Journal of Acute Disease

journal homepage: www.jadweb.org

Original article https://doi.org/10.12980/jad.6.2017JADWEB-2016-0064

Effect of adjuvant therapy with ginkgo-damole on apoptosis, nerve injury and platelet aggregation of patients with acute cerebral infarction

Zhi-Yong Lu*

Department of Neurology, Yingkou Affliated Hospital of Liaoning University of Traditional Chinese Medicine, Yingkou 115000, Liaoning, China

ARTICLE INFO

Article history:
Received 1 Sep 2016
Accepted 27 Oct 2016
Available online 23 Jan 2017

Keywords:
Ginkgo-damole
Adjuvant therapy
Platelet aggregation
Apoptosis
Acute cerebral infarction

ABSTRACT

Objective: To investigate the effect of adjuvant therapy with ginkgo-damole on apoptosis, nerve injury and platelet aggregation of patients with acute cerebral infarction.

Methods: A total of 74 patients with acute cerebral infarction treated in our hospital from March 2014 to December 2015 were retrospectively analyzed, and they were divided into ginkgo-damole group and conventional treatment group according to a therapeutic schedule that whether ginkgo-diyidamolum were included. At Week 2 and Week 4 after treatment, contents of apoptosis molecule, nerve injury molecule and index of platelet aggregation in serum were detected.

Results: At Week 2 after treatment, contents of soluble Fas, soluble Fas ligand, soluble tumor necrosis factor related apoptosis inducing ligand, S100β, neuron specific enolase, glial fibrillary acidic protein, myelin basic protein, malonaldehyde, endothelin-1, fibrinogen and D-dimer in patients' sera of ginkgo-damole group were significantly lower than those of conventional treatment group. Contents of nitric oxide in sera were obviously higher than that of conventional treatment group. At Week 4 after treatment, contents of soluble Fas, soluble Fas ligand, soluble tumor necrosis factor related apoptosis inducing ligand, S100β, neuron specific enolase, glial fibrillary acidic protein, myelin basic protein, malonaldehyde, endothelin-1, fibrinogen and D-dimer in patients' sera of ginkgo-damole group were significantly lower than those of conventional treatment group. Contents of nitric oxide in sera were obviously higher than that of conventional treatment group.

Conclusions: Adjuvant therapy with ginkgo-damole can inhibit the apoptosis of neuron cells and neurogliocyte and reduce the neural function injury and the situation of platelet aggregation.

1. Introduction

In recent years, with the development of aging of population in China, the morbidity of cardiovascular and cerebrovascular diseases continuously raises, among which the incidence of cerebrovascular disease rapidly raises, leading to an adverse effect on both the health and life security of mid-aged population[1-3]. Acute cerebral infarction is the most common cerebrovascular disease in clinic, namely, cerebral arterial thrombosis, which accounts for 60%–80% of all cerebral apoplexy with a higher fatality rate and disability rate. How to effectively prevent and treat acute cerebral infarction has become an important issue of concern for clinicians. The pathological basis of occurrence of acute cerebral infarction lies in the atherosclerosis and injury of vascular endothelium function, based on which adherence and activation of platelet, thrombosis, vessel stenosis and decreased blood flow occur, further leading to hypoxic-ischemic damage on local tissue[4-6]. Anti platelet and anticoagulation are conventional ways to treat acute cerebral infarction in clinic, which are assisted with reducing intracranial pressure, neurotrophy and rehabilitation exercise, leading to a restriction effect on the development of cerebral infarction, reduction of hypoxic-ischemic damage on neural function and an improvement of fate of disease. Nevertheless, the current state of the treatment of acute cerebral infarction is not so pleased. Infarction lesions following the treatment of conventional anti platelet and anticoagulation on part of patients with acute cerebral infarction

*Corresponding author: Zhi-Yong Lu, Department of Neurology, Yingkou Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Yingkou 115000, Liaoning, China.
Tel: +86 898 417 2672146
E-mail: 34801850@qq.com
The journal implements double-blind peer review practiced by specially invited international editorial board members.

continuously expand, while secondary infarction occurs in the rehabilitation process on the other part of patients[7,8].

Ginkgo-diyidamolum is used as medicines to treat cardiovascular and cerebrovascular diseases in recent years, including extracts of traditional Chinese medicine, folium ginkgo, and western medicine, dipyridamole, which can inhibit the aggregation and activation of platelet in the developing process of myocardial infarction and cerebral infarction through various different ways and further improve the fate of disease of infarction[9]. In the present study, we analyzed the effect of adjuvant therapy with ginkgo-damole on apoptosis, nerve injury and platelet aggregation of patients with acute cerebral infarction.

2. Materials and methods

2.1. Study subjects

Patients with acute cerebral infarction treated in our hospital from March 2014 to December 2015 were retrospectively analyzed. The inclusive criteria of cases were as follow: (1) Diagnosis standard of acute cerebral infarction in Internal Medicine (8 edition)[10] and diagnosis standard of stroke in TCM Standardized Sheet of Apoplexy Syndromes Diagnosis were met[11]; (2) Patients of first onset were hospitalized within 24 h after onset; (3) CT and MRI scan were carried out to confirm the nidus of cerebral infarction after hospitalization; (4) Case data were complete. The exclusive criteria were as follow: (1) Patients were diagnosed cerebral hemorrhage or transient cerebral ischemia; (2) Patients were combined with disturbance of consciousness, myocardial infarction, thrombotic diseases and coagulation disorders; (3) Case data were incomplete. There were in total 74 cases included, whose case data were retrospectively analyzed and they were divided into ginkgo-damole group and conventional treatment group according to different therapeutic schedule.

2.2. Therapeutic methods

Patients of both groups were given the symptomatic and supportive treatment of early venous thrombolysis, dehydration of intracraninal pressure, maintenance of water electrolyte balance, lipid-decreasing, anti-infection and neurotrophy, and were given 300 mg aspirin enteric-coated tablets and 300 mg clopidogrel hydrogen sulfate tablets for chewing after hospitalization. Then 100 mg aspirin enteric-coated tablets and 75 mg lopidogrel hydrogen sulfate tablets were orally taken once daily as anticoagulant therapy. Patients in ginkgo-damole group were treated with ginkgo-diyidamolum based on the above conventional treatment. The methods were as follow: 10 mL of ginkgo-diyidamolum added with 250 mL normal saline was injected intravenously once daily for continuously 4 weeks' treatment.

2.3. Detection methods of serum index

At Week 2 and Week 4 after treatment, 5 mL of peripheral blood specimens from both groups were collected and centrifuged into sera. ELISA was used to detect the contents of S100β, neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), malonaldehyde (MDA), soluble Fas (sFas), soluble Fas ligand (sFasL), soluble tumor necrosis factor related apoptosis inducing ligand (sTRAIL), nitric oxide (NO), endothelin-1 (ET-1), fibrinogen (FIB) and D-dimer (D-D).

2.4. Statistical methods

Software SPSS version 20.0 was used to input and analyze data. Measurement data between groups were analyzed by *t*-test. Difference was considered as statistically significance when $P < 0.05$.

3. Results

3.1. Index of apoptosis

At Week 2 after treatment, contents of sFas [(93.42 ± 10.25) *vs.* (157.65 ± 17.65) μg/L], sFasL [(132.46 ± 16.51) *vs.* (193.14 ± 22.37) μg/L] and sTRAIL [(63.57 ± 9.35) *vs.* (97.18 ± 10.78) ng/L] in sera of patients from ginkgo-damole group were significantly lower than those of conventional treatment group ($P < 0.05$). At Week 4 after treatment, contents of sFas [(78.35 ± 9.25) *vs.* (121.31 ± 14.67) μg/L], sFasL [(108.34 ± 13.24) *vs.* (164.45 ± 19.32) μg/L] and sTRAIL [(50.38 ± 6.62) *vs.* (79.65 ± 8.34) ng/L] in sera of patients from ginkgo-damole group were significantly lower than those of conventional treatment group ($P < 0.05$).

3.2. Index of nerve injury

At Week 2 after treatment, contents of S100β [(1.03 ± 0.15) *vs.* (1.67 ± 0.19) ng/mL], NSE [(25.14 ± 2.98) *vs.* (42.35 ± 5.68) ng/mL], GFAP [(1.98 ± 0.24) *vs.* (3.49 ± 0.49) pg/mL], MBP [(3.47 ± 0.49) *vs.* (5.92 ± 0.71) ng/mL] and MDA [(5.14 ± 0.78) *vs.* (9.14 ± 1.18) nmol/mL] in sera of patients from ginkgo-damole group were obviously lower than those of conventional treatment group ($P < 0.05$). At Week 4 after treatment, contents of S100β [(0.86 ± 0.11) *vs.* (1.35 ± 0.15) ng/mL], NSE [(19.37 ± 2.29) *vs.* (34.21 ± 4.92) ng/mL], GFAP [(1.47 ± 0.18) *vs.* (2.89 ± 0.35) pg/ mL], MBP [(2.85 ± 0.35) *vs.* (3.69 ± 0.45) ng/mL], MDA [(4.02 ± 0.55) *vs.* (7.35 ± 0.97) nmol/mL] in sera of patients from ginkgodamole group were obviously lower than those of conventional treatment group ($P < 0.05$). Difference of contents of S100β, NSE, GFAP, MBP, MDA in sera between Week 2 and Week 4 after treatment was considered as statistically significance ($P < 0.05$).

3.3. Index of platelet aggregation

At Week 2 after treatment, content of NO [(77.51 ± 9.35) *vs*. (48.91 ± 5.35) μmol/L] in sera of patients from ginkgo-damole group was significantly higher than that of conventional treatment group ($P < 0.05$). Contents of ET-1 [(67.34 ± 8.17) *vs*. (113.58 ± 14.94) μg/L], FIB [(3.24 ± 0.52) *vs*. (4.77 ± 0.65) μg/L] and D-D [(141.39 ± 18.24) *vs*. (209.42 ± 24.52) ng/L] were significantly lower than those of conventional treatment group ($P < 0.05$). At Week 4 after treatment, content of NO [(89.25 ± 9.98) *vs*. (55.34 ± 7.24) μmol/L] was higher than that of conventional treatment group ($P < 0.05$). Contents of ET-1 [(58.14 ± 7.39) *vs.* (87.26 ± 9.71) μg/L], FIB [(2.78 ± 0.43) *vs*. (4.18 ± 0.65) μg/L] and D-D [(118.47 ± 13.93) *vs*. (164.22 ± 19.35) ng/L] were significantly lower than those of conventional treatment group ($P < 0.05$).

4. Discussion

Platelet aggregation caused by atherosclerotic plaque rupture and vascular endothelial injury is the vital pathological link of acute cerebral infarction[12-14]. The ginkgo-diyidamolum used in the present study has an anti-platelet effect through different ways. The effective components of ginkgo-diyidamolum include extracts of traditional Chinese medicine, folium ginkgo, and western medicine, dipyridamole. The former one can be specifically against the platelet activating factor[15-17], while the latter one can inhibit adenosine diphosphate and thromboxane[18]. Both of them have an effect for inhibiting the activation and aggregation of platelet and improving cerebral blood flow filling following the cerebral infarction. The interruption of cerebrovascular blood flow will lead to a hypoxicischemic situation for brain tissue, which further causes the damage on nerve cells through the multiple ways of activation of apoptosis. Fas/FasL and TRAIL are important molecules to mediate the apoptosis of neuron cells and neurogliocytes. Fas is a class of transmembrane protein which is classified in tumor necrosis factor receptor. It can activate the cascade reaction of downstream caspase combined with FasL and induce the apoptosis[19-21]. TRAIL is also included in tumor necrosis factor receptor which can induce apoptosis through two cascade amplifier channels, nuclear factor-kappa B and caspase[22-24]. In the occurrence process of cerebral infarction, Fas, FasL and TRAIL can fall out of the cells and combine together as soluble molecules, which are sFas, sFasL, sTRAIL, respectively. By analyzing the contents of the above apoptosis molecules in sera of patients from both groups, we know that contents of sFas, sFasL, sTRAIL in sera of patients of ginkgodamole group were significantly lower than those of conventional treatment group, which indicated that adjuvant therapy with ginkgodiyidamolum can inhibit the neural apoptosis caused by hypoxicischemic situation of patients with acute cerebral infarction.

Apoptosis of neuron cells and neurogliocytes caused by hypoxicischemic situation will lead to the cell rupture and the release of various structural molecules and functional molecules in endochylema out to the cells and further into the blood circulation through blood brain barrier. S100β, NSE, GFAP and MBP are important structural and functional molecules in neuron cells and neurogliocytes. Contents of the above molecules in sera indicate the severity of nerve injury. S100β is a class of acid calcium-binding protein in neural cells, which participates in the dynamic change of cytoskeletal components and the regulation of intracellular calcium homeostasis. NSE is a metabolic enzyme in neuron cells, which involves in the regulation and control of energy metabolism in cells. GFAP and MBP are important molecules participating in the cytoskeletal composition of neuroglia[25-27]. By analyzing the contents of the above marker molecules of nerve injury, we can know that contents of S100β, NSE, GFAP, MBP in sera of patients from ginkgo-damole group were significantly lower than those of conventional treatment group. In the process of hypoxic-ischemic damage in neural cells, except the release of various molecules in endochylema into blood circulation, the lipid molecules in cytomembrane and organelle membrane will have oxidizing reaction and then produce lipid peroxidation products MDA[28,29]. We can know by analyzing the content of MDA that the content of MDA in sera of patients from ginkgo-damole group was significantly lower than that of conventional treatment group. The above analysis indicated that adjuvant therapy with ginkgo-diyidamolum can reduce the damage on nerve function.

The therapeutical effect of ginkgo-diyidamolum works through the inhibition of the activation and aggregation of platelet and further improves blood perfusion in brain tissue. To find out the aggregation degree of platelet after the treatment of patients with acute cerebral infarction, we analyzed the contents of NO, ET-1, FIB and D-D in serum. NO is an endothelium-derived relaxing factor produced by vascular endothelial cell, which has a significant inhibition effect on activation and aggregation of platelet. While ET-1 is an important endogenous vasoconstriction peptide, which can improve the local aggregation of platelet and the thrombogenesis. In the process of the activation of platelet and the thrombogenesis, FIB can combine with platelet through platelet membrane glycoproteins compounds IIbIIIa and further improve the aggregation of platelet. While D-D is the production of degradation following the formation of fibrin monomer from fibrinogen and cross linking through activation factor X, which will further indicate the hyperfibrinolysis and hypercoagulability. We can know by analyzing the index of the above platelet aggregation, that the content of NO in sera of patients from ginkgo-damole group was obviously higher than that of conventional treatment group, while contents of ET-1, FIB and D-D were remarkably lower than those of conventional treatment group, which indicated that adjuvant therapy with ginkgo-damole can inhibit the platelet aggregation for patients with acute cerebral infarction.

In conclusion, adjuvant therapy with ginkgo-damole can inhibit the apoptosis of neuron cells and neurogliocytes and reduce the damage on nerve function and the aggregation of platelet.

Conflict of interest statement

The authors report no conflict of interest.

References

[1] Lee SW, Kim HC, Lee HS, Suh I. Thirty-year trends in mortality from cerebrovascular diseases in Korea. *Korean Circ J* 2016; **46**(4): 507-14.

[2] Dharmasaroja PA, Muengtaweepongsa S, Pattaraarchachai J. Clinical course, prognostic factors, and long-term outcomes of malignant middle cerebral artery infarction patients in the modern era. *Neurol India* 2016; **64**(3): 436-41.

[3] Lorente L, Martín MM, Pérez-Cejas A, Abreu-González P, Ramos L, Argueso M, et al. Association between total antioxidant capacity and mortality in ischemic stroke patients. *Ann Intensive Care* 2016; **6**(1): 39.

[4] Kristoffersen DT, Helgeland J, Waage HP, Thalamus J, Clemens D, Lindman AS, et al. Survival curves to support quality improvement in hospitals with excess 30-day mortality after acute myocardial infarction, cerebral stroke and hip fracture: a before-after study. *BMJ Open* 2015; **5**(3): e006741.

[5] Nolte CH, Audebert HJ. [Management of acute ischemic stroke]. *Dtsch Med Wochenschr* 2015; **140**(21): 1583-6. German.

[6] Li S, Sun X, Bai YM, Qin HM, Wu XM, Zhang X, et al. Infarction of the corpus callosum: a retrospective clinical investigation. *PLoS One* 2015; **10**(3): e0120409.

[7] Park JY, Rha SW, Choi B, Choi JW, Ryu SK, Kim S, et al. Impact of low dose atorvastatin on development of new-onset diabetes mellitus in Asian population: three-year clinical outcomes. *Int J Cardiol* 2015; **184**: 502-6.

[8] Hsu JC, Maddox TM, Kennedy K, Katz DF, Marzec LN, Lubitz SA, et al. Aspirin instead of oral anticoagulant prescription in atrial fibrillation patients at risk for stroke. *J Am Coll Cardiol* 2016; **67**(25): 2913-23.

[9] Chen KS, Xu GP, Wang JP, Yu RX, Yang Y, Huang X. [Effect of ginkgo-diyidamolum injection on cerebral blood flow and vascular endothelial function of patients with acute cerebral infarction]. *Chin Tradit Pat Med* 2014; **36**(12): 2479-82. Chinese.

[10] Ge JB, Xu YJ. *Internal medicine*. 8th ed. Beijing: People's Medical Publishing House; 2013, p. 473-4.

[11] Collaboration Group of Encephalopathy Emergency of State Administration of Traditional Chinese Medicine. [A draft for diagnosis and effect evaluation criteria of stroke]. *Beijing Zhong Yi Yao Da Xue Xue Bao* 1996; **19**(1): 55-6. Chinese.

[12] Goddard A, Leisewitz AL, Kristensen AT, Schoeman JP. Platelet activation and platelet-leukocyte interaction in dogs naturally infected with Babesia rossi. *Vet J* 2015; **205**(3): 387-92.

[13] Chen Y, Liu Y, Luo C, Lu W, Su B. Analysis of multiple factors involved in acute progressive cerebral infarction and extra- and intracranial arterial lesions. *Exp Ther Med* 2014; **7**(6): 1495-505.

[14] Zhao M, Zhang L,Wang Z, Wang X, Wang Y, Wei H, et al. Dynamic analysis of blood pressure changes in progressive cerebral infarction. *Int Health* 2015; **7**(4): 293-7.

[15] Wu C, Zhao X, Zhang X, Liu S, Zhao H, Chen Y. Effect of *Ginkgo biloba* extract on apoptosis of brain tissues in rats with acute cerebral infarction and related gene expression. *Genet Mol Res* 2015; **14**(2): 6387-94.

[16] Li W, Luo Z, Liu X, Fu L, Xu Y, Wu L, et al. Effect of *Ginkgo biloba* extract on experimental cardiac remodeling. *BMC Complement Altern Med* 2015; **15**: 277.

[17] Ran K, Yang DL, Chang YT, Duan KM, Ou YW, Wang HP, et al. *Ginkgo biloba* extract postconditioning reduces myocardial ischemia reperfusion injury. *Genet Mol Res* 2014; **13**(2): 2703-8.

[18] F igueredo VM, Okusa C, Kaneda K, Inamura Y, Miyamae M. Regular dipyridamole therapy produces sustained protection against cardiac ischemia-reperfusion injury: is it time to revisit PARIS? *Int J Cardiol* 2014; **176**(3): 822-7.

[19] Meng HL, Li XX, Chen YT, Yu LJ, Zhang H, Lao JM, et al. Neuronal soluble fas ligand drives M1-microglia polarization after cerebral ischemia. *CNS Neurosci Ther* 2016; **22**(9): 771-81.

[20] Chen B, Wu Z, Xu J, Xu Y. Calreticulin binds to fas ligand and inhibits neuronal cell apoptosis induced by ischemia-reperfusion injury. *Biomed Res Int* 2015; **2015**: 895284.

[21] Schleicher RI, Reichenbach F, Kraft P, Kumar A, Lescan M, Todt F, et al. Platelets induce apoptosis via membrane-bound FasL. *Blood* 2015; **126**(12): 1483-93.

[22] Tisato V, Gonelli A, Voltan R, Secchiero P, Zauli G. Clinical perspectives of TRAIL: insights into central nervous system disorders. *Cell Mol Life Sci* 2016; **73**(10): 2017-27.

[23] Pan X, Pang M, Ma A, Wang K, Zhang Z, Zhong Q, et al. Association of TRAIL and its receptors with large-artery atherosclerotic stroke. *PLoS One* 2015; **10**(9): e0136414.

[24] Dessein PH, Lopez-Mejias R, Ubilla B, Genre F, Corrales A, Hernandez JL, et al. TNF-related apoptosis-inducing ligand and cardiovascular disease in rheumatoid arthritis. *Clin Exp Rheumatol* 2015; **33**(4): 491-7.

[25] Lai PM, Du R. Association between S100B levels and long-term outcome after aneurysmal subarachnoid hemorrhage: systematic review and pooled analysis. *PLoS One* 2016; **11**(3): e0151853.

[26] Ye H, Wang L, Yang XK, Fan LP, Wang YG, Guo L. Serum S100B levels may be associated with cerebral infarction: a meta-analysis. *J Neurol Sci* 2015; **348**(1-2): 81-8.

[27] Li K, Jia J, Wang Z, Zhang S. Elevated serum levels of NSE and S-100β correlate with increased risk of acute cerebral infarction in Asian populations. *Med Sci Monit* 2015; **30**(21): 1879-88.

[28] Altintas O, Kumas M, Altintas MO. Neuroprotective effect of ischemic preconditioning via modulating the expression of adropin and oxidative markers against transient cerebral ischemia in diabetic rats. *Peptides* 2016; **79**: 31-8.

[29] Yaidikar L, Thakur S. Arjunolic acid, a pentacyclic triterpenoidal saponin of *Terminalia arjuna* bark protects neurons from oxidative stress associated damage in focal cerebral ischemia and reperfusion. *Pharmacol Rep* 2015; **67**(5): 890-5.