Beneficial synergistic effects of microdose lithium with pyrroloquinoline quinone in an Alzheimer’s disease mouse model

Lei Zhao a,1, Neng Gong b,1, Meng Liu c, Xiaoli Pan a, Shaoming Sang c, Xiaojing Sun a, Zhe Yu b, Qi Fang b, Na Zhao a, Guoqiang Fei a, Lirong Jin a, Chunjiu Zhong a,c,*, Tianle Xu b

a Department of Neurology, Zhongshan Hospital, Fudan University, Shanghai, China
b Institute of Neuroscience, State Key Laboratory of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
c State Key Laboratory of Medical Neurobiology, Institute of Brain Science, Shanghai Medical College, Fudan University, Shanghai, China

1. Introduction

Alzheimer’s disease (AD) is a complicated, neurodegenerative disorder involving multifactorial pathogenesis and still lacks effective clinical treatment. Recent studies show that lithium exerts disease-modifying effects against AD. However, the intolerant side effects at conventional effective dosage limit the clinical use of lithium in treating AD. To explore a novel AD treatment strategy with microdose lithium, we designed and synthesized a new chemical, tri-lithium pyrroloquinoline quinone (Li3PQQ), to study the synergistic effects of low-dose lithium and pyrroloquinoline quinone, a native compound with powerful antioxidation and mitochondrial amelioration. The results showed that Li3PQQ at a relative low dose (6 mg/kg) exhibited more powerful effects in restoring the impairment of learning and memory, facilitating hippocampal long-term potentiation, and reducing cerebral amyloid deposition and phosphorylated tau level in APP/PS1 transgenic mice than that of lithium chloride at both low and high dose (5 and 100 mg/kg). We further found that Li3PQQ inhibited the activity of glycogen synthase kinase-3 and increased the activity of β-amyloid-binding alcohol dehydrogenase, which might underlie the beneficial effects of Li3PQQ on APP/PS1 transgenic mice. Our study demonstrated the efficacy of a novel AD therapeutic strategy targeting at multiple disease-causing mechanisms through the synergistic effects of microdose lithium and pyrroloquinoline quinone.

1 These authors contributed equally to this work.

© 2014 Elsevier Inc. All rights reserved.
Aldrich Corporate (St. Louis, MO, USA). Twenty-week-old mice chemicals in these experiments were purchased from Sigma—Eisai Pharmaceutical Ltd (Tokyo, Japan). All other drugs and by Shanghai Rixin Biomedical Company. Donepezil was purchased administrated by gastric gavage daily in 8-week-old C57BL/6 mice on a 12/12-hour light-dark cycle in a temperature-controlled room littermate wild-type mice). The mice were individually housed in (42.07 polymerase chain reaction analysis of tail biopsies. The weights of type control were produced by the Model Animal Research Cen-

2. Methods

2.1. Mice

All mouse care and experimental procedures were approved by Medical Experimental Animal Administrative Committee of Fudan University, and by the Institutional Animals Care and Use Committee of the Institute of Neuroscience, Shanghai Institutes for Biological Science, Chinese Academy of Science. APP/PS1 transgenic mice used in this study were described previously (Pan et al., 2010), which were obtained from the Jackson Laboratory (strain name, B6C3-Tg [APPswe, PSEN1dE9] 85Dbo/J; stock number 004462). All male APP/PS1 transgenic mice and littermate wild-type control were produced by the Model Animal Research Center of Nanjing University, China. The genotype was confirmed by polymerase chain reaction analysis of tail biopsies. The weights of mice before the drugs administrations were from 32.3 g to 52.5 g (42.07 ± 0.54 g for APP/PS1 transgenic mice, 38.62 ± 0.70 g for littermate wild-type mice). The mice were individually housed in Plexiglas cages with free access to food and water and maintained on a 12/12-hour light-dark cycle in a temperature-controlled room (22 °C).

2.2. Lithium level test by inductively coupled plasma mass spectrometry

Li3PQQ (6 and 12 mg/kg, dissolved in ddH2O), LiCl (5 and 100 mg/kg, dissolved in ddH2O), and ddH2O were respectively administrated by gastric gavage daily in 8-week-old C57BL/6 mice (SLAC Laboratory animal, Shanghai, China, male, n = 8 for each group) for single and continuous treatment. Single treatment means that mice were sacrificed 1 hour after drug administration in the first time, and continuous treatment means that mice were sacrificed 1 hour after drug administration in the 10th day. After using 55% nitric acid (TAMAPURE-AA-100, Japan) to decompose and microwave digested the blood and brain, we detected the concentration of lithium by inductively coupled plasma mass spectrometry (ICP-MS) (using Agilent 7100XICPMS, Palo Alto, CA, USA).

2.3. Drugs and treatment

Li3PQQ was provided by Shanghai Rixin Biomedical Company (Shanghai, China) and its structure was determined by mass spectrometry (Supplementary Fig. 1A). The purity used in the present study was over 99.0% pure as determined by high performance liquid chromatography (Supplementary Fig. 1B). Tri-sodium pyrrolo-quinoline quinone (Na3PQQ) was also provided by Shanghai Rixin Biomedical Company. Donepezil was purchased from Eisai Pharmaceutical Ltd (Tokyo, Japan). All other drugs and chemicals in these experiments were purchased from Sigma—Aldrich Corporate (St. Louis, MO, USA). Twenty-week-old mice were randomly assigned to 10 groups, with n = 16 per group including 7 groups of APP/PS1 transgenic and 3 groups of littermate wild-type control mice. Of APP/PS1 transgenic mice, 2 groups received different doses (6, 12 mg/kg/d) of Li3PQQ dissolved in ddH2O at a volume ratio of 0.2 mL/10 g by gastric gavage daily for 8 weeks; 2 groups received LiCl (dissolved in ddH2O, 5 and 100 mg/kg/d) and 1 group received Na3PQQ (dissolved in ddH2O, 14 mg/kg/d); 1 positive control group received donepezil (dissolved in ddH2O, 1.5 mg/kg/d), a cholinesterase inhibitor. Of littermate wild-type mice, 2 groups received Li3PQQ (12 mg/kg/d) and LiCl (100 mg/kg/d), respectively. Solvent control groups of APP/PS1 transgenic and littermate wild-type mice were treated with equal volume of ddH2O. The time for daily drug administration lasted for 8 weeks.

2.4. Morris water maze

The Morris water maze was conducted according to the protocol described in our previous study (Pan et al., 2010). The training session lasted for 5 days (4 trials per day). A trial was terminated when the mouse had climbed onto the escape platform or when 60 seconds had elapsed. Each mouse was allowed to stay on the platform for 30 seconds. The probe test was performed on the sixth day. Swimming paths in probe test were monitored using an automatic tracking system. This system was used to record the swimming trace and calculate the latency to the platform and the time spent in each quadrant. Crossing times represent the times the animal crossed the position where the platform was placed during the learning session. The target quadrant occupancy represents the percent time the mouse spent in the quadrant where the platform was placed during the learning session (represented as “T”), whereas the opposite quadrant occupancy means the percent time the mouse spent in the quadrant opposite to the target quadrant (represented as “O”).

2.5. Electrophysiology

Transverse hippocampal slices (350-μm thick) were prepared from APP/PS1 transgenic mice. The protocol was used as described previously (Zhao et al., 2008). The field excitatory postsynaptic potentials (fEPSPs) were recorded via a glass micropipette filled with artificial cerebrospinal fluid (1–3 MΩ) placed in the stratum radiatum. Stimuli (0.1 ms duration) were delivered every 30 seconds. Test pulses were recorded for 10–20 minutes before data collection to ensure stability of the response. To induce long-term potentiation (LTP), the stimulation intensity under control conditions was adjusted to evoke approximately 30%–50% of the maximum response. Theta burst stimulation was used to induce LTP, consisted of 5 bursts (4 pulses, 100 Hz) delivered at an inter-burst interval of 200 ms and repeated once at 20 seconds. The slope of fEPSPs was determined by Clampfit 9.0 software.

2.6. Immunohistochemistry

Paraformaldehyde-fixed, free-floating brain sections (30 μm) were immuno-chemically stained using anti-Aβ 1-42 antibody (Covance, Princeton, NJ, USA) and anti-phosphorylated tau Ser396 antibody (Cell Signaling Technology, Danvers, MA, USA). The quantitative analysis was conducted as previously described (Oddo et al., 2004) by a computerized image NIKON E600FN NeuroLucida system (Nikon Corporation, Kyoto, Japan). The plaque numbers and area (40×) were measured as the total number and area of per section per mouse by Image-Pro Plus 5.0 (Media Cybernetics, Bethesda, MD, USA). The phosphorylated tau-positive cell numbers were counted in 8 cortex fields (200×)
per section per mouse. There were 6 mice per group and we chose 6 sections per mouse.

2.7. β-Amyloid peptide measurements by enzyme-linked immunosorbent assay

Aβ enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen Human β-Amyloid 1-42 Colorimetric ELISA, Invitrogen, Grand Island, NY, USA) was used according to the manufacturer’s instructions.

2.8. Western blotting

The protocols, samples, and antibodies of the Western blotting experiments for glycogen synthase kinase-3α (GSK3α), GSK3β, phospho-GSK3α, phosphor-GSK3β, Aβ-binding alcohol dehydrogenase (ABAD, 1:1000, abcam, Cambridge, UK) and glyceraldehyde 3-phosphate dehydrogenase were prepared as before described (Pan et al., 2010).

2.9. Enzyme activities assay

Activities of GSK3, GSK3α, GSK3β, and ABAD were determined by the GENMED kit (Genmed, Shanghai, China) according to the manufacturer’s instructions. The activity was measured as the difference between the absorbance value at 0 and 5 minutes. The assay was repeated twice for each sample.

2.10. Statistical analysis

All data are shown as the mean ± standard error of the mean, with statistical significance assessed by Student t-test. All statistical analyses were performed using GraphPad prism 5.01.

3. Results

3.1. Blood and brain lithium levels after Li3PQQ and LiCl administration

We used ICP-MS to test blood and brain lithium levels after single and continuous administration of Li3PQQ (6 and 12 mg/kg) and LiCl (5 and 100 mg/kg) by gastric gavage daily in 8-week-old C57BL/6 mice. The lithium dosage of 12 mg/kg Li3PQQ is similar to that of 5 mg/kg LiCl as calculated by lithium molarity. Blood and brain lithium levels in groups with single and continuous administration of 100 mg/kg LiCl were much higher than those in groups with 5 mg/kg LiCl and 12 mg/kg Li3PQQ (Fig. 1A and B). When standardizing the administration dosage by lithium molarity, blood and brain lithium levels in group with single administration of 12 mg/kg Li3PQQ were not significantly different from those in group with single administration of 5 mg/kg LiCl. However, blood lithium level in group with continuous administration of 5 mg/kg LiCl was significantly elevated as compared with that in group with single administration of 5 mg/kg LiCl. Blood lithium level in group with single administration of 12 mg/kg Li3PQQ was not significantly
different from that in group with continuous administration of 12 mg/kg Li3PQQ. Brain lithium levels in groups with continuous administration of 5 mg/kg LiCl were significantly increased as compared with those in groups with continuous administration of 12 mg/kg Li3PQQ (Fig. 1D).

3.2. Li3PQQ significantly improved cognitive function of APP/PS1 transgenic mice but not littermate wild-type mice

Since the most important requirement for AD treatment is the improvement of cognitive function, we first tested the learning and memory ability of APP/PS1 transgenic mice after Li3PQQ treatment. In the Morris water maze test, after 8-week vehicle administrations, APP/PS1 transgenic mice showed significantly longer escape latency in finding the platform as compared with wild-type littermates from the third training day to the sixth day. (Fig. 2A, the third day, \( p = 0.021 \); the fourth day, \( p = 0.004 \); the fifth day, \( p < 0.006 \); the sixth day, \( p < 0.001; n = 10 \) for each group). During the probe test, APP/PS1 transgenic mice also showed significantly fewer times of crossing the platform location (Fig. 2C) and lower preference for the target quadrant (Fig. 2D), indicating the impaired spatial memory of APP/PS1 transgenic mice.
Li3PQQ dose-dependently (6 and 12 mg/kg, n = 10 for each group) improved the spatial learning and memory of APP/PS1 transgenic mice as represented by the reduced escape latency in finding the platform during the training sessions (Fig. 2A), increased crossing times (p = 0.005 for 6 mg/kg, p = 0.002 for 12 mg/kg, Fig. 2C) and target quadrant occupancy (p = 0.150 for 6 mg/kg, p = 0.023 for 12 mg/kg, Fig. 2D) in the probe test. Although LiCl also showed the beneficial effect both at the dosages of 5 and 100 mg/kg, the effect was significantly inferior to that of 12 mg/kg Li3PQQ (Fig. 2A and B). We found that Li3PQQ (12 mg/kg) showed better performance in the improvement of cognitive function compared with that in LiCl (5 mg/kg, n = 10) group (escape latency p < 0.001, crossing times p = 0.020, Fig. 2A and C), and there were also significant differences in the escape latency for Li3PQQ (12 mg/kg) compared with that in LiCl (100 mg/kg, n = 10) group (p = 0.044). The POQ control group (Na3PQQ 14 mg/kg, n = 10) exhibited only a tendency to improve the learning and memory ability in APP/PS1 transgenic mice, but no significant difference compared with the vehicle group (Fig. 2B–D). As a positive control, donepezil (1.5 mg/kg, n = 10) also restored the impaired spatial learning and memory of APP/PS1 transgenic mice (Fig. 2B–D). These results indicate that, similar to donepezil and LiCl, Li3PQQ has significant beneficial effects in treating the cognitive impairment of APP/PS1 transgenic mice, even in a much lower dosages (6, 12 mg/kg) than that of LiCl (100 mg/kg).

We then tested the effect of Li3PQQ on the learning and memory ability of the littermate wild-type mice by Morris water maze test. Both Li3PQQ (12 mg/kg, n = 7) and LiCl (100 mg/kg, n = 7) showed no significant effects on the escape latency in finding the platform (the sixth day: Li3PQQ, p = 0.377; LiCl, p = 0.187; Fig. 2E), the crossing times (Li3PQQ, p = 0.224; LiCl, p = 0.141; Fig. 2F) and the target quadrant (Li3PQQ, p = 0.074; LiCl, p = 0.675; Fig. 2G), indicating that Li3PQQ does not affect the normal learning and memory ability of wild-type mice.

To further examine the synaptic mechanisms underlying the enhanced learning and memory in APP/PS1 transgenic mice treated with Li3PQQ, we studied hippocampal synaptic plasticity by recording fEPSPs in the CA1 area of hippocampal slices from APP/PS1 transgenic mice. Chronic treatment with Li3PQQ (12 mg/kg) or LiCl (100 mg/kg) had no effects on basal excitatory synaptic transmission, as shown by similar input-output relation of fEPSPs, and similar paired-pulse facilitation of fEPSPs at 3 different inter-pulse intervals (50, 100, 150 ms; Fig. 3A–C). TBS (5 bursts [4 pulses at 100 Hz] delivered at 5 Hz and repeated once at 20 seconds) of Schaffer collateral pathway induced a persistent elevation in the slope of fEPSPs in APP/PS1 transgenic mice, indicating the induction of LTP (Fig. 3D). We found that either chronic Li3PQQ or LiCl administration significantly increased the magnitude of TBS-induced LTP (vehicle, 127% ± 5% of the baseline level, n = 16; Li3PQQ 12 mg/kg, 147% ± 8% of the baseline level, n = 12, p = 0.022; LiCl 100 mg/kg, 143% ± 7% of the baseline level, n = 8, p = 0.044; Fig. 3E and F), suggesting that the enhanced synaptic plasticity may underlie the restored learning and memory.
function of APP/PS1 transgenic mice following Li$_3$PQQ or LiCl treatment.

3.3. Li$_3$PQQ reduced brain Aβ deposition and tau phosphorylation

By immunochemical staining in cortical slices, we analyzed the number and area of amyloid plaques in APP/PS1 transgenic mice after Li$_3$PQQ administration. We found that chronic treatment of Li$_3$PQQ significantly reduced both the number of amyloid plaques ($p = 0.004$ for 6 mg/kg, $p < 0.001$ for 12 mg/kg, Fig. 4A–G) and area of amyloid plaques ($p = 0.002$ for 6 mg/kg, $p < 0.001$ for 12 mg/kg, Fig. 4A–F, H). By ELISA of cortical tissue, we examined the Aβ$_{1-42}$ level. Li$_3$PQQ significantly reduced the level of Aβ$_{1-42}$ ($p = 0.001$ for 6 mg/kg, $p < 0.001$ for 12 mg/kg, Fig. 4A–F, H). Importantly, 12 mg/kg Li$_3$PQQ was significantly more effective than LiCl (100 mg/kg) in reducing the number ($p < 0.001$, Fig. 4G) and area ($p = 0.003$, Fig. 4H) of amyloid plaques, as well as reducing the Aβ$_{1-42}$ level ($p = 0.039$, Fig. 4I). Furthermore, Li$_3$PQQ (both 6 and 12 mg/kg) and LiCl (100 mg/kg) significantly reduced the number of phosphorylated tau-positive cells, as shown by immunochemical staining in cortical slices ($p < 0.001$ for all groups, Fig. 5B–H). By Western blotting, we found that tau phosphorylation at serine 396 was significantly reduced in the Li$_3$PQQ and LiCl administered groups ($p = 0.026$ for LiCl group, $p < 0.001$ for Li$_3$PQQ 6 mg/kg group, $p < 0.001$ for Li$_3$PQQ 12 mg/kg group, Fig. 5I–J).

3.4. Li$_3$PQQ inhibited activities of GSK3α/GSK3β and increased the activity of ABAD

Lithium is a classic inhibitor of GSK3, a multifaceted protein that links Aβ production and tau phosphorylation by dual pathways (Hong et al., 1997; Small and Duff, 2008). To examine the possible mechanisms by which Li$_3$PQQ exerts its effects in preventing cognitive impairment and pathologic alterations of AD, we first examined the expression and activity of GSK3 in the cortex after chronic drug administration. The phosphorylation levels of GSK3α (Ser21) and GSK3β (Ser9) were significantly higher in wild-type vehicle mice than those of APP/PS1 transgenic vehicle mice ($p < 0.001$ for GSK3α, Fig. 6A; $p = 0.004$ for GSK3β, Fig. 6B), indicating reduced activities of both enzymes. Li$_3$PQQ dose-dependently increased the ratio of phospho-GSK3α (Ser21)/GSK3α ($p = 0.032$ for 6 mg/kg, $p = 0.007$ for 12 mg/kg, Fig. 6A) and of phospho-GSK3β (Ser9)/GSK3β (p < 0.001 for both 6 mg/kg and 12 mg/kg, Fig. 6B). Similarly, Li$_3$PQQ at both 6 and 12 mg/kg significantly reduced the enzyme activity of total GSK3 (p < 0.030 for 6 mg/kg, p < 0.001 for 12 mg/kg, Fig. 6E), as well as that of GSK3α (p = 0.037 for 6 mg/kg, p = 0.005 for 12 mg/kg, Fig. 6C) and GSK3β (p = 0.045 for 6 mg/kg, p < 0.001 for 12 mg/kg, Fig. 6D). As a positive control, LiCl also significantly increased the phosphorylation levels of GSK3α (p = 0.008), GSK3β (p = 0.002), and inhibited the enzymatic activities of total GSK3, GSK3α, and GSK3β (p < 0.001 for all groups, Fig. 6). On the contrary, donepezil had no effect on the phosphorylation levels of GSK3α and GSK3β (p > 0.05 for all groups, Fig. 6A and B), and did...
not affect the enzymatic activities of total GSK3, GSK3α, or GSK3β (p > 0.05 for all groups, Fig. 6C and E). These results indicate that chronic Li₃PQQ treatment significantly enhanced the phosphorylation of both GSK3α and GSK3β, and reduced their enzymatic activities, indicative of a possible mechanism underlying the beneficial effects of Li₃PQQ in APP/PS1 transgenic mice.

ABAD is a main mitochondrial alcohol dehydrogenase. Aβ binds to ABAD and inhibits the ABAD activity, underlying Aβ-induced oxidative toxicity (Lustbader et al., 2004; Yao et al., 2011). As a cofactor of alcohol dehydrogenase, PQQ might protect mitochondria from oxidative damage by affecting the activity of ABAD in AD. Thus, we examined the expression and activity of ABAD after drug treatments. There were no differences in ABAD protein expression between wild-type vehicle mice and APP/PS1 transgenic vehicle mice in the vehicle groups, and between groups of vehicle control and drug treatment (all p > 0.05, Fig. 7A). However, the ABAD activity of wild-type vehicle mice was significantly higher than that of vehicle-treated APP/PS1 transgenic mice (p = 0.006, Fig. 7B). Li₃PQQ significantly increased the enzymatic activity of ABAD (p = 0.073 for 6 mg/kg, p = 0.022 for 12 mg/kg, Fig. 7B). LiCl and donepezil did not affect the ABAD activity (p > 0.05 for all groups, Fig. 7B), suggesting a unique lithium-independent mechanism of Li₃PQQ in regulating ABAD activity.

4. Discussion

This study revealed the effects of a newly synthesized compound, Li₃PQQ in APP/PS1 transgenic mice and the possible underlying mechanisms. It has been known that lithium has a potential disease-modifying effect against AD (Forlenza et al., 2011; Kessing et al., 2008), but the clinical use is restricted by the toxicity of high-dose lithium, such as the generally used LiCl and lithium carbonate. Li₃PQQ is composed by lithium and PQQ, and the molarity of lithium at 12 mg/kg Li₃PQQ is similar to that of 5 mg/kg LiCl. By examining blood and brain lithium levels using ICP-MS, the most accurate method of detecting lithium level, we found that single administration of 5 mg/kg LiCl and 12 mg/kg Li₃PQQ did not exhibit significant differences of blood and brain lithium levels, suggesting that Li₃PQQ has a similar absorption rate to that of LiCl. However, continuous administration of 5 mg/kg LiCl had a more
significant effect in increasing blood and brain lithium levels than that of continuous administration of 12 mg/kg, suggesting a lower lithium accumulative effect of Li3PQQ. Excessive high lithium levels in blood and brain underlies the basis of the toxicity. Thus, the tolerance of chronic administration of Li3PQQ may be better than that of LiCl.

**Fig. 6.** The effects of Li3PQQ on the activities of GSK3. (A, B) Representative Western blots and statistical results showing the effects of different drugs on the ratios of brain phospho-GSK3α (Ser21) to total GSK3α and phospho-GSK3β (Ser9) to total GSK3β. (C–E) Statistical results showing the effects of different treatments on the enzymatic activities of total GSK3, GSK3α, and GSK3β. n = 6 for each group. *p < 0.05, **p < 0.01; NS, no significant difference. Values represent means ± SEM; compared with vehicle control group of APP/PS1 transgenic mice (Student t-test). Abbreviations: Li3PQQ, tri-lithium pyrroloquinoline quinone; SEM, standard error of the mean.

**Fig. 7.** The effects of Li3PQQ on the expression and activity of ABAD. (A) Representative Western blot and statistical results showing that none of the treatments affected ABAD expression. (B) Statistical results showing the effects of different drugs on ABAD activity; n = 6 for each group. *p < 0.05, **p < 0.01; NS, no significant difference. Values represent means ± SEM; compared with vehicle control group of APP/PS1 transgenic mice (Student t-test). Abbreviations: ABAD, Aβ-binding alcohol dehydrogenase; Li3PQQ, tri-lithium pyrroloquinoline quinone; SEM, standard error of the mean.
Facing so many drug trials failed, another more important question is the efficacy of Li3PQQ against AD. Our study showed that Li3PQQ reversed cognitive impairment of AD mouse model, as represented by increased crossing times, shortened escape latency, and enhanced target quadrant occupancy in Morris water maze test (Fig. 2), as well as the electrophysiological evidence of hippocampal LTP facilitation (Fig. 3). Importantly, Li3PQQ at a relative low concentration (6 or 12 mg/kg) exhibited much stronger pharmacologic effects than that of 5 mg/kg LiCl in the cognitive improvement, similar to that of LiCl at the conventional used high dose (100 mg/kg). Because Li3PQQ and LiCl did not exhibit significant effects on cognitive function of the wild-type littermates, Li3PQQ and LiCl restored impaired cognitive ability of APP/PS1 mice probably through modifying pathologic alterations of the brains. Our study demonstrated this possibility that Li3PQQ (6 or 12 mg/kg) and LiCl (100 mg/kg) significantly decreased brain Aβ deposition (Fig. 4) and phosphorylated tau level (Fig. 5). Furthermore, the beneficial effects of Li3PQQ at 12 mg/kg are much better than that of high-dose LiCl at 100 mg/kg in alleviating the pathologic alterations. Taken together, Li3PQQ showed a much more powerful beneficial effects in treating the cognitive impairment and pathologic alterations than that of low-dose LiCl with the similar lithium molarity, and even better than that of clinical used high-dose LiCl in some respects, indicating a potential AD treating strategy with microdose lithium.

It has been reported that lithium exerts its effect in modifying AD progression through inhibiting GSK3 activity. A growing body of evidence strongly indicates that GSK3 is involved in AD pathogenesis through dual pathway to modulate Aβ production and tau phosphorylation (Hong et al., 1997; Phiel et al., 2003; Rockenstein et al., 2007; Small and Duff, 2008). Indeed, we also found that Li3PQQ significantly inhibited GSK3 activities (Fig. 6). Mitochondrial dysfunction has also been demonstrated to contribute to AD pathogenesis (Bishopol et al., 2010). The inhibition of ABAD enzymatic activity because of ABAD-Aβ interaction is one of main causes of mitochondrial dysfunction in AD (He et al., 2003; Kasahara and Kato, 2003). Furthermore, oxidative stress is also an important contributor to AD pathogenesis (Smith et al., 1996). The previous study has showed that PQQ, a newly identified redoxcofactor vitamin (Kasahara and Kato, 2003), plays a potential role in scavenging reactive oxygen species and attenuating oxidative stress (Misra et al., 2004; 2012). We also found that Li3PQQ significantly enhanced ABAD activity (Fig. 7), suggesting the possible role of PQQ. However, LiCl (5 mg/kg) or Na3PQQ (14 mg/kg) alone had little effects in treating AD, but Li3PQQ (12 mg/kg) with the similar molarity for both lithium and PQQ showed much significant beneficial effects, suggesting that besides their respective effects on GSK3 and ABAD, lithium and PQQ may exert synergistic effects through other unknown mechanisms, which need to be further studied. Taken together, Li3PQQ should be a promising candidate drug targeting at multiple disease-causing mechanisms through the synergistic effects of microdose lithium and PQQ.

Disclosure statement

The corresponding author Chunjiu Zhong holds shares of Shanghai Rixin Biomedical Company and other authors declare no conflicts of interest. Other authors have no actual or potential conflicts of interest.

Acknowledgements

The authors thank Dr Xiang Yu for critical reading and discussions of the manuscript. This study is supported by 973 project (grant no 2011CB904000), the National Natural Science Foundation of China (grant no 81071019), key fund for developing new drugs from State Scientific & Technological Ministry of China (grant no 2012ZX09102-003), fund for outstanding academic leaders in Shanghai (11XD1401500), and fund for International cooperation in Shanghai (10430709600).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2014.06.003.

References


