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New mechanisms in preventive effect of ellagic acid on cognition in mice with Alzheimer's disease type dementia

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Antioxidant mechanisms in protective effects of some natural compounds on progression of Alzheimer's disease (AD) were reported during last years. Our previous data revealed significant improving effect of a natural polyphenol Ellagic Acid (EA) on rodent cognitive functions. The goal of this study was to evaluate the effect of EA on cognition of mice with chemically induced dementia from AD type. This animal model was produced via Scopolamine treatment of male Albino mice and was verified by cognitive and biochemical methods. After 5-days treatment with EA both the changes in the cognitive functions of animals and biochemical correlates were evaluated. Significant preventive effect of EA on the processes of learning and memory (Step-through test) of dement animals was established. The high percent (50%) of memory prevention by EA was accompanied by significant antioxidant effect (decreased lipid peroxidation) and inhibited activity of acetylcholine esterase in the brains of EA-treated animals. An increase of dopamine uptake in the brains of EA-treated dement animals was also found. Our results reveal some of the complex mechanisms underlying the EA preventive effect on the cognition in mouse model of AD-dementia.

Key words: Ellagic acid, Alzheimer's disease, Memory, Antioxidants, Acetylcholine esterase, Dopamine

INTRODUCTION

Alzheimer disease (AD) is the most common dementia with yet disputable etiology [1]. Along with the leading Aβ-amyloid hypothesis, the degeneration in the cholinergic system and the brain oxidative stress are other important players in the AD pathogenesis [2].

The cholinergic system plays a crucial role in learning and memory [3] and therefore treatment with AChE-inhibitors relieves some of the key AD symptoms [4]. Recent studies show that along with the cholinergic system the dopaminergic system can also be affected in AD in a complex way. Changes in the dopamine (DA) levels, expression of the DA-receptors and DA transporter (DAT) are often observed in the course of AD [5].

The brain oxidative stress is another key player in AD pathology. The increased accumulation of free radicals gradually leads to depletion of the antioxidant system of the brain cells which leads to damage of the lipid membrane constituents [6, 7].

Protective effects of some polyphenol compounds on the progression of AD were reported during last years [8]. Our previous unpublished data revealed a significant improving effect of the natural polyphenol ellagic acid (EA) on the cognitive functions of rodents.

EA is a dimeric derivative of gallic acid, which spontaneously forms a dilactone [9]. Due to the unique chemical structure of EA its carbon and oxygen atoms form a planar complex extended π-electron system [10, 11], which allows EA to serve as an efficient free radical scavenger [12], as well as a chelating agent to some polyvalent metal ions [13, 14].

The goal of this study was to study some mechanisms of the protective effect of EA on the cognition of mice with chemically induced dementia of AD type.

EXPERIMENTAL

Experimental animals and treatment scheme

Male albino ICR mice (18-20 g) were used, divided in the following experimental groups: Control (saline); Sco-treated (Scopolamine-treated); EA+Sco-treated.

The animal model of AD was induced *via* treatment with daily i.p. injection of 1 mg/kg scopolamine for 11 days. The method was verified by cognitive and biochemical tools and markers, i.e. learning and memory tasks, lipid peroxidation and acetylcholine esterase (AChE) activity in brain.

Control animals received saline in the same volume and way of treatment for 11 days.

The possible preventive effect of EA was studied by treating the animals from the EA+Sco-

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group with scopolamine (1mg/kg b.w. i.p.) for 11 days and 400 mg/kg dose of the natural polyphenol (p. o.) for five consecutive days.

Behavioral and biochemical analysis

On the 24th hour after the last treatment all groups were tested behaviorally and afterwards decapitated and the brains were removed on ice and collected for biochemical evaluation.

The behavioral changes were estimated with the Step-through test (for estimation of the changes in the emotional learning and memory of animals at the end of treatment) by Jarvik and Kopp [15]. The step-through task is a one-trial emotional memory task combining fear conditioning with an instrumental response [16] in a special apparatus [15].

Neuromuscular coordination and the effect of learning new locomotor skills and memory was tested on Rot-a-Rod set up [17, 18]. Initially the animals were trained on new motor skills on the Rot-a-Rod apparatus and after the treatment period were retested again.

Next biochemical parameters were evaluated in a 10% supernatant of brain homogenates in 0.1 M potassium phosphate buffer with pH 7.4:

Acetylcholinesterase activity (AChE) was estimated by the colorimetric reaction of the products of the AChE catalyzed decomposition of acetylthiocholine according to Ellman's method [19].

For the evaluation of antioxidant activity of EA, the lipid peroxidation was measured by determination of the TBARS (thiobarbituric acid reactive substances) through the color products from their reaction with thiobarbituric acid according to the protocol by Buege and Aust [20].

The DA uptake was measured with the protocol by Nicklas *et al.* [21]. In brief, brain synaptosomal fractions were incubated in Krebs-Ringer medium with radioactive DA. At suitable intervals portions of the suspension were filtered, afterward the filters were washed and the washings' radioactivity was measured to determine the DA uptake.

Statistical analysis

Experimental data were analyzed statistically by Student's t-test. Results were expressed as means ± SEM. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

After 5-days treatment of Sco- mice with EA both the changes in the cognitive functions of animals and the biochemical parameters were evaluated in all groups.

Significant preventive effect of EA on the processes of learning and memory was found *via* the step-through test. A very high percent (50%) difference in avoidances between Sco and EA+Sco groups was observed signifying significant memory prevention by EA ($P < 0.001$) (Fig. 1).

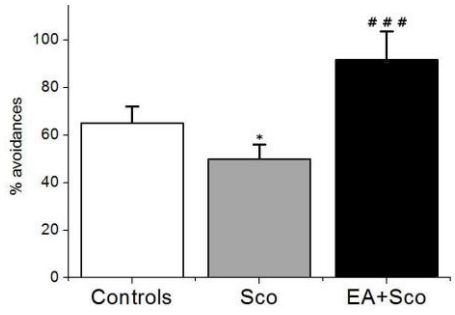

Fig. 1. Step-through test performance (avoidances) of the control, Sco-treated (Sco) and EA+Sco treated (EA+Sco) animals (*P<0.05, *vs* controls; ###P<0.001, *vs* Sco-treated).

The preventive effect of EA was also available in the second parameter of the Step-through test – the latency time – which is comparable with the saline-treated controls (Fig. 2).

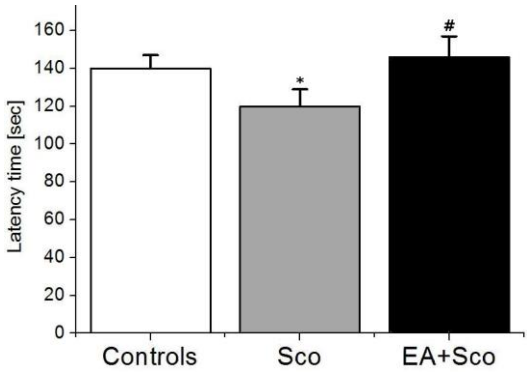

Fig. 2. Step through test performance (latency) of the control, Sco-treated (Sco) and EA+Sco-treated (EA+Sco) animals (*P<0.05, vs controls; #P<0.05, vs Sco-treated).

Preventive effect of EA after the last treatment on the motor learning and memory and neuromuscular coordination of dement animals also was established via Rot-a-Rod test (Fig. 3), where the motor learning effect appears as the elongated falling latency in the test performance [18]. There is over 30% increase in the latency time in EA+Sco-treated over Sco-treated group.

The treatment with EA of Sco-animals also showed beneficiary effects upon the measured biochemical parameters. The measured AChE activity in the brain was reduced by over 50% in the EA+Sco-treated group compared to the Sco-treated group (Fig. 4).

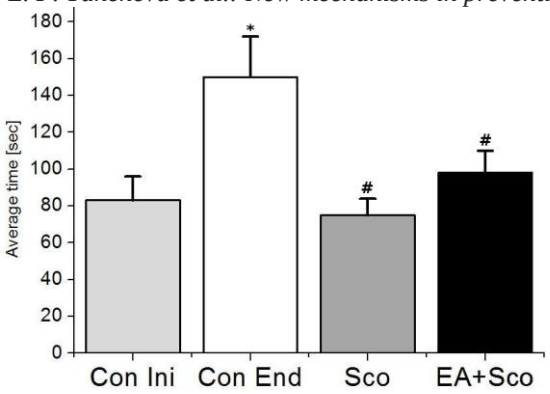

Fig. 3. Rot-a-Rod test performance: initial state of controls (Con Ini) and the effect of learning in the controls at the end of treatment (Con End), Sco-treated (Sco) and EA+Sco-treated (EA+Sco) animals (*P<0.05, *vs* Con Ini; #P<0.05, *vs* Con End).

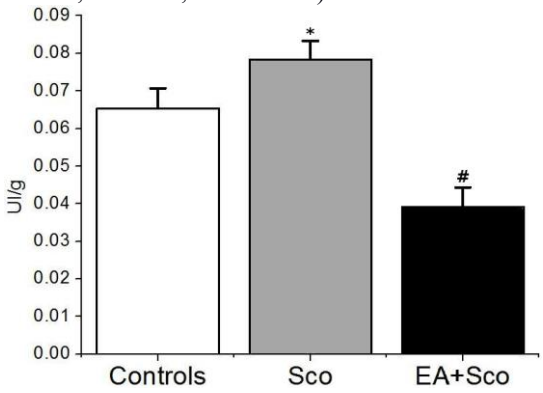

Fig 4. Brain AChE activity in control, scopolamine-treated (Sco) and EA+Sco-treated (EA+Sco) animals (*P<0.05, *vs* controls; #P<0.05, *vs* Sco-treated).

Similar trend was established for the measured products of the lipid peroxidation (Fig. 5), showing significant antioxidant activity of EA reducing the level of TBARS by 13% compared to the Sco-treated group. A decrease in DA uptake in brains of EA+Sco-treated AD animals was also found (Fig. 6). The DA uptake was reduced by over 17% in the EA+Sco-treated animals compared to the Sco-treated group.

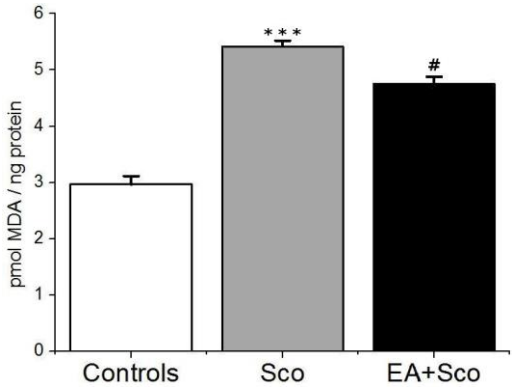

Fig. 5. TBARS in the brain in control, Sco-treated (Sco) and EA+Sco-treated (EA+Sco) animals (***P<0.001, *vs* controls; #P<0.05, *vs* Sco-treated).

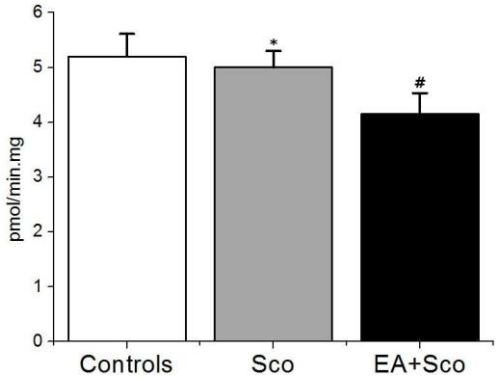

Fig. 6. Dopamine uptake in the brain in control, Sco-treated (Sco) and EA+Sco-treated (EA+Sco) animals (*P<0.05, *vs* controls; #P<0.05, *vs* Sco-treated).

Our results revealed some of the complex mechanisms underlying the EA preventive effect on the cognitive disorders in mouse model of dementia.

The high percent (50%) of prevention by EA on the emotional **learning and memory** which relies on cholinergic neurons rich pathways as the thalamo-amygdalo-cortical one [22, 23], as shown on the step through test, was accompanied by decreased lipid peroxidation which precedes amyloid formation leading to neurons death [24]. Also there is good preventive effect of EA on the motor learning and memory as shown on the Rot-a-rod test performance, which in the recent years has also been shown to be affected in AD [25]. This means that EA may impact not only on the commonly evaluated in AD rich in cholinergic neurons learning memory pathways but also on the motor learning related cerebellar-thalamic-cortical networks poorer in such neurons [26]. However, unlike the results in emotional learning where EA acts as a rather strong memory enhancer/protector whose effect may be attributed to the protective effect on the cholinergic system, here EA has less pronounced capacity to restore the evaluated impaired motor-cognitive functions. This may be due to the complexity of the underlying networks which have, receive and project terminals to structures with varying degree of impair in AD, where the greatly benefiting from EA-treatment cholinergic neurons [27, 28] is a minor portion of it such as the basal ganglia [29]. Portion of the cholinergic basal ganglia acts as map "reorganizer" in the motor learning processes [29] and it seems that once impaired, their complex dynamic functionality is hard to be repaired sufficiently within the duration of the study.

EA can decrease **lipid peroxidation** in either direct or indirect routes in AD. EA being amphipathic molecule and having better solubility in lipids than in water [30] may bind non-

covalently to the lipid membranes or embed in them [31]. AD has pronounced oxidative stress caused damage of lipid membranes and membrane-related cell and biochemical processes [32, 33]. A possible direct mechanism for EA membrane protection is that EA "shields" the membranes from the undesired oxidation and peroxidation processes related to the oxidative stress. Being both radical scavenger and chelating agent *via* indirect route EA can inhibit NADPH and ADP-Fe^{3+}-dependent lipid peroxidation [34].

EA also inhibited the activity of **acetylcholine esterase** in the brains of animals. AChE is a group of enzymes with complex molecular polymorphism of quaternary structure. Although the enzyme forms display similar catalytic activity, they differ in their hydrodynamic parameters and ionic or hydrophobic interactions; for example some of the forms with a hydrophobic terminal can bind selectively to amyloid plaques in AD [27].

A recent study using enzyme assay for AChE (from electric eel) reports that EA can act as AChE inhibitor at *in vitro* conditions with IC_{50}=45.63 μM [35]. However, in the complex environment of the body's cells and fluids the correlation between EA and AChE activity may not be that straightforward, since EA can also act as an antioxidant reducing some of the triggering factors for AChE overexpression like stress and Aβ-amyloid formation processes [27]. Also EA can reduce the formation of Aβ oligomers by inhibiting BACE1 and by promoting the Aβ fibrilization [36]. It is known that the most common form of AChE G4 can aggregate and co-localize with Aβ oligomers and fibrils [27]. In this way EA can also reduce the AChE availability and activity.

A decrease of **dopamine uptake** in the brains of EA-treated dement animals was also found. EA significantly reduces the levels of the DA uptake in the brain. DA is uptaken mostly by the DA transporter (DAT), however, another monoamine or other less specific organic ion transporters can also participate in its membrane transport [37]. In EA-treated animals there is only 17% decrease in the DA uptake which probably means that EA acts upon this parameter by some indirect mechanism or by inhibiting the secondary uptake routes. A recent study reports that EA is a very potent inhibitor of some of the organic anion transporters in the brain like OAT1. It was found that EA has IC_{50}=207 nM for this particular transporter [38]. These transporters are involved in the transport of the metabolites from the DA pathway and along with kidney are specifically expressed in the brain [39]. So the reduction of the DA uptake in EA-treated animals may be attributed to inhibition of some of the secondary routes for DA transport.

This reduced DA uptake is related to the improvement in the motor memory according to a recent study [18], where inhibition of DA transport enhances motor learning.

CONCLUSIONS

The present study reveals the complex mechanisms of EA preventing effect on the memory of dement mice, namely antoxidant activity, AChE inhibition and DA modulation in the brain. Further studies will elucidate new details in the established complex effects of EA on the learning and memory of animals with scopolamine-induced dementia.

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НОВИ МЕХАНИЗМИ В ПРЕВАНТИВНИЯ ЕФЕКТ НА ЕЛАГОВАТА КИСЕЛИНА ВЪРХУ КОГНИТИВНИТЕ ФУНКЦИИ ПРИ МИШКИ С МОДЕЛ НА БОЛЕСТТА НА АЛЦХАЙМЕР

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(Резюме)

През последните години бяха съобщени различни антиоксидантни механизми в защитните ефекти на някои природни съединения върху прогреса на болестта на Алцхаймер (БА). Наши предишни данни разкриха значително подобряващ ефект на естествения полифенол - елаговата киселина (ЕК) - върху когнитивните функции на гризачите. Целта на това изследване е да се оцени ефектът на ЕК върху когнитивните способности на мишки с химически индуцирана деменция от тип БА. Животинският модел на БА бе предизвикан чрез третиране със скополамин на мъжки мишки и бе потвърден с поведенчески и биохимични методи. След 5-дневно третиране с ЕК бяха оценени както промените в когнитивните функции на животните, така и биохимичните корелати. Беше установен значителен превантивен ефект на ЕК върху процесите на учене и паметта на животни с деменция. Високият процент (50%) на превенция върху паметта чрез ЕК е придружен от значителен антиоксидантен ефект (понижена липидна пероксидация) и инхибиране на активността на ацетилхолинестеразата в мозъците на третирани с ЕК животни. Установено е също така увеличение на поемането на допамин в мозъка на животни, третирани с ЕК. Нашите резултати разкриват някои от сложните механизми, които стоят в основата на превантивния ефект на ЕК върху когнитивните функции в модел на деменция тип БА при мишки.