Neuroprotective mechanism of Kai Xin San: upregulation of hippocampal insulin-degrading enzyme protein expression and acceleration of amyloid-beta degradation

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Graphical Abstract

Pathway of Kai Xin San on protecting nerves and improving cognitive function

Kai Xin San: Radix Ginseng + Poria + Radix Polygalae + Acorus Tatarinowii Rhizome

Accelerating amyloid-β degeneration

Increasing insulin-degrading enzyme protein expression

Alzheimer’s disease rat model

Abstract

Kai Xin San is a Chinese herbal formula composed of Radix Ginseng, Poria, Radix Polygalae and Acorus Tatarinowii Rhizome. It has been used in China for many years for treating amnesia. Kai Xin San ameliorates amyloid-β (Aβ)-induced cognitive dysfunction and is neuroprotective in vivo, but its precise mechanism remains unclear. Expression of insulin-degrading enzyme (IDE), which degrades Aβ, is strongly correlated with cognitive function. Here, we injected rats with exogenous Aβ(42) (200 μM, 5 μL) into the hippocampus and subsequently administered Kai Xin San (0.54 or 1.08 g/kg/d) intragastrically for 21 consecutive days. Hematoxylin-eosin and Nissl staining revealed that Kai Xin San protected neurons against Aβ-induced damage. Furthermore, enzyme-linked immunosorbent assay, western blot and polymerase chain reaction results showed that Kai Xin San decreased Aβ(42) protein levels and increased expression of IDE protein, but not mRNA, in the hippocampus. Our findings reveal that Kai Xin San facilitates hippocampal Aβ degradation and increases IDE expression, which leads, at least in part, to the alleviation of hippocampal neuron injury in rats.

Key Words: nerve regeneration; neurodegeneration; traditional Chinese medicine; Kai Xin San; insulin-degrading enzyme; amyloid-β; Alzheimer’s disease; Chinese herbal compound; Aβ-degrading enzymes; neurons; Radix Ginseng; Radix Polygalae; Acorus Tatarinowii Rhizoma; neural regeneration

Introduction

Amyloid-β (Aβ), a key biomolecule in senile plaques, plays a central role in the pathology of Alzheimer’s disease (AD) (Amici et al., 2016; Chai et al., 2016; Zhang et al., 2016). In the brain, under physiological conditions, Aβ is constantly generated from amyloid precursor protein and cleaved by β- and γ-secretases (Li et al., 2016; Niu et al., 2016). It is widely accepted that abnormal accumulation of Aβ, containing Aβ(42) and Aβ(40), participates in the pathology of AD (Awasthi et al., 2016; Wang, 2016). Aβ(42), which is hydrophobic and prone to aggregation, seems to be more neurotoxic than Aβ(40) in AD. It is mostly degraded by Aβ-degrading enzymes, but an imbalance between its generation and degradation leads to pathological Aβ accumulation in AD (Saido, 2013). Howev-
nder a 12-hour dark/light cycle. The rats received a standard
in an animal laboratory at 22 ± 2°C and 60 ± 3% humidity,
males), weighing 200–240 g, were provided by the Laboratory
Committee, Heilongjiang University of Chinese Medicine (No.
KXS has been shown to improve learning and memory in
several paradigms, including models of AD (Hu et al., 2013;
Chu et al., 2016). KXS ameliorates the cognitive dysfunc-
tion induced by Aβ in vivo (Li et al., 2013) and seems to be
involved in the protective effects targeting the neuropatho-
logical cascade after Aβ deposition. However, it is not known
whether KXS directly mediates the catabolic mechanism of
Aβ to reduce Aβ accumulation, though research on the cata-
obolic mechanism of Aβ is ongoing. Identifying whether KXS
can affect the expression of Aβ-degrading enzymes will pro-
vide important insight into treatment options for AD. To this
end, we investigated the efficacy of KXS on Aβ degradation,
and explored its mechanism in light of the catabolic role of
IDE in Aβ metabolism. We demonstrate that KXS accelerates
Aβ degradation by increasing IDE expression in the brain.

Materials and Methods

Animals
230 specific-pathogen-free Wistar rats (115 males, 115 fe-
males), weighing 200–240 g, were provided by the Laboratory
Animal Center, Heilongjiang University of Chinese Medicine,
China (license No. SYXK (Hei) 2013-012). Rats were housed
in an animal laboratory at 22 ± 2°C and 60 ± 3% humidity,
under a 12-hour dark/light cycle. The rats received a standard
diet and free access to water. The animal facilities and proto-
cols were approved by the Institutional Animal Care and Use
Committee, Heilongjiang University of Chinese Medicine (No.
2014HZYLL-018). All procedures conformed with the Na-
tional Institutes of Health Guide for the Care and Use of Lab-
oratory Animals (NIH Publications No. 8023, revised, 1978).

Drugs and KXS extraction
Radix Ginseng (Renshen), Poria (Fuling), Radix Polygalae
(Yuanzhi) and Acorus Tatarinowii Rhizoma (Shichangpu)
were purchased from Harbin Tongrentang Drug Company
(Harbin, China). From these components, we prepared KXS
as described previously (Liu et al., 2014). In brief, the four
dried raw herbs were mixed together in a weight ratio of 3:3:2:2
(Radix Ginseng 60 g, Poria 60 g, Radix Polygalae 40 g, Acorus
Tatarinowii Rhizoma 40 g), and decocted/extracted by reflux-
ing for 1.5 hours in 2,000 mL boiling 60% ethanol (1:10, w/v).
The extracts were passed through filter paper, dried under a
vacuum and stored at −80°C. The extracts were mainly char-
acterized by 75 chemical constituents, identified by ultra-per-
formance liquid chromatography tandem mass spectrometry.

Aβ and KXS administration
200 rats were equally and randomly allocated to four groups:
control, model, low-dose KXS (KXSL) and high-dose KXS
(KXSH; Table 1). Aβ42 (Wako Pure Chemical Industries,
Lt, Japan) was dissolved in dimethyl sulfoxide and diluted
with 0.9% saline. For intrahippocampal Aβ42 injection (all
groups except control), rats were anesthetized with 3% pen-
tobarbital sodium (50 mg/kg) and fixed in a stereotoxic
instrument as previously described (Asle-Rousta et al., 2013).
Aβ42 solution was injected into the hippocampus bilaterally
at the following coordinates: 3.6 mm posterior to the breg-
ma, 2.2 mm lateral to the midline and 4.0 mm below the
top of the skull. A total of 10 μL per rat (5 μL each side, 200
μM) was injected over 10 minutes. The needle was slowly
withdrawn from the brain, the surgical incision was sutured,
and penicillin sodium was administered to prevent infec-
tion. Control rats underwent the same procedure, but same
volume of saline was injected instead of Aβ42. Subsequently,
rats received KXS (KXSL group, 0.54 g/kg/d; KXSH group,
1.08 g/kg/d) or an equivalent volume of saline (control
and model groups) intragastrically once a day for 21 consecu-
tive days. Forty rats were used for histology and Nissl staining (n
= 10 per group) and 160 for the enzyme-linked immunosor-
cept assay (ELISA) (n = 40 per group).

Histological staining
Rats were anesthetized with 10% (3.5 mL) chloral hydrate
and decapitated after perfusion with 10% formalin and the
brain was removed immediately. Brains from the rats in each
group (Table 1) were fixed with 10% formalin for he-
matoxylin-eosin and Nissl staining. The tissue containing
the hippocampus was dehydrated through a graded series of
alcohol (75%, 85%, 95%, and 100%), embedded in paraffin,
and sliced into 4-μm-thick sections. These sections were
deparaffinized and subjected to hematoxylin-eosin and Nissl
staining (Ooigawa et al., 2006; Zhou et al., 2012). Under a
microscope (Olympus, Tokyo, Japan), a 300 × 100 μm2 field
of view was selected at random to count the number of in-
jured cells (showing nuclear shrinkage or disappearance)
and total cells in the hippocampal dentate gyrus, and the
percentage of injured cells was calculated.

ELISA assay for injected Aβ12
At five time points after injection (12, 24, 48, 96 and 168
hours; n = 8 rats per time point), brain Aβ12 concentration
was measured. Rats were anesthetized with 10% (3.5 mL)
chloral hydrate and decapitated. The brains were quickly
dissected on ice at the allocated time-point. For ELISA,
brains were homogenized in 70% formic acid buffer (1:10
w/v) for 1 hour. The lysates were centrifuged at 12,000 × g
for 30 minutes. The supernatants were collected and
neutralized with 1 M Tris-base solution. The solution con-
taining Aβ12 was measured using a specific and sensitive
sandwich ELISA kit (Wako Pure Chemical Industries, Ltd.,
Japan). Total protein concentration was measured using the
BCA method.
Rats were anesthetized with 10% (3.5 mL) and the forward: AGA GGC ATC CTG ACC CTG AAG -1.08 -1.08 -1.08 -1.08 Forward: AGT TAA AAG AAG CCC TCG ATG -0.54 -0.54 -0.54 -0.54 Reverse: TGC TCA ATA AGG GTG TCT TCT AC -1.08 -1.08 -1.08 -1.08 Reverse: GGT TGG CCT TAG GGT TCA GAG -1.08 -1.08 -1.08 -1.08 β-Actin Forward: AGA GGC ATC CGT ACC CTG AAG -163 -163 -163 -163 IDE: Insulin-degrading enzyme.

Western blot assay

Thirty rats were equally and randomly allocated to three groups (Table 1). Rats were anesthetized with 10% (3.5 mL) chloral hydrate and decapitated. Brains were removed on ice and hippocampi were dissected out for western blot and real-time-polymerase chain reaction (PCR) assays. To measure IDE content, hippocampal tissue was lysed in radioimmunoprecipitation assay buffer containing protease inhibitor. Protein content was determined using the Bradford method. Samples were mixed with an equal volume of 2× loading buffer and proteins were denatured by boiling at 100°C for 5 minutes. Protein samples (2 μg/μL) were resolved using 10% gel and transferred to polyvinylidene fluoride membranes. Blots were blocked in 5% non-fat milk for 2 hours at room temperature, then incubated with primary polyclonal antibody against human IDE (1:400; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. Immunoreactive bands were probed with a horseradish peroxidase-linked secondary polyclonal antibody (1:800; Beyotime Institute of Biotechnology, Haimen, China) for 2 hours. Enhanced chemiluminescence detection (Beyotime Institute of Biotechnology, Dalian, China) was used to prepare cDNA by reverse transcription using a PrimeScript RT reagent kit (TaKaRa Biotechnology (Dalian) Co., Ltd.). The PCR protocol consisted of denaturation at 95°C for 30 seconds followed by 40 denaturation and annealing cycles (95°C for 5 seconds, 60°C for 34 seconds). β-Actin was used as an internal reference. IDE mRNA expression was quantified using the 2-ΔΔCt method.

Statistical analysis

All data, expressed as the mean ± SD, were analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA). One-way analysis of variance was used for comparisons of multiple groups and the least significant difference test was used between two groups. P < 0.05 was considered significant.

Results

Effect of KXS on neuronal injury induced by hippocampal Aβ42 injection

Hematoxylin-eosin and Nissl staining were performed to investigate the neuroprotective effect of KXS. As expected, Aβ42 injection induced hippocampal neuronal injury, which was prevented by KXS. Hematoxylin-eosin staining revealed irregular neurons and neuronal death around the injection site (Figure 1B). However, neuronal injuries were markedly less severe after treatment with KXS (Figure 1C, D), and the number of injured neurons in the KXS group was significantly lower than in the model group (F = 23.5; P = 0.03 and P < 0.005 Figure 1E). Accordingly, Nissl staining also revealed typical neuronal pathological changes, including neuronal loss and nucleus shrinkage or disappearance, in the hippocampal den-
tate gyrus in the model group (Figure 2B), and were reversed significantly after treatment with KXS (Figure 2C, D). The proportion of injured neurons was significantly lower in the KXSL group \( (F = 20.6; P = 0.04) \) and KXSH group \( (P = 0.007) \) than in the model group (Figure 2E).

**KXS effect on degradation of Aβ\(_{42}\)**
We explored why KXS reduces Aβ\(_{42}\)-induced histological injury. Aβ\(_{42}\) levels in all groups declined between 6 and 96 hours, indicating that some Aβ\(_{42}\) clearance occurred without treatment. However, at 12, 24, 48, 96 and 168 hours, Aβ\(_{42}\) levels were significantly lower in the KXS groups than in the model group, and lower in the KXSH group than in the KXSL group (Figure 3).

**Effect of KXS on IDE mRNA and protein expression**
To further explore the KXS-induced reduction in Aβ\(_{42}\) levels, hippocampal IDE protein expression was investigated by western blot assay. Rats in the KXS groups showed significant upregulation of IDE protein after 168 hours compared with the model group \( (P < 0.01) \). IDE protein expression increased more in the KXSH group than in the KXSL group (Figure 4A). However, no significant difference in IDE mRNA expression was found between the KXS groups and the model group (Figure 4B). These results indicate that KXS accelerated Aβ degradation by upregulating IDE expression in the brain.

**Discussion**
KXS treatment improves the cognitive dysfunction induced by intrahippocampal Aβ injection (Li et al., 2013). Moreover, rat plasma containing KXS alleviated Aβ\(_{42}\)-induced damage in PC12 cells (Wen et al., 2012). These results tightly link the pharmacological targets of KXS to Aβ\(_{42}\). The present study showed that KXS prevented neuronal injury induced by Aβ\(_{42}\) and suggested that the therapeutic efficacy of KXS was related to Aβ in treating cognitive dysfunction.

KXS improves learning and memory in several paradigms, including experimental AD (Hu et al., 2013). However, whether KXS can affect Aβ degradation and clearance is not clear. The present results show that Aβ\(_{42}\) levels were significantly reduced after treatment with KXS. Because the excessive accumulation of Aβ is regarded as an essential upstream process in the AD pathogenesis cascade, we speculated that KXS might promote Aβ degradation.

Although insulin was long considered the main substrate for IDE, it was recently shown that IDE also degrades Aβ into peptides in the brain (Baranello et al., 2015). In addition, the process of substrate degradation by IDE has been elucidated: a proteolytic chamber, formed by the N and C terminal units of IDE, cleaves only peptides of up to 70 amino acids (Shen et al., 2006). In the brain, IDE is secreted from neurons and microglia, and Aβ degradation takes place extracellularly as well as in the cell membrane (Qiu et al., 1998; Zhang et al., 2013). Some studies reported an association of polymorphisms in the IDE locus with the risk of AD and plasma Aβ levels (Vekrellis et al., 2000; Carrasquillo et al., 2010; Cheng et al., 2015). Most clinical studies confirmed that IDE activity or expression is lower in AD and in individuals at high risk of AD (Perez et al., 2000; Del Campo et al., 2015). Moreover, an increase of cerebral endogenous Aβ was found after IDE inhibition or knockout (Shen et al., 2006). These studies suggest that IDE participates in the degradation of Aβ, and that its dysfunction contributes to the increase of Aβ in the brain. Therefore, in our study, we explored whether KXS would alter IDE expression.

Indeed, there was a significant increase in IDE protein expression after treatment with KXS, suggesting that this is an important reason for the decrease in Aβ\(_{42}\). Consistent with this, some active components from KXS have the ability to increase the protein level and activity of IDE. For instance, ginsenoside Rg1, one of the major active ingredients of KXS, can increase IDE expression by upregulating PPARγ, leading to lower levels of Aβ\(_{42}\) in the hippocampus (Quan et al., 2013). Ginsenosides Rg3 and Rb1, which act on the insulin signal transduction pathway, also can upregulate IDE protein expression, implying that the insulin pathway may be involved in regulating IDE expression (Gu et al., 2014; Jang et al., 2015). Together, these findings suggest that KXS and its active constituents can increase IDE expression. However, according to our PCR results, the mRNA level of IDE did not change. There are a number of possible reasons for these inconsistent changes. One is that rapid transcription coupled with rapid degradation will result in no change in RNA survival and availability for translation, and therefore cannot account for increased protein production. Conversely, KXS may regulate post-transcriptional changes, such as increased translation efficiency or reduced protein degradation, without affecting the mRNA level of IDE (Rodnina, 2016). These complicated molecular mechanisms represent an even greater challenge and should be addressed in subsequent studies.

In summary, the results of the present study demonstrate for the first time that KXS accelerates Aβ degradation by upregulating IDE protein expression and improving cognitive dysfunction. KXS is a promising agent for the treatment of AD.
Baranello RJ, Bharani KL, Padmaraju V, Chopra N, Lahiri DK, Greig NH, Pappolla MA, Sambamurti K (2015) Amyloid-beta protein clearance and degradation (ABCD) pathways and their role in Alzheimer's disease. Curr Alzheimer Res 12:32-46.

Campion D, Dumanchin C, Hannequin D, Dubois B, Belliard S, Puel M, Thomas-Anterion C, Michon A, Martin C, Charbonnier F, Raux G, Camuzat A, Penet C, Mesnage V, Martinez X, Clerget-Darpoux F, Brice A, Frebourg T (1999) Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. Am J Hum Genet 65:664-670.

Carrasquillo MM, Belbin O, Zou F, Allen M, Ertekin-Taner N, An- sari M, Wilcox SL, Kashino MB, Ma L, Younkin LH, Younkin SG, Younkin CS, Dincman TA, Howard ME, Howell CC, Stanton CM, Watson CM, Crump M, Vitart V, Hayward C, et al. (2010) Concordant association of insulin degrading enzyme gene (IDE) variants with IDE mRNA, Abeta, and Alzheimer's disease. PLoS One 5:e8764.

Chai GS, Wang YY, Yasheng A, Zhao P (2016) Beta 2-adrenergic receptor activation enhances neurogenesis in Alzheimer's disease mice. Neural Regen Res 11:1617-1624.
Figure 4 KXS upregulates IDE protein expression but has no effect on IDE mRNA expression.

Cheng H, Wang L, Shi T, Shang Y, Jiang L (2015) Association of insulin degrading enzyme gene polymorphisms with Alzheimer’s disease: a meta-analysis. Int J Neurosci 125:328-335.

Chu H, Zhang A, Han Y, Lu S, Kong L, Han J, Liu Z, Sun H, Wang X (2016) Metabolomics approach to explore the effects of Kai-Xin-San on Alzheimer’s disease using UPLC/ESI-Q-TOF mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 1015-1016:50-61.

Del Campo M, Stargardt A, Veerhuis R, Reits E, Teunissen CE (2015) Accumulation of BRB2-BRICHOS ectodomain correlates with a decreased clearance of Abeta by insulin degrading enzyme (IDE) in Alzheimer’s disease. Neurosci Lett 589:47-51.

Gu WJ, Liu D, Zhang MR, Zhang H (2014) Effect of ginsenoside Rb1 on insulin signal transduction pathway in hippocampal neurons of high-glucose-fed rats. Zhongguo Zhongyao Zazhi 39:1064-1068.

Hu Y, Liu M, Liu P, Yan JJ, Liu MY, Zhang GQ, Zhou XJ, Yu BY (2013) Effect of kai xin san on learning and memory in a rat model of paradoxical sleep deprivation. J Med Food 16:280-287.

Jang SK, Yu JM, Kim ST, Kim GH, Park da W, Lee DI, Joo SS (2015) An Ap42 uptake and degradation via Rg3 requires an activation of caveolin, clathrin and Apβ-degrading enzymes in microglia. Eur J Pharmacol 758:1-10.

Leissring MA (2016) Abeta-degrading proteases: therapeutic potential in Alzheimer disease. CNS drugs 30:667-675.

Li L, Luo J, Chen D, Tong JB, Cao YQ, Xiang J, Luo XG, Shi J, Wang H, Huang JP (2016) BACE1 in the retina: a sensitive biomarker for monitoring early pathological changes in Alzheimer’s disease. Neurogen Res 11:447-453.

Li Y, Liu B, Liu XW, Huang SM (2013) Mechanism research of Kai Xin Granule on Aβ-induced cognitive impairment in rats. Zhongyiayao Xinhui 33:34-38.

Liu XW, Liu S, Huang SM (2016) Plasma pharmacomecy study of effective extract from kai-xinsan on Alzheimer’s disease. Zhongguo Shuyan Fangxiu Zazhi 20:179-183.

Niu Jw, Zhang B, Chen H (2016) Safety and efficacy of human umbilical cord-derived mesenchymal stem cells in patients with Alzheimer’s disease: study protocol for an open-label self-control trial. Clin Trials Degener Dis;1:1-8.

Ootigawa H, Nakashiro H, Fukui S, Otani N, Osumi A, Toyooka T, Shima K (2006) The fate of Nissl-stained dark neurons following traumatic brain injury in rats: difference between neocortex and hippocampus regarding survival rate. Acta Neuropathol 112:471-481.

Perez A, Morelli L, Cresto JC, Castano EM (2000) Degradation of soluble amyloid beta-peptides 1-40, 1-42, and the Dutch variant 1-40Q by insulin degrading enzyme from Alzheimer disease and control brains. Neurochem Res 25:247-255.

Qu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, Rosner MR, Safavi A, Hersh LB, Selkoe DJ (1996) Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. J Biol Chem 271:32730-32738.

Quan Q, Wang J, Li X, Wang Y (2013) Ginsenoside Rgl decreases Apβ(1-42) level by upregulating PPARγ and IDE expression in the hippocampus of a rat model of Alzheimer’s disease. PLoS One 8:e59155.

Rodnina MV (2016) The ribosome in action: Tuning of translational efficiency and protein folding. Protein Sci 25:1390-1406.

Saido TC (2013) Metabolism of amyloid beta peptide and pathogenesis of Alzheimer’s disease. Proc Jpn Acad Ser B Phys Biol Sci 89:321-339.

Shiyan Fangjixue Zazhi 20:179-183.

Zhang Y, Wang B, Wan H, Zhou Q, Li T (2013) Meta-analysis of the effect of Kai Xin San and Ginkgo biloba on Alzheimer disease. Acta Neurobiol Exp 73:1-8.

Zhang SG, Wang XS, Zhang YD, Di Q, Shi JP, Qian M, Xu LG, Lin XJ, Lu J (2016) Indirubin-3'-monoxime suppresses amyloid-beta-induced apoptosis via inhibiting tau hyperphosphorylation. Neural Regen Res 11:988-993.

Zhang Y, Wang B, Wan H, Zhou Q, Li T (2013) Meta-analysis of the insulin degrading enzyme polymorphisms and susceptibility to Alzheimer’s disease. Neurosci Lett 541:132-137.

Zhao Z, Xiang Z, Haroutunian V, Buxbaum JD, Stetka B, Pasinetti GM (2007) Insulin degrading enzyme activity selectively decreases in the hippocampal formation of cases at high risk to develop Alzheimer’s disease. Neurobiol Aging 28:824-830.

Zhou X, Song HH, He W, Yang XY, Zhou ZB, Feng X, Zhou LH (2012) Neonatal exposure to sevofurane causes apoptosis and reduces nNOS protein expression in rat hippocampus. Mol Med Rep 6:543-546.

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