Antioxidant Properties of Galantamine Hydrobromide

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The antioxidant properties of galantamine hydrobromide ((4α,6β)-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol hydrobromide) were studied in vitro, using luminol-dependent chemiluminescence and spectrophotometry. It was found that this compound was a scavenger of reactive oxygen species (ROS). By comparing the antioxidant effects of galantamine ((4α,6β)-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol), galantamine hydrobromide, narwedine (4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-one), and narwedine hydrobromide it was found that the antioxidant activity depended on the enolic OH group in the molecule. The presence of a quaternary nitrogen in the compound increased the strength of the scavenging effect. It is proposed that the antioxidant properties observed in vitro may contribute to the therapeutical effect of galantamine hydrobromide on patients with brain degeneration.

Key words: Galantamine Hydrobromide, Narwedine, Antioxidant Properties

Introduction

Galantamine hydrobromide is traditionally used in the stimulation of the synaptic acetylcholine–mediated neurotransmission (Santos et al., 2002). During the past few years it has been tested as a drug against the most common cause of dementia – the Alzheimer’s Disease (Ventura and Sterron, 2001; Schneider, 2002). As a result the quality of life of patients with mild to moderate AD symptoms remarkably improved (Rockwood et al., 2001; Wilkinson and Murray, 2001). Therefore the opportunity to switch from treatment with other anti-AD drugs to galantamine is discussed (Getios et al., 2001; Gasser and Gasser, 2001).

Latest clinical trials (Pitchumoni and Doraoway, 1998; Tuppo and Forman, 2001) and in vitro research (Arlt et al., 2001) supported an increasing evidence that free radical-induced oxidative damage play a role in the pathogenesis of AD (Pitchumoni and Doraoway, 1998; Babior, 2000; Raha et al., 2000; Blass, 2001; Halliwell, 2001; Ozcankaya and Delibas, 2002; Xie Z. et al., 2002). The brain is especially sensitive to oxidative damage because of its high content of easily oxidized fatty acids, high use of oxygen, and low levels of endogenous antioxidants (Rokyta, 1996; Tuppo and Forman, 2001; Behl and Moosmann, 2002). Any substance capable of penetrating the blood-brain barrier, should affect the brain degeneration by control on the oxidative stress. Therefore, the antioxidant properties of drugs used in the therapy of the...
central nervous system (CNS) are of great importance.

The aim of the present investigation is to measure in vitro the antioxidant properties of galantamine hydrobromide (Gal. HBr), and to examine the influence of the molecular structure on the interactions with reactive oxygen species.

Materials and Methods

Materials

Galantamine, galantamine hydrobromide, narwedine and narwedine hydrobromide were synthesized and purified (Krikorian et al., 2000). Analyses of the samples by infrared spectroscopy, nuclear magnetic resonance and mass spectroscopy, showed a 98–99% content of the desirable products and 1–2% water. The polarimetric analysis indicated 95% L-component in the racemic mixtures.

Sodium hypochlorite and most of the other reagents used were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and were of finest grade.

KO$_2$ was dissolved in anhydrous dimethylsulfoxide (Aldrich-Chemie, Steinheim, Germany) in a concentration of 1 mm. The solution was stored in nitrogen and was used not later than 2 h after preparation.

The chemiluminescence reagent was prepared by dissolving luminol in a small amount of 0.01 m NaOH. Then the solution was diluted to a luminol concentration of 1 mm with a 50 mm phosphate buffer solution (PBS), and the pH was adjusted to 7.4 with 0.01 m HCl.

The compounds tested were dissolved in PBS, pH 7.4 to concentrations of 1, 10 and 100 µm and their effect on the luminol-dependent chemiluminescence (CL) and on the reduction of Nitroblue Tetrazolium to formazan (NBT test) was examined.

The reagent for NBT test was prepared by dissolving Nitroblue Tetrazolium (0.04 mm), EDTA (0.005 wt%) and Na$_2$CO$_3$ (0.008 wt%) in PBS (pH 7.4).

Methods

The luminol-dependent chemiluminescence (CL) was used for registration of ROS (Allen, 1975; Allen and Loose, 1976), with an LKB 1251 luminometer (Bioorbit, Turku, Finland) set at 310 K, and connected with an AT-type computer.

Data collection was performed by MultiUse program version 1.08 (Bioorbit, Turku, Finland). Three types of CL assays were used. The ratio of CL in the presence and absence of the compound investigated was named CL scavenging index (CL-SI) and presented in percent. To compare the results for different compounds and different assays the concentrations of the compounds causing 50% ($C_{50}$) decrease of the parameters (CL-SI) were calculated.

By measuring the reduction of Nitroblue Tetrazolium to formazan any possible interactions of the molecules with the luminol and/or the light emitted were monitored. The intensity of the absorbance of a sample divided by the intensity of the absorbance of the control sample, is presented in percent; this is the spectrophotometrical scavenging index (SPh-SI).

**Assay I: Luminol-dependent CL in the system of potassium superoxide (KO$_2$)-produced superoxide**

The assay was carried out using 1 ml samples of PBS, pH 7.4, containing 0.1 mm luminol and the drug (in control sample, drug was omitted). The CL was measured immediately after the addition of 20 µl KO$_2$ solution. In this case the release of the superoxide is a fast process. Therefore CL was measured using the "flash assay" option of the MultiUse program every 50 msec.

**Assay II: Luminol-dependent CL in the system of iron-dependent hydroxyl radical formation**

One ml samples of PBS, pH 7.4, containing: 0.1 mm luminol, 0.1 mm Fe$^{3+}$ (FeCl$_3$), 0.1 mm EDTA, 0.1 mm ascorbate, 1 mm H$_2$O$_2$ and any of the tested drug at concentrations between 1 and 100 µm, or a buffer for the controls. The CL was measured using the "flash assay" option of the MultiUse program, every 50 milliseconds.

**Assay III: Luminol-dependent CL in the system of NaOCl-generated HClO**

The sample contained the following substances in 1 ml PBS: 0.1 mm luminol 0.06 mm NaOCl and the tested drug at concentrations between 1 and 100 µm, or a buffer for the controls. The chemiluminescence was registered after addition of
NaOCl using the “flash assay” option of the MultiUse program, every 50 milliseconds.

Assay IV: Reduction of NBT to formazan in the presence of K₂O-generated \( \cdot \text{O}_2^- \) radicals

The inhibitory effect of each synthesized compound on the reduction of NBT to formazan in presence of \( \cdot \text{O}_2^- \) was tested using Perkin-Elmer 552 UV-VIS spectrophotometer, which was connected to a PC. For this purpose, in a 1-ml sample vessel we introduced 20 \( \mu \text{l} \) 1 mm solution of KO₂ in dimethylsulfoxide and 980 \( \mu \text{l} \) of 50 mm PBS, pH 7.4, containing 0.04 mm NBT and the compound to be tested. The reaction mixture was vigorously mixed after the addition of the KO₂ solution and the absorbance at 560 nm measured. Five measurements of the compounds at each concentration were performed to calculate the experimental errors. The experiments were performed as it is described in Traykov et al. (1997).

Results and Discussion

The measurements of the luminol-dependent chemiluminescence in presence of \( \cdot \text{O}_2^- \), \( \cdot \text{OH} \) and HOCl showed that galantamine hydrobromide is an antioxidant with strong influence of the concentration on the CL-SI values (Fig. 1). The spectrophotometric NBT-test proved that the effect of Gal. HBr on CL was not related to an interaction with exited luminol and/or with the light emitted. The calculated \( C_{50} \) values were 15 ± 2 \( \mu \text{m} \), 83 ± 12 \( \mu \text{m} \) and 25 ± 4 \( \mu \text{m} \) for \( \cdot \text{O}_2^- \), \( \cdot \text{OH} \), and HOCl, respectively. Apparently galantamine hydrobromide is a scavenger of \( \cdot \text{O}_2^- \), \( \cdot \text{OH} \) and HOCl. The values of \( C_{50} \) suggested that the strength of the radical-scavenging effect decreased in the order \( \cdot \text{O}_2^- > \text{HOCl} > \cdot \text{OH} \).

Antioxidant activities of four compounds: galantamine, narwedine, galantamine hydrobromide and narwedine hydrobromide were assayed. Galantamine (Fig. 2, structure 1) and narwedine (structure 2) were identical except for the enol group in galantamine transformed into a carbonyl group in narwedine. The corresponding hydrobromides (Fig. 2, structures 3 and 4) showed the same difference. The difference between molecules of the galantamine (structure 1) and galantamine hydrobromide (structure 3) is in the partial charge, coordination symmetry and bonding of the nitrogen. The same difference shows up between narwedine (structure 2) and its hydrobromide (structure 4). The radical-scavenging properties were compared by measuring the luminol-dependent chemiluminescence in a system containing Fe³⁺-EDTA, H₂O₂-generated \( \cdot \text{OH} \) radicals with 1, 10 and 100 \( \mu \text{m} \) PBS solutions of these compounds (Fig. 3). The antioxidant activity of the molecule disappeared after transformation of the enol group (galantamine) into a carbonyl group (nar-
The radical-scavenging property of galantamine hydrobromide was observed in vitro. The value of this result is that it enables one to investigate the possibility of direct antioxidant effect of the compound in vivo. The fact that such effect could be feasible (as shown in the in vitro testing) does not mean that it actually occur in vivo, and further studies are underway to examine this question.

Another important question is whether the concentrations at which the galantamine hydrobromide exerts antioxidant effects are relevant to the concentrations present in vivo. Taking into account the pharmacokinetics of galantamine hydrobromide (Michailova et al., 1989) and its optimum therapeutical dose (between 16 and 24 mg) for AD patients (Wilkinson and Murray, 2001; Tariot, 2001; Lilienfeld, 2002), concentrations of about 10–15 µM of the drug should be expected in the blood plasma of the average adult person (Diem et al., 1973; Lambev et al., 2002). In addition, the ability of lipophilic drugs to concentrate within hydrophobic regions, such as the interior of membranes, must not be ignored too. It may be expected that the concentration range of galantamine hydrobromide used at the present in vitro investigation (1–100 µM) is probably close to the range of the possible therapeutical concentrations achieved in the clinically treated AD patients.

As the antioxidant activity was related to the enol group of the galantamine and galantamine hydrobromide, any chemical transformation of the OH group should affect the ability of the resulting compound to scavenge the ROS. If a substantial antioxidant effect of galantamine hydrobromide is proved in vivo, the enol group should be kept intact during the synthesis of new drugs based on galantamine.

Finally, if an in vivo effect is proved, other possible aspects of the antioxidant activity of the galantamine hydrobromide (such as inactivation of the enzyme systems implicated in the production or elimination of the free-radicals, control on phagocytosis, etc) should be investigated too.

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