OPEN

Arecoline attenuates memory impairment and demyelination in a cuprizone-induced mouse model of schizophrenia

Zhizhong Xu[a,c,*], Abulimiti Adilijiang[a,c,*], Wenqiang Wang[a], Pan You[a], Duoduo Lin[a], Xinmin Li[a,c] and Jue He[a,b,c]

Cerebral demyelination is possibly one of the main pathological factors involved in the development of schizophrenia. Our previous studies have showed that *Areca catechu* nut extract could ameliorate cognitive decline by facilitating myelination processes in the frontal cortex in a cuprizone (CPZ)-induced mouse model of schizophrenia. The aim of the present study was to evaluate the effects of arecoline, one of the alkaloids in *A. catechu* nut extract, on memory impairment and cerebral demyelination in CPZ-treated mice. Mice were treated with CPZ (0 or 0.2%) in chow food and arecoline hydrobromide (0, 2.5, or 5 mg/kg/day) in drinking water for 12 weeks before Y-maze behavioral test. After the behavioral test, the mice were sacrificed for the measurement of myelin basic protein in the frontal cortex. We showed that arecoline-attenuated spatial working memory impairment, concurrent with attenuated demyelination related to vehicle-treated CPZ mice for the first time. Arecoline is one of the primary active ingredients in *A. catechu* nut responsible for attenuating memory impairment and demyelination in CPZ mice, cerebral demyelination may have a role in memory impairment, and modulation of cerebral demyelination could be a useful strategy in schizophrenia treatment. *NeuroReport* 30:134–138 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.

NeuroReport 2019, **30**:134–138

Keywords: areca catechu nut, arecoline, cuprizone, demyelination, memory, schizophrenia

[a]Xiamen Xian Yue Hospital, Xiamen, Fujian, [b]Department of Neurology, First Affiliated Hospital, Institute of Neurological Disease, Henan University, Henan, People's Republic of China and [c]Department of Psychiatry, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

Correspondence to Jue He, PhD, Xiamen Xian Yue Hospital, 387-399 Xian Yue Road, Xiamen, Fujian 361012, People's Republic of China
Tel: +86 592 539 2569; fax: +86 592 539 5079;
e-mail: hejue2002@hotmail.com

*Zhizhong Xu and Abulimiti Adilijiang contributed equally to the writing of this article.

Received 26 September 2018 **accepted** 31 October 2018

Introduction

Schizophrenia is characterized by disturbances of perception, emotion, social functioning, and cognition, and its neuropathology is relatively poorly understanding. Recently, increasing evidence suggests that the pathological process underlying schizophrenia may involve an abnormality of brain myelin affecting white matter [1–4]. Thus, neuroprotective agents including antioxidants that target on white matter injury might be used for schizophrenia treatment. *Areca catechu* nuts, popularly known as 'betel nuts,' which possess antioxidant and anti-inflammatory effects [5], have beneficial effects on positive, negative, and cognitive symptoms of schizophrenia [6–8]. Our previous studies have showed that *A. catechu* nut extract could ameliorate cognitive decline by facilitating myelination processes in the frontal cortex in a cuprizone (CPZ)-induced mouse demyelination model of schizophrenia [8,9].

To find out the possible main active ingredients from *A. catechu* nut extract that exert beneficial effects on schizophrenia, arecoline, one of the alkaloids in *A. catechu* nut extract, was investigated in the current study. Arecoline possesses anti-inflammatory effects and has activity on nicotinic acetylcholine receptors, which are related to learning and memory [10,11]. Therefore, our hypothesis is that arecoline is one of the main active ingredients from *A. catechu* nut extract that exert beneficial effects on cognitive impairment and white matter injury in schizophrenia. To test this hypothesis, the effects of arecoline on the alternation behavior impairment in a Y-maze test and on the decrease of myelin basic protein (MBP) expression in the frontal cortex were evaluated in a CPZ-induced demyelination mouse model of schizophrenia.

Materials and methods

Animals

All procedures with animals were performed in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Alberta. In total 40 male C57BL/6 mice (Charles River Canada, Montreal, Quebec, Canada), housed four or five per cage,

0959-4965 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/WNR.0000000000001172

of 5 weeks old at the beginning of the experiments were used.

Drug treatment

Arecoline hydrobromide (ARE) and CPZ were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Milled chow with 0.2% CPZ (w/w) was prepared in a standard rodent chow (LabDiet; PMI Nutrition International LLC, Brentwood, Missouri, USA) [8]. ARE was prepared in distilled drinking water (1 or 2 mg/100 ml water with target dose: 2.5 or 5 mg/kg/day), and average amount of water or solution consumed was about 7.5 ml/30 g/day (250 ml/kg/day), with no difference in fluid consumption between normal chow and CPZ-contained chow mice, and between water and ARE solution consumption [12]. Starting from 5 weeks old, the mice were fed with CPZ (0 or 0.2%) and the CPZ mice were administrated with ARE (0, 2.5, or 5 mg/kg/day) in drinking water for 12 weeks. These procedures produced the following four groups of mice: Con (normal chow + water), CPZ (0.2% CPZ chow + water), CPZ + ARE2.5 (0.2% CPZ chow + 2.5 mg/kg/day ARE in drinking water), CPZ + ARE5 (0.2% CPZ chow + 5 mg/kg/day ARE in drinking water).

Y-maze test

At the age of 17 weeks old following drug treatments, spatial working memory performance in mice was assessed by recording spontaneous alternation behavior in a Y-maze [12]. The maze was made of black-painted wood. Each arm of the Y-maze was 40-cm long, 12-cm high, 3-cm wide at the bottom, and 10-cm wide at the top, and positioned at an equal angle. The mice were individually placed at the end of one arm and allowed to move freely through the maze during an 8-min session. Mice tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the mice know which arm they have already been visited. The sequence of arm entries was recorded visually. Spontaneous alternation behavior was defined as the entry into all three arms on consecutive choices in overlapping triplet sets. The percent spontaneous alternation behavior was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries − 2) multiplied by 100 [8].

Western blot analysis

On the day after behavioral testing, the mice were sacrificed for MBP protein analysis. The mice were perfused with 0.1 M PBS (pH 7.4), and the frontal cortex from right hemisphere was dissected, frozen in dry-ice powder, and stored at − 70°C until used [8]. In detail, the frontal cortex from the right hemisphere was dissected on the back surface of an iced tissue culture dish covered by a PBS-wetted filter paper: (i) freeing the frontal cortex from the surrounding tissues including rhinencephalon and striatum from brain sagittal midline along the lateral ventricle; (ii) further defining the frontal cortex by cutting along bregma 0 coronal plane and excluding the rostral part of the cortex from bregma. The frontal cortex samples from each mouse were homogenized at 4°C in a lysis buffer [50 mM Tris–HCl, 150 mM NaCl, 10 mM EDTA, 10 mM NaF, 1 mM sodium orthovanadate, 1% NP-40, 10 mM sodium pyrophosphate decahydrate, 0.5 mM DTT, 0.2 mM PMSF, and Complete Mini Protease Inhibitor Cocktail (pH 7.4; Roche Diagnostics, Laval, Quebec, Canada). The lysates were centrifuged twice at 10 000*g* for 10 min. The protein concentration of the supernatant was determined using a BCA protein assay kit (Pierce, Rockford, Illinois, USA). An aliquot of each sample containing equal amounts of total protein (50 μg/8–9 μl) was denatured in a protein-loading buffer, separated on a 12% polyacrylamide gel, and subsequently transferred to a polyvinylidene difluoride membrane (Biorad, Hercules, California, USA). After being blocked by 5% skim milk powder in TBST (10 mM Tris–HCl, 150 mM NaCl, 0.05% Tween 20; pH 7.4), the membranes were incubated overnight at 4°C with an anti-MBP (18 kDa, 1 : 2000, chicken polyclonal; Aves Labs Inc., Tigard, Oregon, USA) antibody or anti-β-actin (42 kDa, 1 : 5000, monoclonal; Sigma-Aldrich) antibody. The membranes were then washed with TBST and probed with horseradish peroxidase-conjugated anti-chicken or anti-mouse secondary antibody for 2 h at room temperature. The immune complexes were detected by an ECL chemiluminescence system (Amersham, Buckinghamshire, UK) and exposed to high-performance chemiluminescence film (Amersham). The MBP or β-actin band in the film was scanned using Vista Scan software (UMAX Technologies, Taibei, Taiwan). Then the band intensities (optical density) of MBP or β-actin were analyzed by densitometry (density × area) using an Image-Pro Plus image analysis system (Media Cybernetics, Silver Spring, Maryland, USA), and the MBP/β-actin ratio was calculated. The results of the MBP/β-actin ratio in each group were standardized to the measurement results of the control (Con) group (as 100%).

Statistical analysis

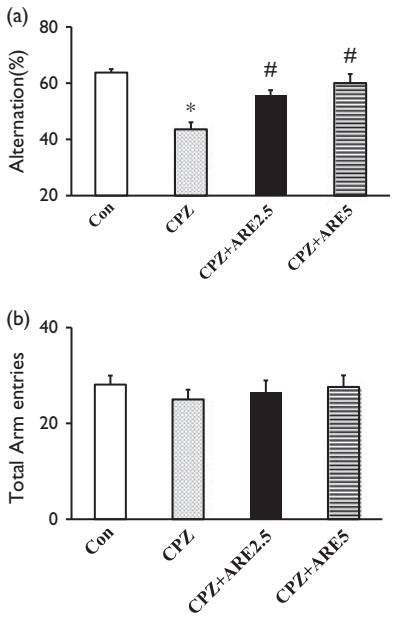
All results were expressed as mean ± SEM. The significance of differences was determined by one-way analysis of variance (ANOVA), followed by a Newman–Keuls post-hoc test for multiple comparisons. A *P*-value of less than 0.05 was regarded as statistically significant.

Results

Arecoline-attenuated spatial working memory impairment in the cuprizone mice

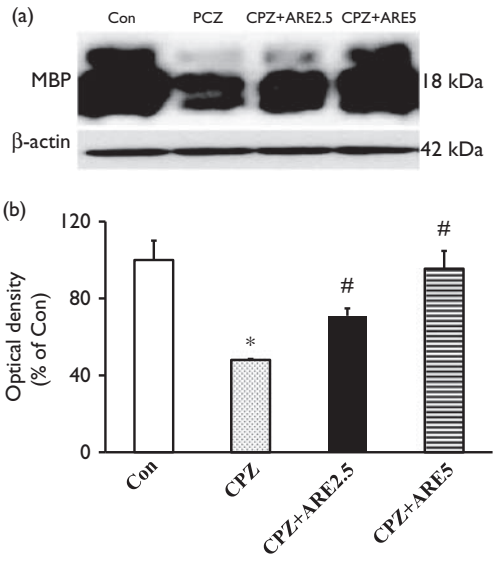
Spatial working memory performance in mice was assessed by recording spontaneous alternation behavior in a Y-maze test after CPZ or (and) arecoline treatment [12]. As shown in Fig. 1a, one-way ANOVA showed CPZ and arecoline produced a significant change in the alternation performance [$F(3,36) = 15.15$, $P < 0.0001$;

Fig. 1



Spatial working memory in the mice of Con, CPZ, CPZ + ARE2.5, and CPZ + ARE5 groups. Arecoline attenuated the decrease of spontaneous alternation behavior in the CPZ mice (a), and arecoline and CPZ had no effect on the number of total arm entries (b) during the Y-maze test in mice. Starting from 5 weeks old, the mice were fed with 0.2% CPZ or normal chow and the CPZ mice were administrated with arecoline (0, 2.5, or 5 mg/kg/day) in drinking water for 12 weeks. At the age of 17 weeks old following drug treatments, spatial working memory in mice was assessed by recording spontaneous alternation behavior in the Y-maze. Results are expressed as mean ± SEM ($n = 10$ in each group). ARE, arecoline hydrobromide; Con, control; CPZ, cuprizone. $^{*}P < 0.05$ versus Con; $^{\#}P < 0.05$ versus CPZ.

Fig. 2



(a) Representative western blot bands of MBP in the frontal cortex of mice in Con, CPZ, CPZ + ARE2.5, and CPZ + ARE5 groups. (b) Histogram showing the quantification of the immmunochemically reactive bands in the western blot of MBP in the frontal cortex of mice in Con, CPZ, CPZ + ARE2.5, and CPZ + ARE5 groups. Arecoline attenuated the decrease of MBP level in the frontal cortex of CPZ mice. Starting from 5 weeks old, the mice were fed with 0.2% CPZ or normal chow and the CPZ mice were administrated with arecoline (0, 2.5, or 5 mg/kg/day) in drinking water for 12 weeks. On the day after behavioral testing, the mice were sacrificed for MBP protein analysis. Results are expressed as mean ± SEM ($n = 7$ in each group). ARE, arecoline hydrobromide; Con, control; CPZ, cuprizone; MBP, myelin basic protein. $^{*}P < 0.05$ versus Con; $^{\#}P < 0.05$ versus CPZ.

Fig. 1a]. A post-hoc analysis revealed that alternation behavior in the CPZ mice was less than that in the Con mice and that arecoline significantly attenuated the decrease of alternation behavior in the CPZ mice (Fig. 1a).

There is no difference in the number of total arm entries among the Con, CPZ, CPZ + ARE2.5, and CPZ + ARE5 groups, and CPZ and arecoline had no effect on the number of total arm entries in the Y-maze test (Fig. 1b).

Arecoline increased the expression of myelin basic protein in the frontal cortex of cuprizone mice

MBP is a protein believed to be important in the process of myelination of nerves in the central nervous system, and the cerebral myelination was evaluated by the expression of MBP using western blot in mice [13]. Representative western blot bands of MBP in the frontal cortex of each group are shown in Fig. 2a. The optical densities of western blot bands were quantified. As shown in Fig. 2b, one-way ANOVA showed CPZ and arecoline produced a significant change on the level of MBP expression in the frontal cortex of mice [$F(3,24) = 11.46$, $P < 0.0001$]. A post-hoc analysis revealed that the MBP level in the frontal cortex of the CPZ mice was lower than that in the Con mice and that arecoline significantly increased the decrease of MBP level in the frontal cortex of CPZ mice (Fig. 2b).

Discussion

Consistent with previous studies that CPZ-induced memory impairment and demyelination in animal models [14–16], CPZ administration induced spatial working memory impairment in a Y-maze test and decreased MBP expression in the frontal cortex of mice in the present study. Furthermore, the present study reported arecoline-attenuated spatial working memory impairment, concurrent with attenuated demyelination related to vehicle-treated CPZ mice for the first time. Arecoline had no effect on the number of total arm entries, thus, the beneficial effect of arecoline on spatial working memory was not because of its effect on locomotion activity that may influence initial spontaneous alternation behavior. Our previous studies have showed that *A. catechu* nut extract in which arecoline is one of the main active ingredients could ameliorate cognitive decline by facilitating myelination processes in the frontal cortex in a CPZ-induced mouse demyelination model of schizophrenia [8,9]. *A. catechu* nut extract might ameliorate

schizophrenia symptoms including cognitive decline by targeting oligodendrocytes to prevent demyelination of white matter in the CPZ mice [8].

Copper chelator CPZ is neurotoxicant, which selectively disrupts the respiratory chain of oligodendrocyte, leading to oxidative stress and subsequent apoptosis [17]. CPZ-treated animals are well-accepted demyelination models that could induce schizophrenia-like behaviors including memory impairment, and thus, it can be used to induce mouse demyelination model of schizophrenia [8,14,16]. Preventing white matter injury in the frontal cortex prevented memory impairment in the CPZ mice [8,14,16], and this indicates that there might be a negative relationship between memory and brain white matter injury in the CPZ mice. Cognitive deficit is one of the clinical diagnostic characteristics in schizophrenia [18]. Although white matter injury and myelin abnormalities in the frontal cortex can be found in schizophrenia, the role of brain white matter injury in cognitive deficit in schizophrenia has not yet been resolved [19]. The current study by evaluating the effects of arecoline on spatial working memory and brain white matter injury in the CPZ mouse model of schizophrenia may help to reveal the role of brain white matter injury in cognitive deficit in schizophrenia.

In the present study, arecoline-attenuated spatial working memory impairment in the CPZ mice, and this is consistent with arecoline's capacity of muscarinic receptor 1 cholinergic agonist that are considered to have important roles in cognitive processes and memory improvement [20,21]. *A. catechu* nut extract and arecoline could be considered as a potential analgesic, anti-inflammatory, or antioxidant agents [5,10]. Myelin is produced by mature oligodendrocytes, and oligodendrocyte precursor cells are exceptionally susceptible to oxidative stress [19]. Therefore, arecoline, one of the main active ingredients in *A. catechu* nut extract, might prevent demyelination of cerebral white matter by protecting oligodendrocytes from oxidative stress. However, the protective and antioxidative effects of arecoline have been argued and are still uncertain, although chronic, low-dose intravenous arecoline can improve memory in Alzheimer's disease [10,21,22]. The neuroprotective mechanism of arecoline on the cerebral decrease of MBP and white matter injury, and whether the beneficial effect of arecoline on spatial working memory is directly due to arecoline's neuroprotective effect on cerebral white matter injury in the CPZ mice still need to be further investigated.

Conclusion

Together, the present study suggests that arecoline can attenuate spatial working memory impairment and cerebral demyelination in the CPZ mice, and indicate that cerebral demyelination may have a role in memory impairment in schizophrenia, and modulation of cerebral demyelination may be a useful strategy in schizophrenia treatment.

Acknowledgements

This work was mainly performed in the University of Alberta, and financially supported by Xiamen Municipal Bureau of Science and Technology (3502Z20164049), Xiamen Municipal Health and Family Planning Commission (3502Z20159019), and Fujian Provincial Department of Science and Technology (2017D0007).

Conflicts of interest

There are no conflicts of interest.

References

1 Hageman AT, Gabreëls FJ, de Jong JG, Gabreëls-Festen AA, van den Berg CJ, van Oost BA, *et al.* Clinical symptoms of adult metachromatic leukodystrophy and arylsulfatase A pseudodeficiency. *Arch Neurol* 1995; **52**:408–413.
2 Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, *et al.* White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch Gen Psychiatry* 2003; **60**:443–456.
3 Zhang R, He J, Zhu S, Zhang H, Wang H, Adilijiang A, *et al.* Myelination deficit in a phencyclidine-induced neurodevelopmental model of schizophrenia. *Brain Res* 2012; **1469**:136–143.
4 Grazioplene RG, Bearden CE, Subotnik KL, Ventura J, Haut K, Nuechterlein KH, *et al.* Connectivity-enhanced diffusion analysis reveals white matter density disruptions in first episode and chronic schizophrenia. *Neuroimage Clin* 2018; **18**:608–616.
5 Bhandare AM, Kshirsagar AD, Vyawahare NS, Hadambar AA, Thorve VS. Potential analgesic, anti-inflammatory and antioxidant activities of hydroalcoholic extract of *Areca catechu* L. nut. *Food Chem Toxicol* 2010; **48**:3412–3417.
6 Coppola M, Mondola R. Potential action of betel alkaloids on positive and negative symptoms of schizophrenia: a review. *Nord J Psychiatry* 2012; **66**:73–78.
7 Bales A, Peterson MJ, Ojha S, Upadhaya K, Adhikari B, Barrett B. Associations between betel nut (*Areca catechu*) and symptoms of schizophrenia among patients in Nepal: a longitudinal study. *Psychiatry Res* 2009; **169**:203–211.
8 Adilijiang A, Guan T, He J, Hartle K, Wang W, Li X. The protective effects of areca catechu extract on cognition and social interaction deficits in a cuprizone-induced demyelination model. *Evid Based Complement Alternat Med* 2015; **2015**:426092.
9 Adilijiang A, Guan T, Xu ZZ, Hartle K, Zhang YB, Wang WQ, *et al.* The aqueous fraction of areca catechu nut ameliorates demyelination in prefrontal cortex-induced depressive symptoms and cognitive decline through brain-derived neurotrophic factor-cyclic adenosine monophosphate response element-binding activation. *Chin J Integr Med* 2016. DOI:10.1007/s11655-016-2455-8.
10 Papke RL, Horenstein NA, Stokes C. Nicotinic activity of arecoline, the psychoactive element of 'Betel Nuts', suggests a basis for habitual use and anti-inflammatory activity. *PLoS One* 2015; **10**:e0140907.
11 Verma S, Kumar A, Tripathi T. Muscarinic and nicotinic acetylcholine receptor agonists: current scenario in Alzheimer's disease therapy. *J Pharm Pharmacol* 2018; **70**:985–993.
12 He J, Luo H, Yan B, Yu Y, Wang H, Wei Z, *et al.* Beneficial effects of quetiapine in a transgenic mouse model of Alzheimer's disease. *Neurobiol Aging* 2009; **30**:1205–1216.
13 Zhang Y, Xu H, Jiang W, Xiao L, Yan B, He J, *et al.* Quetiapine alleviates the cuprizone-induced white matter pathology in the brain of C57BL/6 mouse. *Schizophr Res* 2008; **106**:182–191.
14 Zhang J, Yang L, Fang Z, Kong J, Huang Q, Xu H. Adenosine promotes the recovery of mice from the cuprizone-induced behavioral and morphological changes while effecting on microglia and inflammatory cytokines in the brain. *J Neuroimmune Pharmacol* 2018; **13**:412–425.
15 Omotoso GO, Gbadamosi IT, Afolabi TT, Abdulwahab AB, Akinlolu AA. Ameliorative effects of Moringa on cuprizone-induced memory decline in rat model of multiple sclerosis. *Anat Cell Biol* 2018; **51**:119–127.
16 Cui C, Wang J, Mullin AP, Caggiano AO, Parry TJ, Colburn RW, *et al.* The antibody rHIgM22 facilitates hippocampal remyelination and ameliorates

memory deficits in the cuprizone mouse model of demyelination. *Brain Res* 2018; **1694**:73–86.
17 Jakovac H, Grubic Kezele T, Radosevic-Stasic B. Expression profiles of metallothionein I/II and megalin in cuprizone model of de- and remyelination. *Neuroscience* 2018; **388**:69–86.
18 Jahshan C, Rassovsky Y, Green MF. Enhancing neuroplasticity to augment cognitive remediation in schizophrenia. *Front Psychiatry* 2017; **8**:191.
19 Maas DA, Valles A, Martens GJM. Oxidative stress, prefrontal cortex hypomyelination and cognitive symptoms in schizophrenia. *Transl Psychiatry* 2017; **7**:e1171.
20 Chandra JN, Malviya M, Sadashiva CT, Subhash MN, Rangappa KS. Effect of novel arecoline thiazolidinones as muscarinic receptor 1 agonist in Alzheimer's dementia models. *Neurochem Int* 2008; **52**:376–383.
21 Soncrant TT, Raffaele KC, Asthana S, Berardi A, Morris PP, Haxby JV. Memory improvement without toxicity during chronic, low dose intravenous arecoline in Alzheimer's disease. *Psychopharmacology (Berl)* 1993; **112**:421–427.
22 Liu YJ, Peng W, Hu MB, Xu M, Wu CJ. The pharmacology, toxicology and potential applications of arecoline: a review. *Pharm Biol* 2016; **54**:2753–2760.