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Antioxidative properties of galantamine on neuronal damage induced by hydrogen peroxide in SK–N–SH cells

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Abstract

Galantamine, an acetylcholinesterase inhibitor used to enhance memory in AD patients by acetylcholinesterase inhibition, has been tested for its protective properties on an *in vitro* model of H_2O_2-induced oxidative stress. SK–N–SH cells treated with H_2O_2 for 2 h showed an increase in ROS production (54%) and in NO production (52%) together with a marked reduction of the mitochondrial membrane potential (19%). These features, typical of the oxidative injury that accompanies AD, were partly recovered by galantamine. Galantamine reduced the release of reactive oxygen species (up to 50%) and prevented loss in mitochondrial activity. When SK–N–SH cells were treated with H_2O_2 for 24 h, nitrite generation was increased by twice compared with 2 h. Galantamine treatment resulted in a significant inhibition of H_2O_2-induced nitrite generation whatever the concentration tested with a return to control values. Galantamine also concentration-dependently inhibited AChE activity (28–88%) in H_2O_2–SK–N–SH cells after 24 h. This drug, which facilitates cholinergic neurotransmission, is also neuroprotective by lowering oxidative injury. Our study provides a better understanding of the mechanisms of protection of this acetylcholinesterase inhibitor which also has antioxidative properties.

Keywords: Acetylcholinesterase inhibitor; Galantamine; Nitric oxide; Reactive oxygen species; Oxidative injury

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder causing progressive impairment of memory and cognitive function. AD is also characterized by the presence of abnormal deposition of β-amyloid protein (Aβ) to form senile plaques and neurofibrillary tangles and by extensive loss of neurons. This neuronal damage has been regarded as a critical factor involved in the loss of memory and cognitive impairment in AD (Samanta et al., 2006; Tuppo and Arias, 2005). Although the pathogenesis of AD remains unknown, several studies suggested that free radicals were involved as causative factors of the inflammatory injury and oxidative stress which have been implicated in this chronic neurodegenerative disease (Klein and Ackerman, 2003; Lipton et al., 2007; Moreira et al., 2005). Increased production of reactive oxygen and nitrogen species, such as hydrogen peroxide (H_2O_2) and nitric oxide (NO) are generated by Aβ in microglial cells (Butterfield and Lauderback, 2002; Casal et al., 2002) and lead to neuronal cell death (Bal-Price and Brown, 2001). Coinciding with a depletion of antioxidant defences, generated radicals can attack proteins, deoxynucleic acid and lipid membranes, thereby disrupting cellular function and integrity leading to mitochondrial damage. These effects are commonly referred to as ''oxidative stress'' (Hensley et al., 1998). They implicate that the brain of AD patients is under increased oxidative stress (Behl, 1999; Small et al., 2007). Thus, neuronal oxidative damage is a major effector of neurodegeneration.

Therapeutic strategy aimed at removal of free radicals or prevention of their formation might be beneficial in AD.

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Although the neuroprotective effects of antioxidants have been established (Floyd, 1999) and are thought to be due to the removal of reactive oxygen species (ROS) or peroxinitrite, no prevention is known and no curative treatment available. However, because deficits in cholinergic neurotransmitter system have been observed with a resulting acetylcholine level deficiency (Kasa et al., 1997; Oddo and LaFerla, 2006), in addition to the accumulation of amyloid in AD brains, current efforts have been made to develop treatments which phamacologically palliate the cognitive impairments. Acetylcholinesterase inhibitors (AChEIs) which maintain cognitive function at a constant level (Akaike, 2006; Cheng and Tang, 1998; Doody, 2003; Hogan et al., 2004) show the most encouraging results for increasing cholinergic therapy, e.g. tacrine, galantamine, donepezil and huperzines. These AChEIs have been reported to reduce cognitive impairments both in animal models of memory deficiency and in AD patients (Giacobini, 1998; Hogan et al., 2004; Jordá et al., 2004). These AChEIs which have not the same potency to block the enzyme also have a different pharmacological profile but all reduce the ongoing neuronal damage (Arias et al., 2005; Lleo et al., 2006). Several studies have shown that synaptic loss in the cerebral cortex and hippocampus was the major reason for cognitive decline in AD (Law et al., 2001; Mulder et al., 2005; Wang et al., 2004). Indeed, AChE activity present in the brain has been found associated with senile plaques (Mattson, 2004 for review) and the subsequent neuronal damage.

Neuroprotective properties of AChEIs have been demonstrated through various mechanisms. For example, galantamine was recently shown to prevent apoptotic cell death by inducing activation of nicotinic acetylcholine receptors (nAChRs) and up regulation of Bcl-2 (Arias et al., 2004; Coyle et al., 2007; Hansen and Taylor, 2007; Wang et al., 2007). A tacrine derivative, Bis(7)-tacrine, with higher AChE inhibition and memory enhancement potency than tacrine (Li et al., 2005; Liu et al., 2000; Pang et al., 1996; Wang et al., 2003) protects against ischemia-induced injury and hydrogen peroxide-induced apoptosis in brain cells (Wu et al., 2000; Xiao et al., 2000). It also prevents glutamate-induced neuronal apoptosis by directly blocking NMDA receptors (Fu et al., 2007; Li et al., 2004, 2007; Luo et al., 2007), and so do huperzines which have emerged as a novel class of potent AChEIs (Wang and Tang, 2005). The majority of these AChEIs may also modulate the processing of amyloid precursor protein (Lenzken et al., 2007; Nordberg, 2006).

Because oxidative stress is widely implicated in AD, we hypothesized that oxidative injury could affect the neurons leading to a loss of integrity of the cholinergic system. The aim of the present study was thus to evaluate oxidative injury status in a neuronal model and its relation with AChE inhibition. For this purpose, we tested a well-known AChEI, galantamine which is as effective as tacrine in AD treatment but with lower toxicity, in a human neuroblastoma cell line (SK–N–SH) with H_2O_2 as inducer of oxidative stress. Oxidative injury and cytotoxicity were assessed by measuring ROS, mitochondrial activity and NO release using fluorescent or non-fluorescent probes. The dual mechanisms of action of this drug are discussed.

2. Materials and methods

2.1. Materials

Galantamine, H_2O_2, foetal calf serum (FCS), calcium- and magnesium-free PBS, culture medium (DMEM) and additional medium supplements were purchased from Sigma (France). Probes were ordered from Molecular Probes (Leiden, The Netherlands). All other chemicals were of the highest grade of purity and commercially available. Stock solutions of galantamine were prepared in methanol and further diluted in DMEM. The final methanol concentration in the culture medium was 1:100 (v/v). All control cells were incubated with the same concentration in vehicle.

2.2. Cell culture and treatment

The SK–N–SH cell line (ECACC, Sigma) was grown in medium (DMEM) supplemented with 10% Fetal calf serum, 1% penicillin/streptomycin in a 5% CO_2–95% O_2 atmosphere at 37 °C. Cells were seeded in 96-multiwell dishes at a density of 100,000 cells/ml (100 μl in each well) for cytotoxicity and fluorescence detection or in six-well dishes (1 ml in each well) for measuring NO production and acetylcholinesterase (AChE) activity. Cells were treated with the drugs in serum-free DMEM. Experiments were carried out 24–48 h after cells were seeded. Cells were exposed for 2 h, either to H_2O_2 (500 μM), or galantamine alone (0.1–100 μM), or to the combination of H_2O_2 plus galantamine. As no change in AChE activity was seen after 2 h, SK–N–SH cells were also incubated with galantamine for 24 h. Together with AChE activity measurement, it was decided to check one of the oxidative stress markers and NO production was chosen. The comparison of effects was performed at a range of concentrations including blood concentration found in humans after oral administration (around 5 μM) (Mannens et al., 2002). H_2O_2 was used in the usual range of concentrations and duration of exposure (Li et al., 2003; Ye et al., 2007; Zhang and Tang, 2000). LPS (1 μg/ml) was used as a positive control.

2.3. Cytotoxicity and membrane integrity assessment

SK–N–SH cells were incubated either with H_2O_2 or H_2O_2 plus galantamine in DMEM without FCS for 2 h. Cytotoxicity was assessed by colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue; MTT), following incubation. The plates were read at 535 nm on a spectrophotometer (BIO-TEK Instruments Elx 808). Results for the dose-response cytotoxicity assays are expressed as a percentage of the controls and IC_{50} values were calculated.

2.4. Chromatin condensation assay; Hoechst 33342

Hoechst 33342 (Molecular Probes) is a noncytotoxic DNA dye that permits the determination of the total chromatine quantity variations and the degree of chromatin condensation (Maciorowski et al., 1998). It preferentially binds to triplet