PERSPECTIVE

Galantamine protects against beta amyloid peptide-induced DNA damage in a model for Alzheimer's disease

Alzheimer's disease (AD) is the most common type of dementia in elderly population. With a growing aging population not only in the United States but also in the worldwide, AD constitutes an emergent public health problem. Over decades, the prevailing hypothesis was that neurodegeneration might result from one or two of the specific lesions that characterize AD, *e.g.*, accumulation of amyloid plaques (extracellular deposits of amyloid-beta peptide ($A\beta_{1–42}$) and hyperphosphorylation of tau protein. However, molecular mechanisms underlying the pathological lesions in AD are not clarified and the notion that amyloid plaques and phosphorylated tau are pathologic molecules is slowly changing, suggesting that soluble oligomers of Aβ, rather than insoluble in the amyloid plaques are the most toxic due to the induction of oxidative stress, which has emerged as an important event driving neurodegeneration (Anand et al., 2014). In addition, the $A\beta_{1–42}$ contributes to the impaired cholinergic neurotransmission which is a consistent features of AD. The role of cholinergic neurotransmission in memory processing and storage is the basis of the widely accepted cholinergic hypothesis and during the past three decades, acetylcholinesterase inhibition has become the most widely studied and four acetylcholinesterase inhibitors (AChEi), tacrine, donepezil, rivastigmina and galantamine have been approved for treating the symptoms of AD. These drugs provide symptomatic treatment but do not alter the course of the disease.

Galantamine commercialized under the name of Razadyne® is the most recently approved AChEi in many countries for AD patients at mild, moderate, and advanced moderate stages. Lately, its efficacy has also been observed in patients with AD at severe stage. The ability of this drug to cross the blood-brain barrier makes it suitable for AD patients, and unlike other AChEis, galantamine has a weak AChEi effect. Nevertheless, it has a dual mode of action, since it inhibits AChE and modulates allosterically both the nicotinic and muscarinic acetylcholine receptors (AChRs) to potentiate the sensitivity to acetylcholine (ACh); additionally, galantamine and some of its derivatives exert antioxidant activity which has been associated with the presence of enol group and quaternary nitrogen. The antioxidant activity of the molecule disappears after transformation of the enol group (galantamine) into carbonyl group (galantaminone, narvedine). The same effect is observed after transformation of the enol group of galantamine hydrobromide; the presence of quaternary nitrogen is not involved in the radical-scavenging action, but is responsible for the increasing of the strength of the scavenging effect (Tsvetkova et al., 2013). Furthermore, Galantamine modulates non-amyloidogenic processing of amyloid precursor protein and inhibits the aggregation and toxicity of Aβ. In summary, accumulating evidences demonstrate that galantamine exerts neuroprotection against $A\beta_{1–42}$-induced cell loss and neurotoxicity; nevertheless, antigenotoxicity studies were still missing to define the contribution of the drug to neuroprotection mechanism through regulation of DNA damage.

Different types of DNA damage including DNA double-strand breaks (DSBs), DNA single-strand breaks (SSBs), bulky adducts, abasic sites, crosslinking (interstrand and intrastrand), oxidation of specific bases (8-hydroxydeoxyguanosine), insertions and deletions are associated to neurodegenerative diseases (**Figure 1**). Consequently, cells deploy a diverse repertoire of mechanisms to maintain genetic integrity; however, with advanced age there is a decreasing at both antioxidant system and capacity of the cell to counteracting genotoxic stimulus. Unless repaired in an error-free process, DNA instability can result in mutations and altered cellular behavior (Pearl et al., 2015). The understanding of AD is continually changing; for instance, classical hallmarks of AD, earlier thought to be responsible for the disease development, now rather seem to reflect the damage suffered by neurons over a long time as cellular adaptive strategy to oxidative stress, and although the mechanisms are diverse, neuronal death is the inevitable event in AD (Anand et al., 2014).

The DNA damage responses are essential cellular mechanisms for maintaining the genomic integrity, and its disruption is one of the principal hallmarks of chronic and age-related diseases. Increased production of reactive oxygen species, such as H_2O_2 and NO• are generated by Aβ which may can impact different molecules such as proteins, lipids, RNA and DNA. Under this condition, the brain of AD patients is submitted to increased oxidative stress, coinciding with depletion of antioxidant defense system. Each cell in the human body receives tens of thousands of DNA lesions per day by a variety of sources; therefore, cells have evolved a multifaceted response to counteract the potentially deleterious effect of DNA damage. The cellular response to DNA damage involves execution of DNA repair and activation of a repertoire of DNA damage signaling (Narciso et al., 2016).

We recently assess the effects of galantamine on the cell toxicity and DNA strand breaks induced by $A\beta_{1–42}$ using a set of biomarkers such as clonogenic assay (a cell biology technique for studying the effectiveness of specific agents on the survival and proliferation of cells), cytokinesis block micronucleus cytome (a comprehensive system for measuring DNA damage, cytostasis and cytotoxicity) and comet assay (a sensitive technique for the DNA damage detection at the level of the individual eukaryotic cell) in SH-SY5Y human neuroblastoma cell line as *in vitro* model in neurotoxicity research. Consistent with previous studies, we reported that $A\beta_{1–42}$ (10 μM) for 24 hours decreased cell proliferation by inducing cell death by necrosis rather than apoptosis; additionally, $A\beta_{1–42}$ treatment had a stronger impact on genomic stability events compared to untreated control. In contrast, Galantamine post-treatments (0.1, 1.0 and 10 μM) significantly improved the rate of cell survival and exerted a high antigenotoxic activity by reducing $A\beta_{1–42}$-induced DNA damage. Interestingly, the effects of galantamine here reported are at a range of concentrations including blood concentration found in human after oral administration. Overall, our study provided the first experimental evidence indicating that Galantamine also contributes to the neuroprotection mechanisms through regulation of DNA damage in addition to AChEi activity (Castillo et al., 2016). However, there are many questions about DNA damage and cholinergic impairment in the AD brain that should be answered, including which specific mechanisms are involved in cellular changes associated with AD pathogenesis and progression; and which specific enzymes are involved in cellular changes associated with DNA damage.

The lack of a detailed picture of genome repair in the brain could be attributed to the fact that damage repair in the human brain genome can only be studied in postmortem samples, making it difficult to examine these processes individually in specific type cells. Nevertheless,

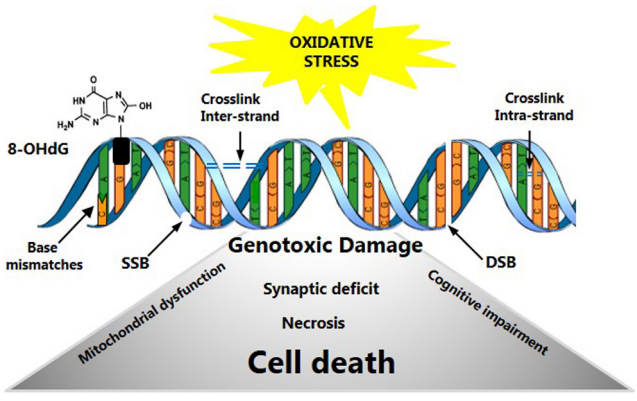

Figure 1 Types of DNA damage due to oxidative stress.
Oxidative stress causes several types of DNA damage, including double strand break (DSB), single strand break (SSB), oxidation of specific bases (8-hydroxydeoxyguanosine) and base mismatch. Defects in DNA repair mechanisms lead to genome instability and consequently to altered cellular behavior or cell death.

SH-SY5Y cells are classical model in neuroscience to understand some events associated with neurodegeneration and neuroprotection. In recent years, novel mechanisms linking DNA instability to neuronal dysfunction have been indentified; in this context, understanding into the molecular mechanisms connecting DNA damage to AD pathology may help to develop novel treatment strategies for patients.

A distinct feature of neurons is that replication associated with DNA repair cannot occur in differentiated, postmitotic cells, and the maintenance of genomic stability becomes a challenge for neuronal survival. Different studies in mice and cell lines have provided insight into the role of repair mechanisms in neurodegeneration and AD. Recently it has been shown that heterozygous mice for the DNA polymerase β, a critical DNA base excision repair enzyme accelerate synaptic and cognitive deficit, including neuronal dysfunction and cell death (Sykora et al., 2015). In addition, studies addressing the involvement of base excision repair (BER) pathways have shown that differentiated human SH-SY5Y cells are more sensitive to oxidative damage than their undifferentiated counterpart (Sykora et al., 2013); according to authors, the attenuated BER in postmitotic neurons seems to be correlated with the decreasing protein levels of long-patch BER components. Still, the literature is scarce and more efforts are needed to explore the relationship between oxidative stress, genotoxicity and repair mechanisms in the brain and its relationship with neurodegenerative diseases such as Alzheimer's disease.

With regard to antigenotoxic effect exerted by Galantamine, we speculate that it might be associated with its antioxidant properties which is supported by the fact that an antioxidant is able to prevent the DNA damage by stimulating certain repair enzymes and induce fidelity DNA repair and replication through an effect denominated bio-antimutagenic or acting as a desmutagenic agent that acts directly on mutagens, or on its precursor, in order to inactivate it. Taken together, our results demonstrated an important contribution regarding galantamine effects against $A\beta_{1–42}$-induced genomic instability. These observations are fully consistent with antioxidant properties of galantamine. It has been reported that drug reduced the release of reactive oxygen species and prevented the loss of mitochondrial activity. The capability of the drug to scavenge the reactive oxygen species has been associated with the enol group and the presence of the quaternary nitrogen in the molecule; in addition, the conversion of galantamine to galantamine hydrobromide is accompanied by a significant increase in the radical-scavenging effect (Tsvetkova et al., 2013).

Our study has reveled an important role of galantamine against $A\beta_{1–42}$-induced genetic instability. In this context, DNA repair mechanisms and modulation of genetic damages might be studied to gain knowledge about the antigenotoxic mechanisms exerted by Galantamine by analyzing pathways and main proteins that are being regulated by the drug.

Nowadays, there is still no effective treatment for AD and no new therapeutic drugs have been approved since 2003. However, several putative drugs have been thoroughly investigated in preclinical studies, but many of them have failed to produce promising results in the clinical scenario. We speculate that lack of effectiveness might be associated with the fact that these drugs do not take into account the genetic instability as potential mechanism in AD pathogenesis. Galantamine is a natural alkaloid, isolated from bulbs and aerial parts of plant from *Amaryllidaceae* family. Numerous studies have shown that bioactive compound belongs to a variety of phytochemical groups such as phenolics, pigments, allysulfides, glucosinolates, tannins, flavonoids and phytosterols are effective phyto-antigenotoxics in addition to their antioxidant, antimutagenic, anticarcinogenic, antoestrogenic, and antiinflamatory properties which might be beneficial in preventing diseases by improving genomic stability. In previous studies, *Amaryllidaceae* alkaloids such as galantamine, Lycorine, Homolycorine and Hemantamine have been shown by exerting a high antioxidant activity and AChEi (López et al., 2002, Castillo et al., 2017). Based on these findings and coinciding with antigenotoxic capacity of Galantamine, it is possible that the modulation of DNA damage exerted by *Amaryllidaceae* alkaloids may represent an important biological property in addition to AChEi activity. We anticipate results obtained in our laboratory, which showed AChEi and antigenotoxic activities of crude extract from a plant belonging to *Amaryllidaceae* family against $A\beta_{1–42}$-induced cytotoxicity and genotoxicity in SH-SY5Y cells when evaluated trough a set of genotoxic and cytotoxic biomarkers. The results showed that $A\beta_{1–42}$ significantly inhibited cell viability through necrosis rather than apoptosis, increased the DNA damage and exerted mitochondrial morphological alterations; however, treatments with the extract led to a significant recovery of cell survival, decreased necrotic cell death and also exerted antigenotoxic effects; additionally, the extract showed an inhibitory activity of AChE (submitted paper). Overall, the modulation of neurodegeneration by agents with antigenotoxic properties might emerge as a new avenue for the development of interventions that may improve the neural defense response against neurodegeneration.

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