, anti-dysimmune and anti-inflammatory treatments are not enduringly effective (Askanas and Engel, 2011; Askanas et al., 2012; Dalakas, 2011; Dimachkie and Barohn, 2013; Engel and Askanas, 2006).

A very interesting aspect of the s-IBM muscle fiber is that its pathologic phenotype shares several molecular abnormalities with AD brain, including abnormal accumulations intracellularly, within s-IBM muscle fibers, of many of the same proteins, including Aβ42 and its oligomers, and phosphorylated tau (Askanas and Engel, 1998; Askanas et al., 1993, 2012; Gouras et al., 2010; Honson and Kuret, 2008; Iqbal et al., 2010; Kannanayakal et al., 2008; Klein et al., 2004; LaFerla, 2010; LaFerla et al., 2007; Mirabella et al., 1996; Selkoe, 2011; Yankner and Lu, 2009). In addition, oxidative stress parameters — including increased iNOS and nitric oxide, increased activation of NF-κB, and increased oxidation of an anti-oxidant DJ-1 — have also been demonstrated in s-IBM muscle fibers, and were proposed to play a pathogenic role (Broccolini et al., 2000; Nogalska et al., 2007; Terracciano et al., 2008; Yang et al., 1996).

Aβ42 is considered the most toxic specie of Aβ because of its high propensity to oligomerize, aggregate, and form amyloid fibrils (De Strooper, 2010; LaFerla et al., 2007). It is present within s-IBM muscle fibers in the form of both oligomers and aggregates (Nogalska et al., 2010a; Vattemi et al., 2009). Aβ is generated from the amyloid-β precursor protein cleaved by β-and γ-secretases (Chow et al., 2010; De Strooper, 2010; LaFerla et al., 2007), both of which are quantitatively increased in s-IBM muscle on the protein and mRNA levels (Nogalska et al., 2010c; Vattemi et al., 2001), as is the activity of γ-secretase (Nogalska et al., 2012), which is responsible for the Aβ42 generation (Chow et al., 2010; De Strooper, 2010).

There is increasing evidence supported by experimental models employing cultured human muscle and transgenic mice (reviewed in Askanas and Engel, 2011; Askanas et al., 2012), that Aβ might be an important pathogenic aspect leading to s-IBM muscle-fiber degeneration, atrophy and weakness (reviewed by Askanas and Engel, 1998, 2011; Askanas et al., 2012; Dalakas, 2008).

Impaired autophagy is another important aspect of the s-IBM pathogenesis, as evidenced by the muscle-fiber vacuolization accompanied by: 1) inhibition of the lysosomal enzyme activities of cathepsins D and B (Nogalska et al., 2010b); 2) increase of LC3-II, a lipidated form of LC3 (Nogalska et al., 2010b), which is considered the most important indicator of increased autophagosome number, also occurring as the result of impaired autophagy (Klionsky et al., 2012); and 3) up-regulation of the proteasome–lysosome shuttle adaptor proteins NBR1 and p62 (D'Agostino et al., 2011; Nogalska et al., 2009). Both NBR1 and p62 bind to the ubiquitin moiety of proteins destined to be degraded (Lamark et al., 2009). Accumulation of selected autophagy-related proteins in s-IBM muscle fibers was also reported by others (Girolamo et al., 2013).

Our very recent studies demonstrated that cultured human muscle fibers (CHMFs) with experimentally-inhibited lysosomal activity have pronounced vacuolization, in addition to significantly increased Aβ42 and its oligomers, accompanied, and probably caused by, the demonstrated increase of γ-secretase activity (Nogalska et al., 2012), and perhaps also by decreased Aβ42 degradation. Therefore, it appears likely that, at least in human muscle fibers, impaired lysosomal activity plays an important pathogenic role by significantly increasing generation of Aβ42 and its toxic oligomers within s-IBM muscle fibers. Accordingly, the lysosomal-activity-inhibited CHMFs might provide a useful experimental model in the search for possible therapeutic agents potentially improving the impaired autophagic functions and their detrimental consequences, such as increased Aβ42 and its oligomers.

Here we describe a novel function of NaPB, namely that in lysosomal-activity-inhibited CHMFs it substantially: (a) improved the phenotype of muscle-fibers by decreasing their vacuolization; (b) increased cathepsins D and B activities, accompanied by a decrease of NBR1, p62 and LC3-II; (c) decreased Aβ42 and Aβ42 oligomers; and (d) decreased γ-secretase activity.

These results, in combination with the results reported in the literature regarding a positive influence of NaPB in various models of neurodegenerative diseases, make NaPB, a relatively safe drug, a strong candidate for treatment of s-IBM patients.

Material and methods

Cultured human muscle fibers (CHMFs)

Primary cultures of normal human muscle were established as routinely performed in our laboratory (Askanas and Engel, 1992) from archived satellite cells of portions of diagnostic muscle biopsies from patients who, after all tests were performed, were considered free of muscle disease. Each experiment was performed on 3–9 different culture sets, each established from satellite cells derived from a different muscle biopsy. 10–15 days after myoblast fusion, the well-differentiated myotubes were, as previously described (Nogalska et al., 2010b, 2012), exposed for 24 h to either: a) *chloroquine* (*Chl*), a lysosomotropic agent raising lysosomal pH and inhibiting activities of cathepsins (Shacka et al., 2006) (50 μM, Sigma-Aldrich, St. Louis, MO); or b) *bafilomycin A1* (*Baf*), an inhibitor of lysosomal V-ATPase causing an increase in lysosomal pH and inhibiting activities of cathepsins (Shacka et al., 2006) (25 nM, Sigma-Aldrich). CHMFs were also treated with *NaPB* (Tocris/R&D Systems, Minneapolis, MN) in doses ranging from 1 to 15 mM, used either alone or together with chloroquine or bafilomycin. After several preliminary experiments, for final-treatment experiments we selected a 15 mM dose of NaPB, because in control CHMFs even this relatively high dose did not produce either adverse morphologic abnormalities or affect muscle-fiber viability, and when applied together with chloroquine or bafilomycin exerted a consistently significant beneficial effect.

To morphologically evaluate accumulation of autophagosomes, three different tissue-culture sets (experiments) were transduced with PremoAutophagy Sensor GFP-LC3 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. 24 h later, CHMFs were treated with chloroquine as described above. Counting of the GFP-LC3 puncta in the muscle fibers was performed on all three different culture experiments. Each experiment involved the same three different conditions: control, chloroquine-exposed, and chloroquine-exposed-plus-NaPB-treated cultures. In each experiment, each condition was composed of two cultures. For the analysis, eight random fields per condition of each culture were photographed (magnification ×40) in each of the three experiments. Overall, in each of the three conditions GFP-LC3 puncta were evaluated in more than 80 fibers, independently by two investigators (AN and CD). In addition, GFP-LC3 transduced CHMFs were also processed for immunofluorescence of NBR1 (1:100, 4BR, Santa Cruz Biotechnology, Santa Cruz, CA).

Immunoblots

CHMFs were harvested in RIPA buffer and immunoblotted, as previously detailed (D'Agostino et al., 2011; Nogalska et al., 2010b, 2012) using antibodies against LC3 (1:500, Novus Biologicals, Littleton, CO), p62 (1:100, A-6, Santa Cruz Biotechnology) or NBR1 (1:700). Aβ42 oligomers were studied using 6E10 antibody (1:500, Covance, Princeton, NJ), as described (Nogalska et al., 2010a). Blots were developed using anti-rabbit or anti-mouse WesternBreeze chemiluminescence kits (Invitrogen). Protein loading was evaluated by the actin band (1:600, C-2, Santa Cruz Biotechnology). Quantification of the immunoreactivity was performed by densitometric analysis using NIH Image J 1.310 software.