Effects of berberine on β-secretase activity in a rabbit model of Alzheimer's disease

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Abstract

Introduction: Relevant aspects of Alzheimer's disease (AD) can be modeled by aluminium-maltolate injection into specific regions of the brain. The possible role of berberine chloride (BC) as an anti-inflammatory agent in the brain has been previously addressed.
Material and methods: Rabbits were divided into control (C), untreated lesion (L) and BC-treated + lesion (L + BC) groups. Animals in L + BC received BC (50 mg/kg) orally 1 day after surgery and daily for 2 weeks. The lesion was induced by injection of 100 µl of either vehicle or water containing 25 mM aluminium-maltol into intraventricular fissure. Weight loss, ataxia, paralysis and tremor were monitored. For histopathology, Bielschowsky silver and H&E staining were employed. β-Secretase activity in hippocampus was finally assessed.
Results: All L animals died on days 12-15 after lesion. Seven to 10 days after lesion, abnormal symptoms as well as cachexia were seen in over 90% of cases. L rabbits lost an average of 0.5 kg which was significant on days 10 and 12 ($p < 0.05$); this was not completely prevented by BC. Up to day 15, all L animals had lost their lives ($p < 0.001$). BC treatment protected the hippocampus from degeneration, altered the behavior and decreased the activity of β-site amyloid precursor protein cleaving enzyme-1 (BACE-1).
Conclusions: Considering the findings in regard to physiological abilities, histological changes and BACE-1 activity in hippocampus changes, it is concluded that BC treatment could be an effective therapy in restoring Al maltol-induced behavioral derangements in the rabbit model of AD.

Key words: Alzheimer's disease, berberine, hippocampus, aluminium maltolate, β-secretase.

Introduction

Alzheimer's disease (AD) is an irreversible, progressive neuro-degenerative disorder that occurs gradually and results in memory loss. Unusual behaviour is accompanied by three main structural changes in the brain including diffuse loss of neurons, intracellular protein deposits termed neurofibrillary tangles (NFT) consisting of hyperphosphorylated tau protein and extracellular protein deposits termed β-amyloid (Aβ) or senile plaques, surrounded by dystrophic neuritis [1-3].

A M S

Considerable research has additionally been performed to investigate a large array of neuroprotective agents using animal models which mimic such disorders. Currently, no effective treatment has been proposed to modify the symptoms; however, understanding the molecular mechanism of AD has opened new opportunities for successful development of medicines that prevent the generation of Aβ. The β-amyloid peptides ($A\beta_{40}$ and $A\beta_{42}$) are implicated in the pathogenesis of AD and are derived from sequential proteolytic cleavage of amyloid precursor protein by β and γ-secretases [4]. Extensive efforts have been devoted to identifying inhibitors of the secretases' activities, aiming to prevent the formation of Aβ. Various mechanisms have been proposed to explain the pathway by which Aβ induces various changes such as neuronal cell death, including intracellular calcium accumulation, disruption of cellular Ca^{2+} homeostasis, reactive oxygen species and nitric oxide production, lipid peroxidation, decreased membrane fluidity, alteration of the cytoskeleton and nucleus, redox-active iron, the inflammatory or autoimmune process and increased sensitivity along an apoptosis–necrosis continuum [5]. Berberine is an isoquinoline alkaloid with a long history of medicinal use in China; it exists in Rhizoma Coptidis (Huanglian), *Hydrastis canadensis* (Goldenseal) and Cortex Phellodendri (Huangbai) as the main active principle of the herbs, with multiple pharmacological effects. It is an acetyl cholinesterase inhibitor similar to galanthamine, a drug used for AD treatment [6].

Due to the inflammation and oxidative stress in the brain which leads to the activation of β-secretase enzyme activity, we decided to investigate the neuroprotective effect of berberine on enzyme activity of an Al-maltol-induced AD model in aged rabbits.

Material and methods

Animals

Twenty-four male New Zealand white rabbits (3-4 kg body weight) were provided by the Experimental Animal Center of Razi Vaccine and Serum Research Institute. They were subsequently housed one per cage with free access to food and water, and kept in a constant environment at the Medical Sciences of Iran University Animal Lab (22 ±2°C, 50 ±5% humidity, and 12-h light/dark cycle). Twelve animals with the same specifications were also used for pathological examinations.

Experimental protocol

The animals were randomly divided into three groups: control (C), untreated lesion (L) and BC-treated + lesion (L + BC). The injection consisted of 100 μl of 25 mM Al maltol [7] intraventricularly by stereotactic surgery as explained by Bertholf *et al.*

[9] under general anaesthesia with sodium pentobarbital (50 mg/kg, *i.v.*). After surgery, a fresh suspension in saline solution of berberine chloride (50 mg/kg) (Sigma, St. Louis, MO, USA) was administered intragastrically once daily for 14 days [6] to the L + BC group and other groups received the vehicle, i.e. saline solution.

On days 4, 7, and 10, 1 control and 3 L animals were sacrificed for histopathological examinations. Animals were anaesthetized by a lethal dose of sodium pentobarbital and, before death, were perfused through the left ventricle with fixative solution (10% phosphate-buffered formalin) [7]. The cranium was dissected in order to remove the brain. The brain was fixed at room temperature in 10% phosphate-buffered formalin for 2 weeks. Sections of frontal cortex, rostral and caudal hippocampus, adjacent temporal cortex, midbrain, pons, medulla, cerebellum, and cervical levels of spinal cord, were trimmed, embedded in paraffin and mounted before being stained using haematoxylin and eosin (H + E) and Bielschowsky silver methods.

Measurements

β-Secretase activity and kinetic analysis were measured using a quenched fluorescence assay, with the synthetic peptide substrate (7-methoxy coumarin-4-yl) acetyl-Ser-Glu-Val-Asn-Leu*Asp-Ala-Glu-Phe-Arg-Lys (2,4-dinitrophenyl)-Arg-Arg-NH_2 for β-secretase [10]. It was purchased from Peptide Institute Inc. Osaka, Japan. The inhibitory activity of the test compounds on β-site amyloid precursor protein cleaving enzyme-1 (BACE-1) was determined based on the percentage decrease of the cleaved substrate by the enzyme (Figure 1). The assay was done with 7 nM BACE-1, 20 μM substrate and 20 mM sodium acetate (CH_3COONa) at a pH of 4.5 provided by 0.1% Triton X-100 as assay buffer in a total reaction volume of 100 μl. After incubation for 60 min at 37°C, the reaction was stopped by addition of trichloroacetic acid. The N-terminal cleavage fragment [(7-methoxy coumarin-4-yl) acetyl-Ser-Glu-Val-Asn-Leu-OH] was analysed by RP-HPLC with the detection of fluorescence intensity (Ex, 328 nm; Em, 393 nm).

Chemicals

All the materials used to isolate and characterize the -secretase enzyme from the hippocampus in the animal model were purchased from Sigma Aldrich Company and used according to the manufacturer's instructions. The procedure was carried out under ice-cold conditions with pre-chilled CelLytic MT mammalian tissue lyses/extraction reagent, protease inhibitor cocktail, pre-chilled micro homogenizer, microcentrifuge (12 000-20 000*g_{max}) for 10 min at 4°C. The supernatant was aliquoted and stored at –70°C until further use.
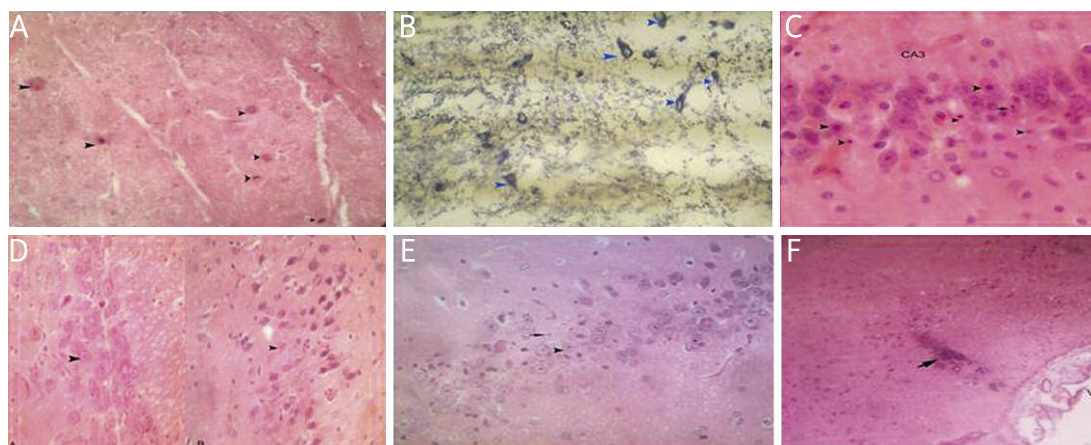
Figure 1. A – Micrographs of H&E 700 section through the hippocampus from animals that received Al(maltol)3 (25 mM) intraventricular injection. Gliosis and encephalitis of the brain in the aged rabbits is also observed in the vascular region (arrows). **B** – Amyloidal plaque and amyloidal deposition by arrows with argyrophilic neurofibrillary degeneration (Bielschowsky's silver method). **C** – Neurofibrillary tangles (arrows) throughout the neuron including the entire length of the dendrites and throughout the axons including the terminals (Bielschowsky silver method). **D** – Apoptotic bodies (arrows) through the hippocampus in CA3 region (H&E*700). **E** – Neuronal loss in CA3 area through hippocampus, L group (A), control group (B). **F** – Neuronal loss (arrowhead) and apoptosis (arrow) in CA2 area through hippocampus in L group (H + E*640)

Statistical analysis

Data were represented as mean ± SEM and the results were subsequently analysed as absolute values (total integrated volume). The significance of differences between control and other groups was statistically analysed by one-way analysis of variance (ANOVA) and, whenever $p < 0.05$, by Bonferroni's t test. Whenever only two groups had to be compared, unpaired Student's t test was used. Differences were considered statistically significant whenever $p < 0.05$.

Results

Behaviour, survival rate and weight loss

All animals in the L group died on days 12-15 after lesion induction while the others survived. In 4 out of 8 animals in the L group, more generalized debilities, including abnormal posture, developed. Seven to 10 days after lesion induction, the following symptoms were seen in over 90% of the animals: ataxia, tremor, forward head tilting, hind limb paralysis and decreased food access due to physical disability. Signs of cachexia (presence of fatty serous tissue) were observed in this group at the time of necropsy. Weight loss was significant in the L group compared to the controls on days 10 and 12 ($p < 0.05$; Table I). L rabbits lost an average of 0.5 kg, whereas the control group gained an average weight of 0.4 kg. Weight loss was not completely prevented by berberine and the decrease in weight reached the level of significance on days 10 and 12 ($p < 0.05$) as well as on day 15, when all L group animals had lost their lives ($p < 0.001$).

Histopathological findings

The intraventricular administration of Al-maltol to the aged rabbits induced neuronal perikaryal neurofibrillary accumulations (hereafter termed neurofibrillary degeneration) in 50% of the animals in 4 days. These accumulations were argyrophilic after 7 days. One hundred percent of the rabbits developed neurofibrillary degeneration in one or more areas of neuroaxis. Multiple levels of neuroaxis were

Table I. Body weight of control, L[a] and L + BC[b] groups of rabbits in kg (mean ± SEM)

Time of measurement	Controls	L group	L + BC group
Day 0 (before surgery)	3.30 ±0.03	3.30 ±0.03	3.30 ±0.03
Day 7	3.35 ±0.04	3.25 ±0.04	3.30 ±0.05
Day 10	3.43 ±0.04	3.03 ±0.02*	3.11 ±0.04*
Day 12	3.48 ±0.04	2.80 ±0.07*	2.90 ±0.08*
Day 15	3.57 ±0.04	All dead	2.77 ±0.12**

*[a]L group – Aluminium maltol-induced lesion group, [b]L + BC group – Aluminium maltol-induced lesion + berberine chloride treated group, *p < 0.05, significantly different compared to the control group (Bonferroni's t test),**p < 0.001, significantly different compared to the control group (Student's unpaired t test)*

examined in all animal groups using H&E and silver staining techniques. The Al-maltol-injected animals showed widespread neurofibrillary degeneration (NFD) in the cortical and subcortical distribution. Proper placement of the intraventricular catheter was histologically verified by sections along the catheter tract; mild to moderate astrocytosis, necrosis, and macrophages were found around the intraparenchymal tract of the catheter (Figures 1 A, 1 B and 1 C). The pyramidal neurons were consistently affected in rostrocaudal whole brain coronal sections in Al-maltol-injected rabbits. The accumulation was maximal near the site of aluminium injection. Aβ deposition, neurofibrillary tangles, apoptosis, apoptotic bodies, neuronal loss and inflammation were also demonstrated close to the cerebral cortex and hippocampus (Figures 1 D, 1 E and 1 F). No abnormality was observed in the brain micrographs of the control animals.

Biochemical findings

BACE-1 activity was detectable by RP-HPLC in hippocampus of the aged rabbits. Measurement of β-secretase activity in the hippocampus revealed a 3.2-fold increase compared to the controls ($p < 0.05$; Figure 2). Approximately 40% of this increase was prevented by berberine treatment ($p < 0.05$; Figure 2).

Discussion

In the present study, it was found that intraventricular Al-maltol injection in the aged New Zealand white rabbit may induce behavioural, biochemical and neuro-pathological changes, similar to what may happen in clinical AD. On the other hand, treatment with BC can protect the hippocampus from degenerative changes, increase the behavioural indices and delay the AD model symptoms (observed in the L group) and decrease BACE-1 activity in the L + BC group compared with the L group.

Rabbits are particularly sensitive to the neurotoxic effects of aluminium and they may develop severe neurological changes depending on the dose, age and route of administration [7-9]. A serious controversy still remains concerning the possible roles of Al in neurodegenerative disorders. However, Savory et al. believe that it is quite reasonable to consider the Al-treated rabbit as an excellent model for mimicking the biochemical composition of the neurofibrillary tangles of AD [9]. Al could also play an important role in the stabilization or aggregation of Aβ peptides in the plaques, loss of neurons and apoptosis in aged rabbits. Overall, the Al-induced experimental AD in rabbits has provided much useful information on several processes that could share a common mechanism with the development of neuro-degeneration in AD; hence, this model may be valuable as more mechanistic schemes are uncovered. Additionally, the model could also be of considerable value in the identification of early diagnostic markers and the development of new strategies in the prevention and treatment of AD.

In the present study, intra-ventricular injection of Al-maltol in aged New Zealand white rabbits was validated as a model for AD due to the neuro-pathological features revealed after Bielschowsky's silver staining under the light microscope. Such characteristic features included neuro-degenerative lesions including Aβ deposition, neuro-fibrillary tangle formation, oxidative stress, apoptosis (apoptotic bodies), neuronal loss and inflammation. Moreover, from the clinical point of view, rabbits showed progressive behavioural symptoms, including forward head tilting, tremor, loss of appetite, weight loss and paralysis. Increased β-secretase enzyme activity was another proof for the validity of the model used in this study.

As found in our study, berberine chloride could delay the behavioural changes and symptoms (weight loss, tremor, ataxia and paralysis) in the rabbit AD model. Berberine could also decrease the cleaved substrate by approximately 40% as detected by RP-HPLC with fluorescence intensity; a significant reduction in enzymatic activities was also observed. Berberine has been used orally as an OTC drug in China and its safety and efficacy after oral administration have been generally accepted [6]. A few studies have demonstrated that berberine activates macrophages and increases phagocytotic function of, and IL-1 production by, murine peritoneal macrophages (PMΦ) [11, 12]. In addition, berberine potentiates the non-specific immunity and, hence, it may be used as a neuroprotective agent in the prevention of AD. Berberine may
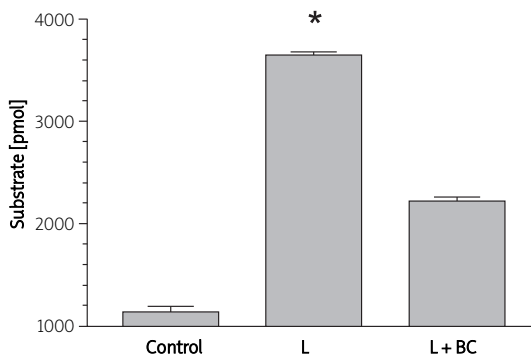
Figure 2. BACE-1 inhibitory activity of the test compounds was determined based on the decrease of the cleaved substrate by the enzyme. The result represents at least three independent experiments; the cleaved substrate level was increased in the L group *Values represent the means ± SEM of 6 male rabbits (> 3 kg); *p < 0.05 when compared with the normal (C) group*

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decrease the expression of peroxisome prolifera-
tor-activated receptor γ isoform 2 (PPARγ$_2$) mRNA
and protein, which is a negative regulator of inflam-
mation [13, 14]. In spite of the fact that it exagger-
ates the inflammatory response, berberine might
be beneficial to AD by ameliorating spatial memo-
ry impairment, improving the expression of inflam-
matory factors, activating microglia and clearing
senile plaque [6].

In conclusion, we conclude that aged rabbits
treated with Al-maltol can serve as a suitable mod-
el of AD. Furthermore, oral administration of berber-
ine chloride can protect CNS cells in AD, caused by
inflammation and oxidative stress; anti-inflamma-
tory effects, inhibition of acetylcholine esterase and,
to some extent, β-secretase inhibition may be the
underlying mechanisms. However, further investi-
gations and clinical research are required to demon-
strate the efficacy of berberine in AD patients.

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