Pyroglutamate Aβ cascade as drug target in Alzheimer’s disease

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INTRODUCTION

For more than three decades, the amyloid cascade hypothesis [1] claiming that amyloid (Aβ) peptides as cleavage products of the precursor amyloid precursor protein (APP) [2] and accumulation in plaques triggers neuron loss and neurofibrillary tangle formation. Since the first report on the peptide sequence of Aβ1-24 of the N-terminus isolated from cerebrovascular amyloid preparations from Alzheimer’s disease (AD) and Down syndrome brain [3] followed by peptide sequencing of amyloid-β isolated from plaques it became evident that amyloid in plaque cores must be predominantly N-truncated [4]. Later, Mori et al. [5] described an N-truncated Aβ variant starting with pyroglutamate at position 3 (AβpE3). Saido et al. [6] was the first to compare the staining pattern of Aβ1-X with AβpE3-X specific antibodies and showed that the N-truncated Aβ variant is a dominant fraction in plaques in AD brain. Russo et al. [7] reported that both Aβ1-X and AβpE3-X can form stable water-soluble aggregates. Using matrix-assisted laser-desorption-time-of-flight mass spectrometry a variety of N-truncated Aβ peptides, including AβpE3-X in amyloid plaques very identified [8]. Wildburger et al. [9] used high-resolution mass spectrometry and identified a wide range of N- and C-truncated amyloid-β peptides from post-mortem brain of AD patients demonstrating no correlation with post-mortem interval. Portelius et al. [10] revealed the relative abundance of full-length and N-truncated variants using immunoprecipitation in combination with mass spectrometric analysis in different brain areas of sporadic and familial AD cases. The major variants were Aβ1-42, AβpE3-42, Aβ42 followed by Aβ1-40 in both sporadic and familial AD cases. Moreover, Upadhyaya et al. [11] demonstrated that AβpE3-X western blotting can be used as an informative biomarker for biochemical amyloid-β staging in post-mortem brain tissue from symptomatic AD and preclinical AD cases. Besides AβpE3-X and a phosphorylated Aβ variant found in plaques, they were also observed as soluble aggregates in a disease-specific manner. The authors concluded that the level of different Aβ variants occur in a hierarchical sequence allowing the distinction of three biochemical amyloid-β stages, with stage 1 and its characteristic marker Aβ1-X, followed by stage 2 with AβpE3-X and stage 3 with a phosphorylated Aβ [11]. In good agreement, Moro et al. [12] observed that deposition of AβpE3-X is closely related to AD, but not with normal ageing and is found in plaques and neurons. In AD mouse models, similar observations were observed. In APP23 mouse brain, AβpE3-X deposits appear during ageing as a maturation process of amyloid plaques starting with Aβ1-X first followed by AβpE3-X deposits [13]. In APP/PS1KI mice, AβpE3-X positive plaques increased with age, on the expense of Aβ1-X [14]. Transgenic mice expressing mutant AβpE3-42 elicit partial conversion of N-terminal Gln-3 into pyroglutamate Glu-3 in a cell-type dependent manner depending on the different mouse line likely due to specific genomic integration of the transgene. Besides abundant loss of Purkinje cells and ataxia [15], degeneration of hippocampus CA1 neurons and cognitive decline [16], or loss of striatal neurons associated with basal locomotor activity and sensorimotor gating (when coexpressed with human QC) [17] was reported in different transgenic lines.

GENERATION OF PYROGLUTAMATE Aβ

Figure 1 shows a schematic presentation of the different steps for generation of N-terminal pyroglutamate peptides involved in AD etiology as well as the cellular pathways involved. In the last years, considerable progress was achieved in elucidating the different molecular steps for generation of pyroglutamate-modified Aβ. β-site APP cleaving enzyme 1 (BACE1) is the first and rate-limiting step in the production of full-length Aβ [18] from its precursor the
There is considerable evidence that QC is the rate-limiting enzyme in the final step of the conversion of N-truncated Aβ into pyroglutamate AβpE3-42 [23, 30–35]. Besides, the normal function of QC to stabilize hormones, peptides, and proteins it may also contribute to neurodegenerative disorders, systemic inflammatory diseases and certain types of oncological conditions (reviewed recently [36]). In the SXFAD mouse model for AD, overexpression of human QC and knock-out of murine QC clearly demonstrated that memory function correlated well with the formation of AβpE3-42 [32]. Although complete loss of QC activity in homozygous QC knock-out mice did not eliminate AβpE3-42 levels completely suggesting that there is a resting QC-like activity by other enzyme(s). Such an activity has been added by Cynis et al. [37] demonstrating that the isoenzyme of QC (isoQC) predominantly modulated chemokine ligand 2 (CCL2; synonyms: monocyte chemotactic protein 1 and small inducible cytokine A2) thereby fostering pE-CCL2 formation and monocyte infiltration. Moreover, pharmacological inhibition of QC/isoQC-activity reduced the pathology in a mouse model for atherosclerosis. The function of QC/isoQC activity in the etiology of AD addresses directly the generation of toxic AβpE3-42 peptides, but equally important may modulate microglia function via recruitment of monocytes from the periphery. Of note, the dual function of microglia cells in AD pathology should be taken into account. They participate in both beneficial amyloid-β clearance, but also in destructive inflammation in AD brain (reviewed in detail [38]).

PROPERTIES OF PYROGLUTAMATE Aβ

Saido et al. [39] hypothesized that the lactam ring of AβpE3-42 peptides and loss of two negative and one positive charges leads to higher hydrophobicity, higher peptide stability, increased aggregation propensity and may escape enzymatic degradation. Another twist in the complex story was published by Nussbaum et al. [40], who pointed out that AβpE3-42 peptides can form soluble oligomers that potentially seed full-length Aβ1-42 and are toxic in a tau-dependent manner. Cross-seeding activities of AβpE3-42 peptides with wild-type Aβ1-42 were also reported by Hu et al. [41, 42]. In addition, AβpE3-42 and AβpE3-42 triggered amyloid-β load and memory deficits when crossed to amyloid-β plaque load and memory deficits when crossed to amyloid-β plaque model [44, 45].

The secondary structure of AβpE3-42, AβpE3-42 and AβpE3-42 peptides was analyzed by far-UV circular dichroism (CD) spectroscopy demonstrating that the CD spectra of monomers were characteristic of a disordered conformation [46]. The aggregation propensities of the Aβ variants analyzed by liquid-state nuclear magnetic resonance (NMR) spectroscopy suggested that the temporal loss of signal intensities was due to conversion of NMR-visible monomers and small aggregates into larger aggregates. The highest loss in signal intensity was found for N-truncated Aβ. Hence, it was reported that AβpE3-42 and AβpE3-42 rapidly formed aggregates with a high aggregation propensity in terms of monomer consumption and oligomer formation [46]. Acute treatment of primary cortical neurons indicated that AβpE3-42 and AβpE3-42 are equally toxic as Aβ1-42. This was further corroborated by induction of working memory deficits after intraventricular injection of AβpE3-42, AβpE3-42 or Aβ1-42 in wild-type mice [46, 47].

In aqueous solution and in 10% sodium dodecyl sulfate micelles AβpE3-40 peptides showed increased β-sheet formation using CD spectroscopy and aggregation behavior by sedimentation analysis when compared with Aβ1-40 [48]. The relative toxicity and abundance of N-truncated and full-length Aβ variants both in vitro and in vivo in AD mouse models was reviewed and discussed previously [21, 49–51].
**DRUG TARGET PYROGLUTAMATE Aβ**

The different steps for therapeutic intervention against the pyroglutamate amyloid-β cascade in the etiology of AD are shown in Fig. 2. It is generated by a two-step process starting by a first cut of the APP between Met at position-1 and Asp at position +1 by β-site APP cleaving enzyme 1 (BACE1), which generates the N-terminus of full-length Aβ1-42 [33]. In addition, meprin-β can cut between Met-1 and Asp+1-2 independent from BACE1 [20]. DPP4 is responsible for the cleavage of the first two N-terminal amino acids of full-length Aβ1-42, generating Aβ3-9 [23, 26]. There is one clinical study published that investigated the influence of DPP4 inhibition in patients with dementia [52]. In this retrospective clinical study the effect of vildagliptin, a DPP4 inhibitor, was investigated on cognitive dysfunction in 60 elderly patients with diabetes with additional diagnosis of mild cognitive impairment (MCI). Fifty percent of the patients were treated with metformin as standard medication control; the other 50% received metformin plus vildagliptin. A significant beneficial effect on stabilizing cognitive scores was observed in the DPP4 inhibitor group [52]. This pilot study did not include however, the assessment of AD biomarkers commonly employed in clinical trials with MCI patients. In addition, it is not clear whether pharmacological inhibition of DPP4 activity is clinically meaningful in nondiabetic MCI and AD patients.

Alternatively, and independent from DPP4 activity, a sequential cleavage of full-length Aβ between Asp at position +1 and Ala at position +2 may also occur by aminopeptidase A activity thereby influencing formation of Aβ3-9 [21, 22].

The next step in the cascade is the conversion of N-terminal Glu-3 of Aβ into Aβ3-9 by QC. In preclinical experiments, pharmacological QC inhibition had significant beneficial effects on Aβ3-9 levels, plaque load, gliosis and memory function [33].

![Pyroglutamate Aβ as a potential drug target against Alzheimer’s disease](Image)

**Fig. 2 Pyroglutamate Aβ as a potential drug target against Alzheimer’s disease.** ① Inhibition of DPP4 prevents N-terminal truncation of the first two amino acid residues of the full-length Aβ monomers (Aβ1-42). ② Inhibition of QC activity prevents the conversion of Aβ3-9 into Aβ3-9 monomers. ③ Neutralizing antibodies react with different conformational and functional variants of Aβ3-9. ④ Once Aβ3-9 monomers are generated, they adopt a pseudo β-hairpin structure at the N-terminus, which is specifically recognized by the TAPAS family of antibodies. The pseudo β-hairpin epitope is neutralized by the TAPAS vaccine and by TAPAS monoclonal antibodies. ⑤ Pan-Aβ3-9 antibodies react with a range of conformations: soluble oligomers, protofibrils and fibrillar forms found in different plaque types. ⑥ Donanemab, a plaque-specific monoclonal antibody, reacts with Aβ3-9 aggregates found in the amyloid-plaque cores. The surface structure of DPP4 [84] and of QC [85] was taken from the Protein Data Bank (PDB). Created with BioRender.com.
2 trial evaluated safety, tolerability and efficacy of passive immunization with donanemab a plaque-specific humanized antibody against AβpE3-X [73]. The study design was unique, as the screening of early symptomatic AD patients was based on a tau threshold screening by brain imaging with flortaucipir PET scanning to assess tangle deposition in vivo. Only patients with intermediate levels of tangle formation were subsequently enrolled in the study. The primary outcome measures were tests to assess cognitive function. The secondary outcome measures assessed in addition amyloid-plaque load and tangle deposition by appropriate PET scans. Donanemab significantly slowed the disease process, slowed slowed cognitive and functional decline on all secondary clinical endpoints, and reduced plaque load, and tau accumulation in a subgroup of patients. Of note, patients with the lowest tau accumulation demonstrated the highest benefit, while patients with the highest tau accumulation did not benefit at all. The safety profile was similar to findings of the phase 1 trial [74]. Although the major endpoints of the phase II donanemab trial were reached and are generally promising, the outcome on the neuropathological level is not entirely clear. For example, neurodegeneration and tau pathology progression were slowed down, but not reversed. Therefore, it is not entirely proven whether AβpE3-X directly triggers tau pathology. One needs to bear in mind, that many neuropathological studies have demonstrated that tau pathology precedes plaque pathology (for example [75, 76]). Of note, the sensitivity of biomarkers used in clinical studies is still lower in comparison with that of post-mortem neuropathological evaluations [77–82].

The meaning of the data of the TRAILBLAZER-ALZ is a matter of ongoing scientific debates as aggregated fibrillar amyloid is generally thought to be pathologically inert as discussed above [59]. Ackley et al. [83] added further evidence to the discussion. The authors reported on a meta-analysis of randomized controlled clinical trials of drugs for the prevention or treatment of AD targeting amyloid mechanism. They concluded that amyloid-plaque reduction strategies did not substantially improve cognition. In conclusion, there is ample evidence that pyroglutamate Aβ is involved in the etiology of AD. The pyroglutamate amyloid-β cascade provides several attractive therapeutic intervention points. DPP4 or QC inhibitors can modulate the stepwise cascade provides several attractive therapeutic intervention involved in the etiology of AD. The pyroglutamate amyloid-beta isoform signatures in familial and sporadic Alzheimer's disease. Acta Neuropathol. 2010;120:185–93.

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AUTHOR CONTRIBUTIONS
The author solely wrote the manuscript and designed the figures using software as indicated in the figures' legends.

FUNDING
Open Access funding enabled and organized by Projekt DEAL.

COMPETING INTERESTS
University Medicine Göttingen holds patents on parts of the discussed research.

ADDITIONAL INFORMATION
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