Does glimepiride provide a novel mechanism of neutralising toxic amyloid-β?

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Alzheimer’s disease is associated with the accumulation within the brain of amyloid-β (Aβ) peptides that damage synapses and affect memory. Our recent study examined Alzheimer’s-related neurodegeneration by incubating cultured neurons with Aβ and measuring synaptic density. We showed that glimepiride, a sulphonylurea used for the treatment of diabetes, protected neurons against Aβ-induced synapse damage by changing the responsiveness of neurons to Aβ. Although glimepiride-treated neurons bound similar amounts of Aβ to control neurons less Aβ accumulated within lipid rafts. More specifically, glimepiride reduced the Aβ-induced activation of cytoplasmic phospholipase A2 (cPLA2), aberrant activation of which causes synapse damage. Glimepiride also reduced the Aβ-induced increase in cholesterol that was required for the activation of cPLA2 suggesting that drug-induced modifications of membrane micro-environments alter the Aβ-induced signalling pathways that lead to synapse damage. Therapeutic strategies in Alzheimer’s disease often include neutralising toxic forms of Aβ. Although several monoclonal antibodies (mAbs) targeting Aβ are currently in clinical trials, there remain concerns over their side effects, their ability to cross the blood brain barrier and their effectiveness. Here we offer an alternative mechanism for neutralising Aβ. Glimepiride caused the release of soluble cellular prion proteins (PrP⁰), a receptor for toxic forms of Aβ. The soluble PrP⁰ bound to and neutralised Aβ thus preventing synapse damage. Such observations raise the possibility that glimepiride could induce soluble PrP⁰ within the brain that will neutralise toxic Aβ, reduce synapse damage and hence delay the progression of cognitive decline in Alzheimer’s disease.

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cultured neurons was measured as an in vitro model of AD-related neurodegeneration. “Natural soluble Aβ oligomers” were isolated from the brain of an Alzheimer’s patient and added to neurons. These Aβ oligomers reduced the amounts of synaptic proteins, including synaptophysin, synapsin-1, cysteine string protein and vesicle-associated membrane protein (VAMP)-1, in cultured neurons indicative of synapse damage [6]. The effects of these Aβ oligomers occurred at picomolar concentrations, similar to the concentrations of Aβ found in brain or cerebrospinal fluid of Alzheimer’s patients [7-9].

The identification of drugs that reduce Aβ-induced synapse damage is a rational strategy to reduce the cognitive decline that is observed in AD patients. Numerous biochemical, epidemiological, pharmacological and genetic studies demonstrated that cholesterol is a risk factor for the development of AD [10-12]. Cholesterol is required for the formation of specific membrane micro-domains called lipid rafts [13] which are thought to affect AD pathogenesis for the membrane protein (VAMP)-1, in cultured neurons indicative of synapse damage [10-12]. Cholesterol is required for the biochemical, epidemiological, pharmacological and genetic decline that is observed in AD patients. Numerous studies demonstrated that cholesterol is a risk factor for the formation of specific membrane micro-domains called lipid rafts [14,15]. (2) Cholesterol depletion protected neurons against Aβ-induced neurodegeneration [16,17]. (3) The coalescence of individual lipid rafts forms signalling platforms [18]. (4) Aβ-induced synapse damage is dependent on activation of cytoplasmic phospholipase A2 (cPLA2) [19]. (5) Aβ causes the translocation of cPLA2 to lipid rafts [19].

Collectively these results indicate that Aβ triggers the events that lead to synapse degeneration from within lipid rafts. Although cholesterol depletion protected neurons, cholesterol synthesis inhibitors are crude pharmacological tools as cholesterol depletion also affects many other neuronal processes. Cholesterol has a key role at the synapse and consequently the inhibition of cholesterol synthesis is not a feasible approach to disrupt Aβ-induced neurodegeneration. The observation that there exist many different, heterogeneous lipid rafts, each containing different proteins and with different functions [20] raised the possibility that compounds that alter the function of the specific lipid rafts involved in Aβ-induced neurodegeneration could be discovered. The factors that affect the formation of lipid rafts are inadequately understood. Rafts contain many proteins attached to membranes via glycosylphosphatidylinositol (GPI) anchors [21]. As GPI anchors promote the formation of rafts [22] we hypothesized that drugs that affect GPI anchors may consequently alter the composition and function of lipid rafts.

**Glimepiride reduced Aβ-induced synapse damage**

Since glimepiride, a sulphonylurea used to treat diabetes, activates an endogenous GPI-phospholipase C (PLC) [23] caused the release of some GPI-anchored proteins, altered the distribution of some lipid raft-resident proteins [24] and reduced cholesterol [25] its effects on neurons were tested. Like many drugs glimepiride has a broad range of actions and while most of the anti-diabetic effects of glimepiride are shared by another sulphonylurea (glipizide), this drug did not affect GPI-PLC and was used as a control. The key observation in our recent paper [26] was that glimepiride-treated neurons were protected against Aβ-induced synapse damage. Neuroprotection was observed at physiologically relevant concentrations [27] but was not seen with the same concentrations of glipizide. The neuroprotection provided by glimepiride was stimulus specific and did not affect synapse damage caused by a phospholipase A2-activating peptide (PLAP).

Some of the effects of glimepiride are mediated by activation an endogenous GPI-PLC [23]. As this effect of glimepiride was not shared by glipizide we hypothesised that activation of GPI-PLC was the key factor responsible for the protective effect of glimepiride. Two methods were used to test this hypothesis. Firstly, neurons treated with phosphatidylinositol (PI)-PLC, to mimic the effects of an endogenous GPI-PLC, showed less synapse damage when incubated with Aβ than control neurons. Secondly, the addition of p-chloromercuriphenylsulphonate (p-CMPS), an inhibitor of GPI-PLC [28], reversed the protective effect of glimepiride showing that its neuroprotective effect was dependent upon the activation of GPI-PLC. Treating neurons with p-CMPS alone did not affect synapses, nor did it affect Aβ-induced synapse damage.

Perhaps the simplest explanation of the glimepiride’s neuroprotective effect would be that it reduced the binding of Aβ to neurons. Since the activation of GPI-PLC releases the GPI-anchored cellular prion protein (PrP<sup>C</sup>), a receptor that mediates Aβ-induced synapse damage [29] from neurons [23], the binding of Aβ to glimepiride-treated neurons was measured. Our finding that there were no significant differences in the concentrations of Aβ that bound to control and glimepiride-treated neurons is consistent with reports that Aβ binds to multiple receptors including the α7 nicotinic acetylcholine and glutamate receptors [30,31]. It suggests that the response of neurons to Aβ depends upon which receptors Aβ interacts with.

Previous studies had indicated that Aβ-induced synapse degeneration was due to events occurring at the synapse [6]. Synaptosomes were isolated from cultured neurons to examine the Aβ-induced signalling events that occurred specifically at the synapse. Treatment of neurons with glimepiride, but not glipizide, reduced the concentrations of
PrPC in synaptosomes. Lipid rafts exist as multiple, heterogeneous sub-sets that have different protein cargos \[20\]. While treatment with the cholesterol synthesis inhibitor squalestatin affected the expression and/or location of several lipid raft-associated proteins including Thy-1, caveolin and VAMP-1, glimepiride treatment had no effect suggesting that glimepiride had a more targeted effects than cholesterol synthesis inhibitors. Glimepiride did not alter the concentrations of Aβ that bound to synaptosomes, indicating that Aβ binds by a PrPC-independent mechanism, but reduced the concentrations of Aβ found within lipid rafts. This observation was of interest as prior studies suggested that synapse degeneration was generated by Aβ from within a cholesterol-sensitive lipid raft \[16\]. These effects of glimepiride were blocked by inhibiting endogenous GPI-PLCs with p-CMPS.

Glimepiride reduced Aβ-induced activation of cPLA2 in synapses

PrPC is associated with several signaling molecules including cPLA2 \[19\]. Several studies suggest that aberrant activation of cPLA2 underlies the pathology of AD; Aβ activates cPLA2 \[32, 33\] and inhibition of cPLA2 reduced the Aβ-induced synapse damage \[6\]. In addition, cPLA2 inhibitors ameliorate the cognitive decline seen in a transgenic model of AD \[34\]. Our study showed that pre-treatment of synaptosomes with glimepiride, but not glipizide, reduced the Aβ-induced activation of cPLA2. Initially it was thought that the glimepiride inhibited cPLA2 directly but this hypothesis was discounted as glimepiride did not affect the activation of cPLA2 by PLAP. So how did glimepiride inhibit cPLA2? The addition of Aβ to synaptosomes causes cPLA2

![Figure 1. Schematic demonstrating how glimepiride might reduce Aβ-induced synapse damage.](image-url)

(A) Soluble Aβ oligomers cross-link membrane bound PrPC leading to the attraction and activation of cPLA2 and synapse damage. (B) In glimepiride-treated neurons Aβ oligomers bind to receptors at the synapse which neither attracted nor activated cPLA2. (C) Soluble PrPC released from glimepiride neurons binds to and neutralised toxic Aβ oligomers.
to translocate from the cytoplasm to lipid rafts and glimepiride reduced the Aβ-induced translocation of cPLA₂ into lipid rafts. As the effects of glimepiride upon both the Aβ-induced activation of cPLA₂ and its translocation to lipid rafts were blocked by p-CMPS we concluded that GPI-anchored proteins were responsible for mediating Aβ-induced activation of cPLA₂. We also observed that the addition of Aβ significantly increased the concentrations of cholesterol within synaptosomes; consistent with reports that increased cholesterol found in Aβ positive synapses in the cortex of AD brains [35]. Such observations are compatible with the hypothesis that Aβ creates the lipid rafts responsible for activation of cPLA₂ and synapse degeneration. The oligomerisation of GPI-anchored proteins triggered lipid raft formation and cell signalling [36, 37] and the increase in synaptic cholesterol was associated with the Aβ oligomer-mediated cross-linkage of lipid raft-associated PrP⁰ and the activation of cPLA₂ [19]. Glimepiride blocked the Aβ-induced increase in cholesterol in synaptosomes; an effect that was also reversed by p-CMPS.

It is notable that although glimepiride did not affect the concentrations of Aβ that bound to synaptosomes, it did block the Aβ-induced increase in synaptic cholesterol and activation of cPLA₂. Clearly the presence of Aβ per se does not increase cholesterol/activate cPLA₂. An emerging paradigm is of cell activation occurring as a consequence of multiple individual lipid rafts coalescing to form a larger membrane platform capable of sustained cell activation [20]. PrP⁰ acts as a scaffold protein that organises signalling platforms [38] and activated cPLA₂ was found within PrP⁰-containing lipid rafts [19]. We hypothesise that critical concentrations of Aβ triggered the formation of a platform capable of the sustained activation of cPLA₂ that leads to synapse damage (Figure 1A). We also conclude that Aβ binding to glimepiride-treated synaptosomes does so via receptors other than PrP⁰ and consequently does not trigger an increase in membrane cholesterol and consequently the lipid rafts that mediate Aβ-induced activation of cPLA₂ do not form (Figure 1B).

**Glimepiride caused the release of soluble PrP⁰ that blocked Aβ-induced synapse damage**

Treatment with glimepiride caused the release of soluble PrP⁰ from neurons [25]. In our recent study soluble PrP⁰ was isolated using an immunoaffinity column followed by reverse phase chromatography. PrP⁰ and soluble PrP⁰ had different migration patterns in high performance thin layer chromatography consistent with a significant change in hydrophobicity after the removal of acyl chains by an endogenous GPI-PLC. The loss of acyl chains did not alter the ability of PrP⁰ to bind Aβ. The concept that disease-relevant conformations of Aβ may constitute only a small proportion of total Aβ [39] raised the possibility that soluble PrP⁰ might bind to the non-toxic forms of Aβ rather than toxic forms of Aβ. This hypothesis was tested by examining the effects of soluble PrP⁰ upon Aβ-induced synapse damage. Firstly we showed that supernatants from glimepiride-treated neurons (containing soluble PrP⁰) blocked Aβ-induced synapse damage. Immunodepletion studies demonstrated that the protective component of these supernatants was PrP⁰. Soluble PrP⁰ purified on C18 columns also blocked the Aβ-induced synapse damage thus confirming that toxic conformations of Aβ bound to soluble PrP⁰. The possibility that soluble PrP⁰ had a direct effect upon neurons was excluded as it did not affect PLAP-induced synapse degeneration. Although complexes of soluble PrP⁰ and Aβ were taken up by neurons they did not increase synaptic cholesterol, nor did they activate synaptic cPLA₂.

We note that soluble PrP⁰ fulfils the same role as the anti-Aβ mAbs designed to neutralise the toxic forms of Aβ and which are the focus of several clinical trials [40] (Figure 1C). However, 2 major questions regarding anti-Aβ mAb therapy remain to be fully explained. The first asks how mAbs to Aβ that are given systemically cross the blood brain barrier to access Aβ within the brain? Glimepiride is thought to cross the blood brain barrier [41] and as PrP⁰ is highly expressed within the brain. This means that whereas mAbs to Aβ given systemically would have to cross the blood brain barrier, soluble PrP⁰ has the advantage of being generated within the brain. In addition, whereas the side effects of anti-Aβ mAbs are partly attributable to their bivalent nature causing the cross-linkage of specific proteins, soluble PrP⁰ is monovalent. In this regard it is of interest that in a transgenic mouse model of AD producing human Aβ and crossed with mice producing anchorless PrP⁰ (which is also soluble [42]) the Aβ-related suppression of long term potentiation (a marker of memory formation) was suppressed [43]. There is a precedent for soluble forms of GPI-anchored proteins being used as treatments. For example CD14 is a GPI-anchored protein [44] that plays a key role in the response of innate immune cells to bacterial components such as lipopolysaccharide (LPS) [45]. The high concentrations of cytokines triggered by LPS can be fatal and soluble forms of CD14 prevented LPS-induced mortality in mice [46].

There is increasing interest in drugs used in the clinic to treat other diseases, and whose pharmacokinetics/side effects are known, to be prescribed “off-label” as AD treatments. Our paper reported that the glimepiride, normally used to treat diabetes, protected neurons against Aβ-induced synapse damage. The protective effect of glimepiride was associated with multiple factors including the loss of PrP⁰ from
synapses, the production of soluble PrP<sup>C</sup>, and the altered distribution of cholesterol, Aβ and cPLA<sub>2</sub> within cell membranes. In glimepiride-treated neurons, Aβ was not targeted to lipid rafts and did not trigger synapse damage. Furthermore, in glimepiride-treated synaptosomes Aβ did not increase cholesterol concentrations and failed to activate cPLA<sub>2</sub>; observations indicating that glimepiride targets rafts involved in Aβ-induced synapse damage. As glimepiride is already used in the clinic it could be considered as a novel adjunctive treatment that could reduce the pathogenesis of AD.

Conflicting interests

The authors have declared that no competing interests exist.

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