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APP Processing in Human Pluripotent Stem Cell-Derived Neurons Is Resistant to NSAID-Based γ-Secretase Modulation

Jerome Mertens,[1,8] Kathrin Stüber,[1,2] Patrick Wunderlich,[3] Julia Ladewig,[1] Jaideep C. Kesavan,[1] Rik Vandenberghe,[4,5] Mathieu Vandenbulcke,[6] Philip van Damme,[4,5,7] Jochen Walter,[3] Oliver Brüstle,[1,2,*] and Philipp Koch[1,2,*]

[1]Institute of Reconstructive Neurobiology, University of Bonn and Hertie Foundation, 53127 Bonn, Germany
[2]Life&Brain GmbH, 53127 Bonn, Germany
[3]Department of Neurology, University of Bonn, 53127 Bonn, Germany
[4]Department of Neurology, University Hospitals Leuven, 3000 Leuven, Belgium
[5]Laboratory for Cognitive Neurology, Department of Neurosciences, University of Leuven, 3000 Leuven, Belgium
[6]Department of Old Age Psychiatry, University Hospitals Leuven, 3000 Leuven, Belgium
[7]Vesalius Research Center, VIB, 3000 Leuven, Belgium
[8]Present address: Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA
*Correspondence: brustle@uni-bonn.de (O.B.), philipp.koch@uni-bonn.de (P.K.)
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SUMMARY

Increasing evidence suggests that elevated Aβ42 fractions in the brain cause Alzheimer's disease (AD). Although γ-secretase modulators (GSMs), including a set of nonsteroidal anti-inflammatory drugs (NSAIDs), were found to lower Aβ42 in various model systems, NSAID-based GSMs proved to be surprisingly inefficient in human clinical trials. Reasoning that the nonhuman and nonneuronal cells typically used in pharmaceutical compound validation might not adequately reflect the drug responses of human neurons, we used human pluripotent stem cell-derived neurons from AD patients and unaffected donors to explore the efficacy of NSAID-based γ-secretase modulation. We found that pharmaceutically relevant concentrations of these GSMs that are clearly efficacious in conventional nonneuronal cell models fail to elicit any effect on Aβ42/Aß40 ratios in human neurons. Our work reveals resistance of human neurons to NSAID-based γ-secretase modulation, highlighting the need to validate compound efficacy directly in the human cell type affected by the respective disease.

INTRODUCTION

Alzheimer's disease (AD) is a common and fatal neurodegenerative disorder. Currently, no effective drugs that can stop, slow, or prevent disease progression are available. Deposition of amyloid plaques consisting of aggregated Aβ peptides in the brain is a hallmark of the disease (Selkoe, 2001). The amyloid cascade hypothesis presumes that the accumulation and oligomerization of Aβ peptides trigger a complex pathological cascade resulting in synaptic dysfunction, tau hyperphosphorylation, and eventually progressive neurodegeneration and dementia (Selkoe et al., 2012). Aβ is a proteolytic derivative of the transmembrane amyloid precursor protein (APP), which is sequentially cleaved by β- and γ-secretases in the amyloidogenic processing pathway (Haass et al., 2012). Intramembranous γ-secretase cleavage of the C-terminal fragments of APP (APP-CTF), which represent the immediate precursors of Aβ, results in multiple length variants of Aβ (Haass et al., 2012). Longer Aβ variants such as Aβ42 and Aβ43 are more prone to aggregation and thus are considered more pathogenic than shorter ones such as Aβ38 and Aβ40 (Karran et al., 2011). Today, the peptide ratio of Aβ42 to Aβ40 in the cerebrospinal fluid (CSF) represents the most sensitive and specific primary biomarker for AD and inversely correlates with the age of disease onset in both sporadic (Blennow et al., 2012) and familial (Kumar-Singh et al., 2006) forms of AD. Mutations in APP or in the γ-secretase subunits presenilin-1 (PS1) and PS2 are the main cause of autosomal-inherited early-onset forms of AD and commonly lead to increased Aβ42/Aß40 ratios and/or overall elevated levels of Aβ. These observations suggest that misprocessing of APP with a consecutive increase of Aβ42/Aß40 ratios is characteristic of and, most probably, causative for sporadic and familial AD (Wiltfang et al., 2001). Based on this hypothesis, several antiamyloidogenic drugs, including compounds that inhibit β- and γ-secretase activity, have been developed (Ghosh et al., 2012; Imbimbo and Giardina, 2011). Interestingly, a subset of nonsteroidal anti-inflammatory drugs (NSAIDs) were identified to act as γ-secretase modulators (GSMs) that specifically lower the production of Aβ42 in favor of shorter Aβ isoforms by targeting γ-secretase PS1 or its substrate APP (Jumpertz et al., 2012; Kukar et al., 2008; Weggen et al., 2001). Unfortunately, and despite solid preclinical data acquired using transgenic animals and APP-transgenic cell lines, NSAIDs such as flurbiprofen and indometacin were not effective in delaying disease progression in

mild-to-moderate AD patients in phase 2 and phase 3 clinical trials (de Jong et al., 2008; Eriksen et al., 2003; Green et al., 2009; Imbimbo and Giardina, 2011; Vellas, 2010). The reasons for these negative outcomes are speculative and have been in part attributed to inappropriate study design, as symptomatic AD patients were treated when the disease may have already been irreversibly advanced (Golde et al., 2011). Also, it remains unclear whether the trialed GSMs indeed lowered Aβ42 levels in the human brain, leaving the important question as to whether γ-secretase modulation is a valid approach in AD therapy unresolved. Further, insufficient brain penetration of the tested compounds, as well as a general failure of the amyloid cascade hypothesis, has been considered (Golde et al., 2011). Remarkably, the efficacy of GSMs in human neurons as the primary target cell type has never been directly explored. Recent advances in neural differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) enable the derivation of authentic neuronal cultures to dissect the pathological mechanisms relevant to AD and drug testing (Israel et al., 2012; Koch et al., 2012; Mattis and Svendsen, 2011; Mertens et al., 2013). Here, we used this approach to determine the efficacy of NSAIDs previously employed in clinical GSM trials in human neurons derived from iPSCs of patients with familial AD and unaffected controls (Ctrl; Figure 1A).

RESULTS AND DISCUSSION

Neurons Derived from Familial AD Patients Show Elevated Aβ42/Aβ40 Ratios

To elucidate APP processing in human neurons from various genetic backgrounds, we took advantage of our recently described and highly standardized pluripotent stem cell-derived, long-term self-renewing neural stem cells (lt-NES cells), which consistently give rise to cultures containing >70% functional human neurons (Falk et al., 2012; Koch et al., 2009). We generated iPSCs from two patients with familial AD (two clones each). Patient AD-1 (AD-1a and AD-1b) carries an A79V substitution in one allele of the PS1 gene, which results in autosomal-dominant AD (Larner and Doran, 2006). Patient AD-2 (AD-2a and AD-2b) carries a K724N mutation in the intracytosolic fragment of APP (for clinical details, see Table S1 available online; Theuns et al., 2006). Characterization of the established iPSC lines revealed sustained silencing of the reprogramming transgenes, a normal karyotype, expression of pluripotency markers, and formation of teratomas upon in vivo transplantation (Figures S1A–S1D). The four AD-patient-derived lines were subsequently differentiated into lt-NES cells according to established protocols (Falk et al., 2012; Koch et al., 2009). We further included lt-NES cells derived from reprogrammed fibroblasts from three unaffected individuals (Ctrl-1: clone a and b; Ctrl-2: clone a and b; Ctrl-3: clone a) (Falk et al., 2012; Koch et al., 2011) and from the hESC lines I3 and I6 (hES-1 and hES-2) (Koch et al., 2009). All Ctrl and AD lt-NES cell lines expressed the rosette-associated neuroectodermal markers PLZF, Nestin, DACH1, SOX2, and apically accentuated ZO-1 (Figure 1B). Familial AD mutations were confirmed by sequencing genomic DNA from patient-derived lt-NES cells (Figure S1E). Following differentiation for 4 weeks, 75%–85% of the cultures consisted of postmitotic neurons that expressed β-III tubulin and MAP2ab, while <10% of differentiated cells were positive for the glial marker glial fibrillary acidic protein (GFAP; Figure 1B). We further detected a consistent neuronal expression of PS1, APP, and phosphorylated Tau protein (PHF1 antibody; Figure 1B). Similarly to Ctrl neurons, which have been described previously (Falk et al., 2012; Koch et al., 2009), the AD lt-NES cell-derived neurons developed mature functional properties, including the generation of action potentials upon depolarization and the establishment of spontaneously active synaptic circuitries (Figure 1C). The neuron-specific APP_{695} variant and β- and γ-secretase-associated genes were expressed at comparable levels in the neuronal cultures (Figure S1F). We detected no apparent variations in neuronal differentiation efficiency, neuronal morphology, basic electrophysiological function, or marker expression in Ctrl and AD lt-NES cell-derived neurons.

Mutations in PS1 and APP are known to result in elevated Aβ42/Aß40 ratios in the CSF of familial AD patients (Borchelt et al., 1996; Kumar-Singh et al., 2006). Hence, we determined the levels of secreted Aβ40 and Aβ42 in conditioned media of the generated Ctrl and AD neurons by ELISA and calculated the Aβ42/Aß40 ratio. No significant difference in the Aβ42/Aß40 ratio between hESC-derived (0.092 ± 0.017) and Ctrl-iPSC-derived (0.096 ± 0.008) neurons was detected (Figure 2A). In contrast, AD-1 and AD-2 neurons showed 37% and 89% increases in Aβ42/Aß40 ratios to 0.126 ± 0.001 and 0.174 ± 0.018, respectively (Figure 2A). Interestingly, the increased Aβ42/Aß40 ratio in AD-1a neurons was solely attributable to decreased secretion of total Aβ40 by 26%, with Aβ42 levels remaining comparable to those generated by hESC- and Ctrl-iPSC-derived neurons (Figure 2B). AD-2a neurons also exhibited a decrease in total Aβ40 secretion by 31%, but in addition showed a 33% increase in Aβ42 secretion, resulting in an overall higher increase in the Aβ42/Aß40 ratio (Figure 2B). Thus, elevated Aβ42/Aß40 ratios in PS1(A79V) mutant neurons are likely due to a partial loss of function in γ-secretase function, while neurons from the patient with the APP(K724N) mutation showed decreased Aβ40 production in combination with a gain of function in Aβ42 secretion (Koch et al., 2012).