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Brain Research 1066 (2005) 10 – 15

BRAIN RESEARCH

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Research Report

Antisense inhibition of acetylcholinesterase gene expression for treating cognition deficit in Alzheimer's disease model mice

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Accepted 24 September 2005
Available online 9 December 2005

Abstract

To examine whether the selected antisense oligodeoxynucleotides (AS-ODN) targeting against human brain acetylcholinesterase (AChE) mRNA could improve the cognitive deficit in the Alzheimer's disease (AD) model mice induced by amyloid-β peptide (Aβ), we determined the time–effect relationship of AChE activity and the learning and memory after AS-ODN delivery. The results showed that the AChE activity decreased gradually along with time, initiating at 8 h and lasting 42 h. The time–effect curves of acetylcholine (ACh) behaved consistency with that of AChE activity. The animal cognition studies showed that in step-through test, the error number of the AS-ODN-treated AD model mice was significantly decreased, and the memory retention was increased. In the water maze performance, the swimming time obviously shortened. Our results indicated that antisense therapy is of potential use in the treatment of cognitive deficit in the Aβ model mice.

Theme: Sensory system
Topic: Pain modulation: pharmacology

Keywords: Antisense oligodeoxynucleotide; Acetylcholinesterase; Alzheimer's disease; Amyloid-β peptide

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that results in loss of memory and cognitive function. The cholinergic theory of AD suggests that the selective loss of cholinergic neurons in AD results in a relative deficit of cholinergic neurotransmitter acetylcholine (ACh) in specific regions of the brain that mediates learning and memory functions and require ACh to do so. The primary approach to treating AD has therefore aimed to augment the cholinergic system [3,20]. Acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7, AChE) is the enzyme responsible for catalyzing hydrolysis of the ACh in the brain. Reduced levels of ACh in the brains of AD patients leave a relative excess of AChE. Pharmacological inhibition AChE has been the most successful strategy to date for reinforcing cholinergic neurotransmission in AD patients [16]. Indeed, the only currently approved drugs for AD are potent AChE inhibitors [11].

However, anti-AChE therapies for AD require high dose of drug and produce side effect associated with systemic cholinergic toxicity. Tacrine, for example, has been associated with liver damage and blood disorders in some patients. Furthermore, current AChE inhibitors interact non-specifically with the AChE homologous serum protein butyrylcholinesterase (BChE) and stimulate regulatory feedback pathways leading to enhanced expression of AChE [13]. Recently, short antisense oligodeoxynucleotides (AS-ODN) were reported to target specific brain AChE mRNA sequences [17]. In this paper, one of the AS-

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0006-8993/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.brainres.2005.09.063

ODN was tested for the efficacy on improving cognition in AD model mice.

2. Materials and methods

2.1. Materials

Lipofectamine™ 2000 was purchased from Invitrogen. Amyloid-β peptide (Aβ, fragment 22–35) was from Sigma. AMV reverse transcriptase was from Promega. Sense and antisense ODNs were synthesized by Bioasia Co. Ltd. (Shanghai, China). The ODN employed was phosphorothioated AS-ODN (5′ ACGCTTTCTTGAGGC 3′), designed to complement the AChE mRNA starting at position 1064, where the mRNA sequences of human and mouse are similar. Phosphorothioated sense oligodeoxynucleotides (S-ODN, 5′ CGGAGTTCTTTCGCA 3′) was synthesized as negative control. ODNs were diluted by PBS and mixed with Lipofectamine™ 2000, incubated for 15 min at 37 °C at a final concentration of 10 μg/ml and ready for injection. All chemicals were of reagent grade.

2.2. Animals

Healthy mice, Kunming species, male, aged 3–4 weeks and weighing 20 ± 1 g at the beginning of the experiments, provided by the Animal Breeding Center Affiliated to AMMS, China, were used throughout the study. Animals were housed in plastic cages, with free access to standard laboratory food and water, and kept in a regulated environment (23 ± 1 °C) under a 12-h light/dark cycle (light on at 8:00 am). Aβ-model mice were made according to the method of Maurice et al. [15]. Briefly, Aβ (22–35) was dissolved in sterile saline (vehicle) at a concentration of 1 mM, sealed and incubated for 96 h at 37 °C to transform Aβ into an "aggregated phase". The unilateral intracerebroventricular (i.c.v.) administration of Aβ was performed according to the procedure established by Laursen and Belknap [12]. Each mouse was injected at bregma with a 50-μl Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.4 mm. The injection volume was 4 μl (equivalent to 4 nmol of Aβ). The memory and cognition tests were performed since the 11th day after the i.c.v injection.

2.3. Animal group assignation

The mice were divided into 5 groups at random: (1) Aβ-model: i.c.v 4 nmol (in 4 μl) aggregated Aβ preparation; (2) Aβ+ AS-ODN group: Aβ-model mice, i.c.v injected (4 μl) AS-ODN (5 μl) 24 h before the test; (3) Aβ + S-ODN group: Aβ-model mice, i.c.v. injected S-ODN (5 μl) 24 h before the test; (4) huperzine A group: Aβ-model mice, intraperitoneal (i.p.) injection huperzine A (Hup, 0.1 mg/kg) 40 min before the test; and (5) control: normal mice, i.c.v. injected sterile saline.

2.4. Histological analysis

After decapitation of mice, the cerebral cortices were dissected and placed in 10% (vol/vol) formalin solution and processed routinely by embedding in paraffin. Tissue sections (10 μm) were stained with hematoxylin and eosin (HE). An experienced histologist who was unaware of the treatment conditions made histological assessments.

2.5. AChE activity measurement

Mice were killed by decapitation, the cerebral cortices were dissected after perfused with ice-cold saline and kept at −70 °C ready for use. Ten percent (1:10, w/vol) cerebral homogenates in cold saline were prepared in ice bath. The homogenates were centrifuged, and the supernatants were used for determination of AChE activity by spectrophotometric method [5,21], using acetylthiocholine iodide (ATCh-I) as substrate. The rate of hydrolysis was measured at the extent of light absorption (wavelength 412 nm). All reactions ran at room temperature in the presence of a butyrylcholinesterase inhibitor (ethopropazine, 10^{-4} M). Protein concentration was determined according to Lowry et al. [14].

2.6. Assay of ACh

ACh was determined by using the method of Hestrin [22]. Briefly, the aliquots (0.8 ml) of cerebral cortex homogenates were mixed with distilled water 1.4 ml, 1.5 mM physostigmine 0.2 ml and 1.84 M trichloroacetic acid 0.8ml blending adequately. After centrifugation, 1 ml of each supernatant was added to 1 ml of basic hydroxylamine. The mixture was incubated for 15 min at 25 °C, then added with 4 M HCl 0.5 ml and 0.37M $FeCl_3$ 0.5 ml. Absorbance was read at 540 nm and calibrated with the blank and the standard (0.2 μmol/ml ACh instead of the cerebral cortex homogenates).

2.7. Step-through test

The number of animals per group was 8–10. The step-through test was performed in the JZZ94 multifunction passive avoidance apparatus (PA M1 O'Hara and Co. Ltd.). The apparatus consisted of two compartments separated by a black wall with a hole in the lower middle part. One of the two chambers is illuminated, and the other is dark. The test was conducted for 2 consecutive days including one training trial (d1), each mouse was placed in the illuminated compartment, facing away from the dark compartment and left for 5 min to habituate to the apparatus, 1 h after the adaptation trial, the mouse received the training trial. The training trial is similar to the adaptation trial except that