PERSPECTIVE

The new $K_V3.4$ inhibitor BDS-I[1–8] as a potential pharmacological opportunity in Alzheimer's disease therapy

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the first cause of dementia in the elderly, with no treatment able to prevent or to block disease progression. AD is characterized by memory impairment and cognitive dysfunction, followed in the late phases of the disease by severe neurodegeneration and neuronal death. The amyloid-β (Aβ) peptide, generated upon the processing of the amyloid precursor protein, is considered the main initiator of AD pathology. Indeed, Aβ peptides, which aggregate and accumulate to form extracellular plaques and intraneuronal deposits, trigger a sequence of pathogenic mechanisms including synaptic dysfunction, neuroinflammation and cell death, leading to cognitive alterations and, subsequently, to dementia. Aβ toxicity also consists in the dysregulation of ionic homeostasis, which contributes to neuronal dysfunction and death. Several studies reported an imbalance of potassium ion (K^+) concentrations in AD brains and the alteration of K^+ channel activity during AD (Etcheberrigaray and Bhagavan, 1999). K^+ channels constitute a large family of ion channels that are involved in determining the resting membrane potential and the action potential waveform and duration and in regulating neurotransmitter release (Rudy et al., 1999). On the other hand, K^+ channels are also implicated in the regulation of cell survival and apoptosis, since cytoplasmic K^+ loss due to the overexpression of K^+ channels has been shown to favor the activation of caspases and nucleases (Yu, 2003), which in turn contribute to the outcome of apoptosis.

Voltage-gated potassium channels (K_V), which allow rapid and selective efflux of K^+ ions across the plasma membrane, are fundamental for the regulation of neuronal excitability and neuronal function. K_V channels usually have a homotetrameric or heterotetrameric structure depending on whether the ion-conducting α-subunits are identical or not, connected with the accessory β-subunits with auxiliary regulatory functions. Among them, the K_V3 Shaw-related subfamily ($K_V3.1$–$K_V3.4$) displays distinct functional properties such as high thresholds of activation, rapid activation and deactivation kinetics and relatively large conductance (Rudy et al., 1999). The $K_V3.4$ channel, which is the only channel among the K_V3 subfamily that carries the fast inactivating potassium currents, has been implicated in several brain disorders such as hypoxia (Kääb et al., 2005) and oxidative stress and in different pathologies, including cancer and cardiovascular diseases (Kääb et al., 2005; Menéndez et al., 2010). Furthermore, the $K_V3.4$ channel has emerged as a relevant player and, hence, as a new target candidate in AD (Angulo et al., 2004; Pannaccione et al., 2007).

Angulo et al. (2004) first suggested that the $K_V3.4$ channel could be the potassium channel subunit involved in AD neurodegeneration. Indeed, they reported that *KCNC4*, encoding for the $K_V3.4$ α subunit, was the only gene with increased expression in both the early and the late stages of AD. In particular, they found that $K_V3.4$ overexpression was mainly confined in Aβ plaques in post-mortem human AD brains and that it correlated with disease severity, thus relating $K_V3.4$ upregulation to amyloid pathology (Angulo et al., 2004). Few years later, we demonstrated that $Aβ_{1–42}$ oligomers induced the upregulation of $K_V3.4$ channels and their accessory subunit, MiRP2, in primary hippocampal neurons through the Ca^{2+}-induced increase of reactive oxygen species and the consequent activation of the nuclear factor-κB (Pannaccione et al., 2007). In addition, we found that the reduction of cytoplasmic K^+ concentrations following the excessive K^+ efflux mediated by upregulated $K_V3.4$ channels after $Aβ_{1–42}$ exposure, induced caspase-3 activation (**Figure 1A**), thus further supporting the correlation between altered intracellular K^+ concentrations and the activation of caspases. In support to this concept, we also demonstrated that the specific pharmacological inhibition of $K_V3.4$ channels by the 43-amino-acids sea anemone toxin BDS-I ("blood depressing substance") prevented the activation of caspase-3 cascade triggered by the $Aβ_{1–42}$ peptide (**Figure 1B**). In this regard, it is worth noting that while the overexpression of activated caspases, including caspase-3, has been documented in AD patients (Pompl et al., 2003), their role in the activation of apoptosis in human AD brains is still controversial. Indeed, despite several studies underlie the involvement of caspase activation in apoptotic processes in *in vitro* and *in vivo* models of AD, their direct correlation with neuronal loss and their role in AD etiology have not yet been determined. Nevertheless, several immunohistochemical and biochemical studies reported the presence of active caspases in neurons, around senile plaques and neurofibrillary tangles, and, in particular, of activated caspase-3 associated to granulovascular degeneration, which is a diagnostic AD

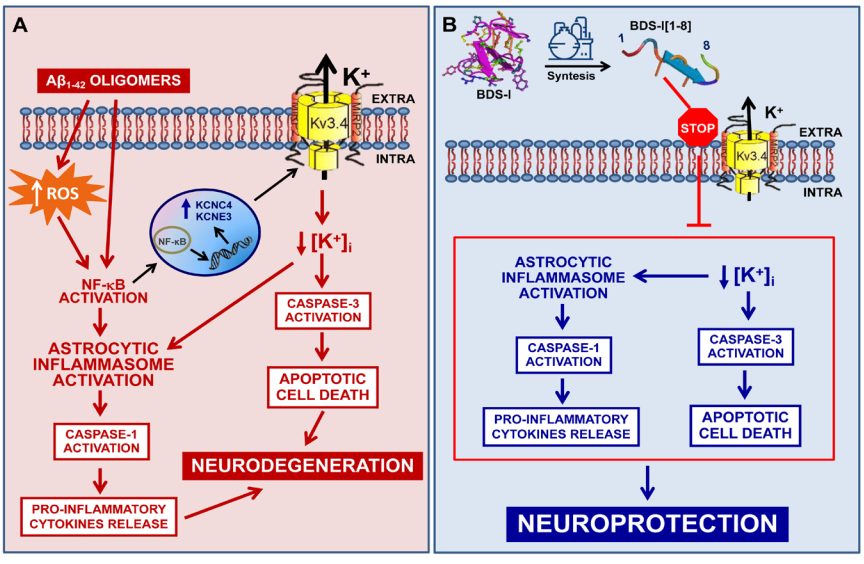

Figure 1 Involvement of $K_V3.4$ upregulation in the neurodegenerative processes triggered by $Aβ_{1–42}$ oligomers.
(A) Proposed mechanisms underlying the neurotoxic effect elicited by the amyloid β1–42 ($Aβ_{1–42}$)-induced upregulation of $K_V3.4$ channels: $Aβ_{1–42}$ oligomers trigger the overexpression of $K_V3.4$ channel (KCNC4) and its accessory subunit Mirp2 (KCNE3) through the activation of the transcriptional factor nuclear factor kappa-B (NF-κB) mediated by Ca^{2+}-induced reactive osygen species (ROS) increase. $K_V3.4$ upregulation, by decreasing intracellular potassium concentrations $[K^+]_i$, induces a cascade of pathogenic events such as mitochondrial membrane depolarization and cytochrome c release, which in turn lead to: (a) caspase-3 activation, thus resulting in neuronal death and synaptic failure and (b) inflammasome activation followed by the cleavage of caspase-1, which is an inflammasome component, and, hence, to its activation, with subsequent release of pro-inflammatory cytokines, thus resulting in neuroinflammation. (B) Schematic representation of the neuroprotective effect elicited by blood depression substance (BDS) fragment by preventing excessive K^+ efflux through $K_V3.4$ channel inhibition.

neuropathological lesion. On the other hand, caspase-3 has been implicated also in non-apoptotic processes, such as amyloid precursor protein metabolism and the formation of tau pathological filaments, but also in spine degeneration and consequent synaptic failure (D'Amelio et al., 2011).

In line with previous studies indicating that K^+ efflux, reactive oxygen specie production and NF-κB activation are required for the induction of both microglial and astrocytic inflammasome, we also demonstrated that K_V3.4 expression and activity were upregulated in a time-dependent manner in primary astrocytes exposed to $A\beta_{1–42}$ oligomers and in reactive astrocytes from Tg2576 mice, a well-known transgenic model of AD (Boscia et al., 2017). Indeed, since several works implicated K^+ flux as a common trigger in regulating inflammasome formation, it is possible to speculate that the astrocytic overexpression of K_V3.4 in the cerebral cortex, hippocampus, and cerebellum of 6-month-old Tg2576 mice and the subsequent increase of K^+ efflux from these astrocytes may be involved in the astrocytic responses to amyloid pathology (**Figure 1A**). Importantly, astrocytes play a crucial role in mediating extracellular K^+ clearance through local K^+ uptake and spatial K^+ buffering, and in the removal of glutamate from the extracellular space, thus contributing to the regulation of neuronal excitability (Verkhratsky et al., 2010). Therefore, is it likely that astroglial failure occurring in the early stages of AD may drive to further neurodegenerative processes leading to synaptic malfunction and loss.

Since the K_V3.4 channel emerged from these findings as a crucial player in several pathogenic processes of AD, we performed a further study to provide a new knowledge about the pharmacological action of a well-known K_V3.4 inhibitor, the marine toxin BDS-I extracted from the *Anemonia sulcata* (Diochot et al., 1998; Pannaccione et al., 2007). In particular, we identified the key amino acidic residues of BDS-I that are essential for its inhibitory action on K_V3.4 channels (Ciccone et al., 2019), thus discovering a small peptide, namely BDS-I[1–8] containing the N-terminal octapeptide sequence of full length BDS-I, as a new K_V3.4 inhibitor (**Figure 1B**). In particular, BDS-I[1–8] is able to inhibit K_V3.4 currents in a concentration-dependent manner with a half-maximal inhibitory concentration value of 75 nM. Moreover, BDS-I[1–8] counteracts the $A\beta_{1–42}$-induced upregulation of K_V3.4 channel activity and subsequent caspase-3 activation in NGF (nuclear growht differentiated)-PC12 cells exposed to $A\beta_{1–42}$ oligomers (Ciccone et al., 2019). Further approaches are needed to elucidate the properties of BDS-I[1–8] and to test its stability and efficacy *in vivo*. Intriguingly, preliminary results obtained in our laboratory showed that K_V3.4 inhibition could be involved in the amelioration of memory performance in young Tg2576 mice, thus suggesting that BDS-I(1–8) could have beneficial effects on synaptic dysfunction and subsequent memory deficits in the early phases of AD. In conclusion, it is possible to think that this octapeptide could provide a potential therapeutic opportunity in AD as well as in other pathological conditions.

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