

● PERSPECTIVE

Targeting the guanine-based purinergic system in Alzheimer's disease

Alzheimer's disease (AD) is the main cause of dementia worldwide and affects approximately 5% of people with 65 years or older. The estimated increase in the elderly population suggests that cases of AD will rise in the next years. AD is a neurodegenerative disease characterized mainly by synaptic and neuronal loss. *Post-mortem* analysis of AD brains revealed the presence of senile plaques and neurofibrillary tangles. Tangles are mainly composed by hyperphosphorylated tau protein and senile plaques contain aggregates from amyloid- β ($A\beta$) peptides. $A\beta$ peptides are originated after misprocessing of the amyloid precursor protein that leads to generation of toxic peptides with 40 ($A\beta_{1-40}$) or 42 ($A\beta_{1-42}$) amino acids (Selkoe, 2001; Karran and De Strooper, 2016). Although recent studies have reported that the oligomeric forms of $A\beta$ are more toxic (Klein, 2013), aggregated forms of $A\beta$ peptides are used to induce toxicity and represent an important strategy for understanding AD-related processes.

In 2004, it was reported that guanosine – the guanine-based nucleoside – inhibited the apoptosis induced by $A\beta_{25-35}$ (a synthetic and toxic form of $A\beta$ peptides) in a neuroblastoma cell line, SH-5YSY (Pettifer et al., 2004). Later, another study showed that guanosine also inhibited the apoptosis and the increase in reactive oxygen species induced by $A\beta_{1-42}$, and remarkably decreased the extra and intracellular contents of $A\beta$ peptides. This effect was probably due to the decrease in β -secretase activity induced by guanosine in this cell culture (Tarozzi et al., 2010).

Guanosine is recognized as a neuromodulator with several *in vitro* and *in vivo* biological effects, and protects neural cells against glutamatergic toxicity (Lanznaster et al., 2016a). In the brain, guanosine can act as an intercellular signaling molecule, being released from astrocytes in physiological and pathological conditions (for example, after an ischemic event). Guanosine is also involved in the modulation of glutamatergic transmission, mainly through promoting a decrease in glutamate release and an increase in glutamate uptake in excitotoxic events (Schmidt et al., 2007; Lanznaster et al., 2016a). Therefore, a neuroprotective role has been attributed to guanosine, although its exact mechanism of action is not completely understood.

We recently reported that guanosine displayed a protective role in an *in vivo* AD-like mouse model. Mice received one single intracerebroventricular infusion of aggregated $A\beta_{1-40}$ peptide (400 pmol/site) and presented short-term cognitive deficit (16 days after $A\beta_{1-40}$ infusion) and an anhedonic-like behavior (20 days after $A\beta_{1-40}$ infusion) that were prevented by guanosine treatment. These effects were accompanied by a recovery in glutamatergic transmission impairment in the hippocampus caused by $A\beta_{1-40}$, mainly observed in glutamate uptake. Interestingly, high-performance liquid chromatography (HPLC) analysis showed an increase in hippocampal adenosinetriphosphate (ATP) and adenosine diphosphate (ADP) content caused by $A\beta_{1-40}$, while $A\beta_{1-40}$ inhibited guanosine-induced increase in GDP (Lanznaster et al., 2016b). This is the first demonstration of guanosine effect over a mouse model of AD and the first analysis of hippocampal purinergic content in an AD-like mouse model (Figure 1). These results add to the recent consensus that purinergic system, mainly through guanosine, could represent an important target for the treatment of neurodegenerative conditions like AD.

AD and other dementias are the top causes of disabilities worldwide, and social and economic costs with AD are incredibly high. Cognitive deficit and memory loss – the main symptoms in AD – severely impair quality of life and are often accompanied by mood-re-

lated symptoms, as depression. *Post mortem* analysis of patients with depression and AD patients revealed a reduction in hippocampal volume, a brain area deeply involved with memory formation. In animal models of AD, we can observe memory impairment (with simple tests that require the hippocampus) and mood-related behaviors, like anhedonia. In our study, we used two tests to assess memory impairment in mice: the novel location test and the Y-maze test. In the first test, we found that $A\beta$ -treated animals spent an equal amount of time exploring the old and the relocated object, thus implying that they did not recognize the old object as a familiar one. Importantly, guanosine prevented this effect in $A\beta$ -treated animals, as animals spent more time exploring the relocated object. Interestingly, we did not observe any cognitive impairment in $A\beta$ -treated animals in the Y-maze test. This lack of effect could be explained by the fact that this task evaluates working memory that is more related to the prefrontal cortex function, but more studies need to be done to clarify this point. In the regard of anhedonia-like behavior, guanosine prevented the increase in the latency to grooming after a splash of sucrose solution in the dorsum of mice. Indeed, the antidepressant effect of guanosine was showed before (Bettio et al., 2012). Importantly, the anti-anhedonic-like effect induced by guanosine in our study was observed after 4 days without any guanosine treatment (4 days of washout), suggesting a persistent antidepressant effect of guanosine.

Impairment in glutamatergic transmission is linked to several neurodegenerative conditions, including AD, and $A\beta$ peptides are known to impair glutamatergic transmission, increasing glutamate release and decreasing its uptake, causing a neurotoxic effect known as glutamatergic excitotoxicity. We showed that glutamatergic transmission is impaired in hippocampal slices from $A\beta$ -treated mice: there was a slight increase in glutamate release, and, in the regard of glutamate uptake, $A\beta$ increased Na^+ -independent uptake. Na^+ -independent uptake is mainly performed by X_C^- system, the cystine-glutamate transporter that releases glutamate and provide cystine for cysteine synthesis in the intracellular space. In our study, we showed that guanosine prevented the alterations in glutamatergic transmission induced by $A\beta$, including the alteration in Na^+ -independent uptake. Our findings are in agreement with a study from Albrecht et al. (2013), where they showed that guanosine could regulate X_C^- activity after an *in vitro* glutamate challenge.

Guanosine release from astrocytes was shown to occur in basal conditions and, importantly, after ischemic events – in concentrations much higher than the adenine-based nucleoside, adenosine. Although adenosine is the best-known purine, its neuroprotective effect is hampered by its main side effect: adenosine induces a significant decrease in arterial and blood pressure, ending all possibilities for adenosine application in clinic. Guanosine, on the other hand, does not produce such side effects (Jackson and Mi, 2014). Of course, more studies need to be done to set guanosine as a therapeutic agent for neurodegenerative diseases. However, one fact needs to be taken into consideration: guanosine is an endogenous molecule with important biological and neuroprotective effects, but little effort has been made to properly identify and characterize the “guanosinergic system”, as science has been doing for so long with adenosine.

Our research group is putting effort in this field, as we have identified some membrane proteins that may be targets for guanosine action. Guanosine effect over glutamatergic transport is well recognized, and we showed that synthetic inhibitors of glutamate transporters abolished guanosine-induced decrease in glutamate release (Dal-Cim et al., 2016), although a direct interaction of guanosine with glutamate transporters was still not reported. Adenosine receptors-containing oligomers, mainly adenosine A_1 receptor (A_1R)/adenosine A_{2A} receptor ($A_{2A}R$) heteromers could represent another site of action for guanosine. Guanosine modulates both A_1R - and $A_{2A}R$ -linked intracellular signaling cascades when the receptors are expressed in the heteromeric configuration, and guanosine-induced neuroprotective effect against an *in vitro* ischemia model is not observed in $A_{2A}R$ -knockout mice (unpublished data). Finally, high-conductance calcium-activated potassium (BK) channels blockade inhibits guanosine neuroprotective effect, and whole-cell recordings in human embryonic kidney

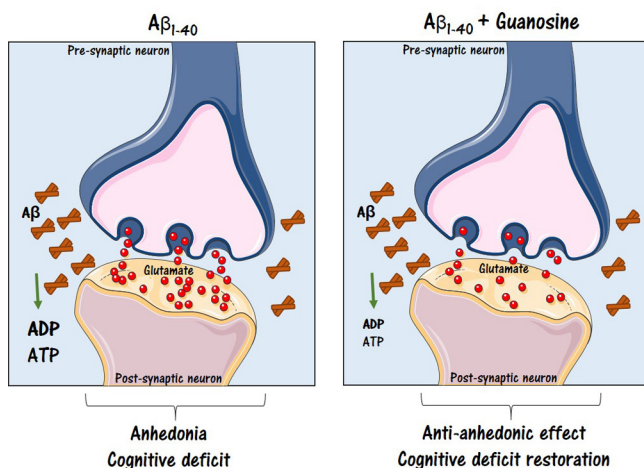


Figure 1 Guanosine prevents the anhedonic-like behavior and the cognitive deficit induced by intracerebroventricular infusion of A β_{1-40} in mice.

We showed that A β_{1-40} impairs glutamatergic transmission in mice hippocampus and increases the content of ADP and ATP. Guanosine treatment (8 mg/kg, i.p.) for 14 days was able to prevent these hippocampal alterations induced by A β_{1-40} (Lanznaster et al., 2016b). This figure was produced using Servier Medical Art (<http://www.servier.com>). A β : Amyloid- β ; ADP: adenosine diphosphate; ATP: adenosinetriphosphate.

293 (HEK293) cells transfected with the functional subunit of BK channels showed that guanosine promoted an increase in potassium conductance in transfected cells (Lanznaster et al., 2016a). Therefore, the exact protein targets and signaling pathways involved in the mechanisms of action of guanosine remain to be identified (**Figure 2**).

In addition to the substantial neuroprotective effect induced by guanosine, several studies reported other biological effects such as neurotrophic, anti-inflammatory, antinociceptive and beyond (Lanznaster et al., 2016a). Our study regarding the effects of guanosine in an AD-like mouse model adds important information to the literature, but additional studies are necessary to unravel the potential therapeutic effect of guanosine in neurodegenerative diseases like AD. It is time to put guanosine in the spotlight.

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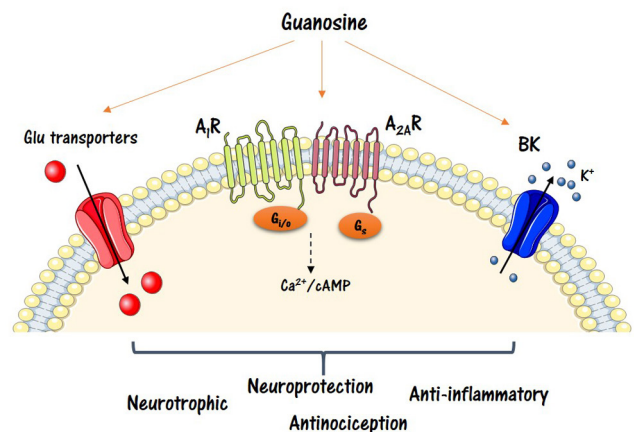


Figure 2 Guanosine presents neuroprotective, neurotrophic, anti-inflammatory and anti-nociceptive effects.

The guanosine receptor was still not reported, but several membrane proteins are involved with guanosine effects and may represent guanosine targets. Guanosine modulates glutamate (Glu) transporters activity, promoting glutamate uptake and decreasing glutamate release. Guanosine also modulates intracellular pathways (as calcium (Ca²⁺) and cyclic adenosine-monophosphate (cAMP) increase) associated with adenosine receptors (A₁R/A₂AR) heteromers interaction. Guanosine increases high-conductance calcium-activated potassium channels (BK) conductance in human embryonic kidney 293 (HEK293) cells (Dal-Cim et al., 2016; Lanznaster et al., 2016a). This figure was produced using Servier Medical Art (<http://www.servier.com>).

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