Original Article

Neural Fuyuan Formula promotes neural plasticity through BDNF/Trkβ signaling pathway

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Background: Depression after stroke is usually a chronic process, which was associated with many health problems. This study was aimed to investigate the underline mechanism of the effect of Neural Fuyuan Formula (NFF) in post-stroke depression and the role of brain-derived neurotrophic factor (BDNF) in the signal pathway regulated by NFF.

Methods: A rat post-stroke depression model was established. Synaptic plasticity of rat was detected by Electron microscopy. The expression of BDNF signaling proteins and synapse related proteins were measured by Western blot. The expression of Synapsin-1 (SYN1) in rat and the culture neurons was detected by Western blot and immunofluorescence. Dendritic complexity was also measured.

Results: NFF could attenuate the synapse change in the post-stroke depression (PSD) model rat. NFF increased the expression of BDNF signaling proteins and synapse related proteins of the PSD model rat ($P<0.05$). NFF increased the expression of SYN1 in rat and the culture neurons ($P<0.05$). NFF could also increase the dendritic complexity in culture neurons ($P<0.05$).

Conclusions: NFF promoted recovery of neurological function through BDNF signaling pathways, which further affirm the curative effect of NFF for treatment of post-stroke depression.

Keywords: Neural Fuyuan Formula (NFF); Post-stroke depression (PSD); synapse; brain-derived neurotrophic factor (BDNF); tropomysin-related kinase B (TrkB)

Introduction

Post-stroke depression (PSD) is considered as one of the most commonly complications of stroke, which accounts for about one-third of stroke survivors experience major depression (1). PSD is usually a kind of chronic disease and related to many other heathy outcomes (2,3). Antidepressant treatment in the first time after stroke may prevent the progress of PSD.

The basic treatment on PSD was focused on stroke and depressive symptom (4). The effect currently antidepressants is single, moreover, many other adverse effects caused by antidepressants would further lead to the decrease of drug compliance. Fluoxetine is the most commonly used antidepressants, which prevents the degeneration of nigrostriatal dopaminergic neurons, however, it would also cause the repression of microglia activation, neutrophil infiltration and the increase of inflammatory factors (5). Thus, we expect that the combined use of antidepressants will minimize these side effects and the pro-inflammatory cytokines releasing.

Traditional Chinese medicine, which is used as complementary and alternative therapy, has attracted much attention for its safety and effectiveness in improving the symptom of depression (6). Treatment of PSD using Chinese medicine compounds could promote the blood circulation by removing blood stasis, relieve mental pressure and enhance renal function. For example, Xing nao Jie yu capsules upregulate synaptotagmin expression in hippocampi of rats with PSD (7), while Wuling capsule appeared to be effective for PSD treatment (8).

Brain-derived neurotrophic factor (BDNF) is a 119-amino acid residue nonglycosylated polypeptide (9). B DNF molecule consists of an 18-aa residue signal sequence, a 110-aa residue prosequence (Pro BDNF), and a 119-aa residue mature segment. BDNF belongs to neurotrophin family, which has varieties of functions including neuronal survival (10), neuronal development and synaptic plasticity (11). BDNF works through two receptor systems, high-affinity tropomysin-related kinase B (TrkB) receptor and the low affinity receptor p75, which is a common nonspecific receptor in neurotrophins (12). Neural Fuyuan Formula (NFF) is a Chinese traditional medicine which effective for treating PSD model rat, however, the cellular and molecular mechanism it plays in PSD is still unknown. Therefore, in this study, we investigated the underline mechanism of the action of NFF and whether BDNF was involved in the signal pathway regulated by NFF.

We present the following article/case in accordance with the ARRIVE Reporting Checklist (available at http://dx.doi.org/10.21037/apm-19-533).

Methods

Animals

Sprague-Dawley (SD) rats of 8 weeks old, weighted 150–200 g, were obtained from the Laboratory Animal Center of Shanghai Municipal Hospital. The rats were housed in clean cages and maintained at 22±2 ℃ with a constant 12-hour light/dark schedule. The animals were allowed free access to food and water. Before operation, rats were fasted for 12 h, anesthetized with 1% pentobarbital sodium intraperitoneal injection (30 mg/kg). Hippocampal neurons were isolated from embryos on neonatal day (P0). All Wistar rats were euthanized by cervical dislocation. Experiments were performed under a project license (NO. 2017SHL-KY20) granted by institutional ethical committee board of Shanghai Municipal Hospital, in compliance with

Animal Care and Use Committee of Shanghai Municipal Hospital guidelines for the care and use of animals.

Establishment of a post-stroke depression model

The stroke model was established by ligation of unilateral internal carotid artery according to a previous research (7). All rats were housed separately and stimulated for 21 days, including restriction for 2 hours, ice water swimming for 5 minutes, fasting for 24 hours, fasting water for 24 hours, tail clamping for 1 minute, light-dark reverse for 24 hours, electric shock on a footplate for10 minutes (1 mA electric current, 30 V voltage, once every 10 seconds, each for 1 second, total 60 times), the home cage inclined at 45° for 24 hours, moist padding for 24 hours, an empty bottle for 2 hours and shaking (50 Hz) at 45 ℃ for 5 minutes. One kind of stimulus was administered per day, and each stimulus appeared once or twice. A successful model was confirmed when rats showed obvious depression, depression, dark hair, decreased horizontal exercise, decreased sugar consumption, decreased vertical movement, decreased body weight and appetite. After establishment of PSD, the rats were intragastrically perfused with fluoxetine or NFF of 45, 15, 7.5 mg/100 g, once a day.

Drugs

NFF consisted of 9 g Wide turmeric, 12 g Acorus gramineus, 9 g Codonopsis pilosula, 15 g Rhizome of rehmannia, 3 g Scorpion, 9 g Bombyx Batryticatus and 9 g Radix Salviae Miltiorrhiae (all purchased from Tianjiang Pharmaceuticals Co., Ltd, Jiangsu Province, China). All components were decocted at 100 ℃ in water of 10 times of their total weight for 1–2 hours, filtered by gauze, followed by decoction at 100 ℃ in water of eight times of the total weight for 1–2 hours. Fluoxetine capsules (20 mg) were purchased from Eli Lilly and Company, Suzhou, China (Approval No. GYZZ J20080016; batch No. 204362). The administration dose was 2 mg/kg.

Electron microscopy

The brains of the anesthetized rat were removed and dissected. The CA3 area were separated and about 1–1.5 mm^3 tissue were placed in a fixative containing 2.5% glutaraldehyde for 2 h, then rinsed in phosphate buffer, and postfixed for 2 h in 1% osmium tetroxide in phosphate buffer at 4 ℃. The brains were dehydrated and

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embedded in resin. Ultrathin sections were cut using a Reichert ultramicrotome, and examined under an electron microscope at ×11,000 at last.

Western blot assay

The hippocampal tissue was obtained and put in cold radioimmunoprecipitation assay buffer with fresh 2 mM phenylmethylsulfonyl fluoride (Beyotime Bio, Nantong, Jiangsu Province, China). Tissues were placed on ice for 30 minutes, and centrifuged at 13,000 ×g for 30 minutes at 4 ℃. Before loading onto a 12% sodium dodecylsulfate-polyacrylamide gel, equal amounts of protein were boiled with 10× loading buffer for 5 minutes. Electrophoresis was performed at 80 V for 30 minutes and 120 V for 120 minutes. Separated proteins were transferred onto a polyvinylidene difluoride membrane with 120 V for 120 minutes. Membranes were firstly blocked with 5% non-fat dry milk at 4 ℃ overnight, and then incubated with primary antibody, rabbit polyclonal anti-BDNF (1:1,000; Sigma), anti-TrkB (1:1,000; abcam), anti-SYNA (1:1,000; abcam), anti-GAP-43 (1:1,000; abcam) and anti-SYN1 (1:1,000; abcam) overnight at 4 ℃. β-actin immunoblots (1:1,000; Sigma) was used as internal control. Immunodetection was performed by electrochemiluminescence (Pierce, Rockford, IL, USA). After incubated with horseradish peroxidase-conjugated goat anti-rabbit or rabbit anti-mouse (1:5,000; Jackson ImmunoResearch, USA) antibody for 1 hour at 37°C, the X-ray films were photographed.

Immunofluorescence

Rats were anesthetized with 1% pentobarbital sodium intraperitoneal injection (30 mg/kg) and intracardially perfused with a saline solution and following a 4% solution of paraformaldehyde in phosphate buffer saline (PBS). Brains of the rats were then fixed for 24 h, stored in 30% sucrose/PBS solution and sectioned on a sliding freezing microtome (Leica, Germany). Twenty micrometre coronal sections were coated on the slides. Immunofluorescence (IF) stainings were performed using anti-SYN1 (1: 1,000; abcam). After wished with PBS for 4 times (5 min per time), slices were incubated with secondary antibody goat anti-mouse, then incubated with DAPI for 15 min, wished with PBS for 4 times. After drying in the air, slices were dropped with coated with mounting medium, and coated with cover glasses.

Dissection and dissociation of primary hippocampal neurons

Hippocampal neurons were isolated from the embryos of rats on neonatal day (P0). Approximately 10 neonatal rats were obtained from each rat. The whole brains were isolated. Twenty hippocampi were resected from the brains under microscope. The meninges were resected. All fetuses and tissues were put in minimum essential medium (MEM, Sigma) and chilled on ice. Other operations were performed in a laminar flow hood. After dissection, the hippocampi were washed by phosphate-buffered saline (PBS, Wako) for three times. Then, 5 ml papain solution and 20–60 μL deoxyribonuclease I (DNase I, Japan) were added to the hippocampi, and incubated at 37 ℃ for 12 min. After incubation and digestion with the enzymes, hippocampi were slowly pipetted using a glass Pasteur pipette for 12 times, and then filtered with a wetted cell strainer (40-μm mesh, BD Biosciences) into a 50 mL conical tube. The cell strainer was pre-wetted with 10 mL MEM (Sigma) containing 20% FBS (Gibco) and 1% N2 supplement (100×, Invitrogen, Life Technologies) to prevent nonspecific neuronal cell attachment. The entire hippocampi suspension was poured through the cell strainer, and then 10 mL 20% FBS/N2/MEM was poured on top to collect neurons remaining on the filter. It was shaken, left for 1 min, and then centrifuged at 180 ×g for 10 min. The supernatant was aspirated, and the pellet was resuspended in the culture medium with the cell density of 3×10^4 cells/mL. Subsequently, the cell suspension was plated into the 12-well multiplates with each volume of 1 ml. Each well was prepared with 0.5 mL of 25% PuraMatrix. Rat embryonic hippocampal neurons were cultured at 37 ℃ in a humidified atmosphere with 5% CO_2/95% air.

Incubation and transfection of primary hippocampus neuron

At the fourth day *in vitro* (DIV 4) culturing, neurons were transfected with lentivirus pLVX-IRES-ZsGreen1. Drugs: H_2O_2, the serum of high dose NFF treated rat and H_2O_2, K252a and H_2O_2, the serum of high dose NFF treated rat and H_2O_2 and K252a were added into the medium at DIV 7. At last, neurons were fixed at DIV 9 and used for the following observation.

Statistical analysis

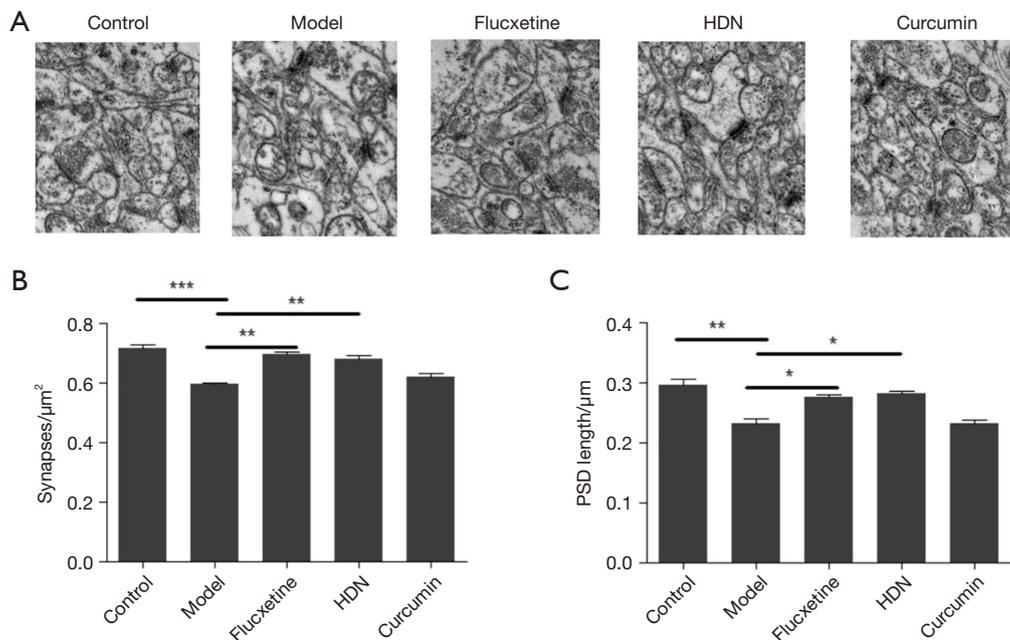The data were presented as means ± SEM. All statistical

Figure 1 TEM ultrastructural results of PSD model rat and the drug therapy group. (A) TEM ultrastructural images of hippocampal DG synapses in control rat, PSD rat, Fluoxetine treated rat, high dose NFF treated rat and Curcumin treated rat, ×400. (B) The statistical data of synapse density in these groups. (C) Mean postsynaptic density length in these groups. (4 rat per group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. PSD, post-stroke depression; DG, dentate gyrus.

analyses were performed using SPSS 19.0 statistics software. Group comparisons were analyzed with one-way ANOVA. $P<0.05$ was considered as significant difference.

Results

NFF rescued the synapse change in the PSD model rat

The effect of NFF on the synaptic plasticity in PSD model rat was first detected. Electron microscopic results revealed a significant reduction of the synapse density in the PSD model rat compared to the control ($P<0.05$), whereas Fluoxetine and high dose NFF all could rescue the change (*Figure 1A,B*). Furthermore, the postsynaptic density length presented significantly shorter compared to the control ($P<0.05$), and Fluoxetine and high dose NFF also could rescue it (*Figure 1A,C*).

NFF increased the expression of BDNF signaling proteins and synapse related proteins of the PSD model rat

The expression of BDNF was significantly suppressed in the PSD model rat compared with control group ($P<0.05$),

but Fluoxetine and high dose NFF could significantly increase the expression of BDNF in PSD model rat ($P<0.05$) (*Figure 2A,B*), while curcumin couldn't change its level. Interstingly, the expression of TrkB which is the receptor of BDNF showed a significant increase in PSD model rat, Fluoxetine, high dose NFF and curcumin all could significantly suppress the expression of TrkB ($P<0.05$) (*Figure 2A,C*). Furthermore, the expression of synapse-associated protein (SYNA), growth-associated protein-43 (GAP-43) and Synapsin-1 (SYN1) were detected. It was shown that the expression of SYNA and GAP-43 were both increased in PSD model group, while Fluoxetine, high dose NFF and curcumin could suppressed the expression of SYNA and GAP-43 ($P<0.05$) (*Figure 2A,D,E*). Furthermore, the expression of SYNI presented a significant decrease in PSD model group, while Fluoxetine and high dose NFF and curcumin could rescue the expression change of SYN1 (*Figure 2A,F*).

NFF increased the expression of SYN1 in rat and the culture neurons

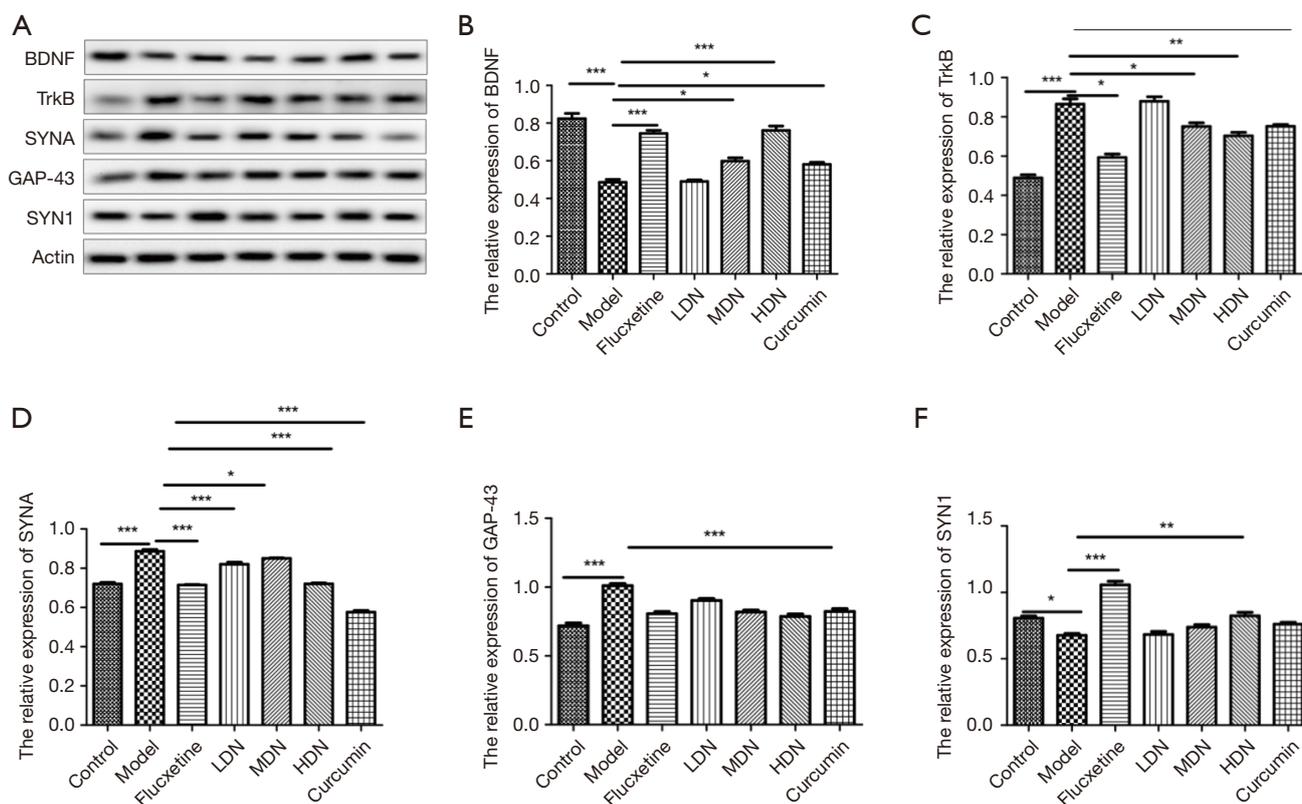Due to the important role of SYN1 in neuritogenesis and

Figure 2 The expression of BDNF signaling pathway proteins in PSD model rat and the drug treating groups. (A) Expression of the BDNF signaling pathway proteins BDNF and TrkB and the synapse related proteins SYNA, GAP-43 and SNY1 in control rat, PSD rat, Fluoxetine treated rat, high dose NFF treated rat and Curcumin treated rat. (B,C) The statistical data of BDNF and TrkB in these groups. (D,E,F) The statistical data of SYNA, GAP-43 and SNY1 in these groups. (4 rat per group). *, P<0.05; **, P<0.01; ***, P<0.001. BDNF, brain-derived neurotrophic factor; PSD, post-stroke depression; SYNA, synapse-associated protein; NFF, Neural Fuyuan Formula.

synaptogenesis (13), we further detected the expression and distribution of SYN1 in the hippocampus using immunofluorescence. The expression of SYN1 was significantly lower in the DG area of the hippocampus (P<0.05) (*Figure 3A*), however, Fluoxetine and high dose NFF could increase the expression of SYN1 in this area (*Figure 3A*). Then the expression of SYN1 in the culture neuron treated with the serum of high dose NFF treated rat and the blocker of BDNF/TrkB signaling K252a under the H_2O_2 condition were measured. H_2O_2 significantly suppressed the expression of SYN1, the serum of high dose NFF treated rat could increase the expression of SYN1 suppressed by H_2O_2 (P<0.05) and K252a could further reduce the expression of SYN1 suppressed by H_2O_2 (P<0.05). Furthermore, when treated with the serum of high dose NFF treated rat and K252a together under the H_2O_2 condition, the expression of SYN1 was similar with

the H_2O_2 treated alone group (P>0.05) (*Figure 3B,C*).

NFF increased the dendritic complexity in culture neurons

The role of NFF in the neuron complexity under the H2O2 treating condition was investigated. H2O2 significantly inhibited the neuron complexity (P<0.05), and NFF could rescued the suppression, when blocked the BDNF signaling in the NFF treating group, the rescued effect was eliminated (*Figure 4*).

Discussion

Post-stroke depression is an important psychological outcome of ischemic stroke, which greatly affects the morbidity and mortality (14), therefore the treatment strategy on post-stroke depression is very important. The
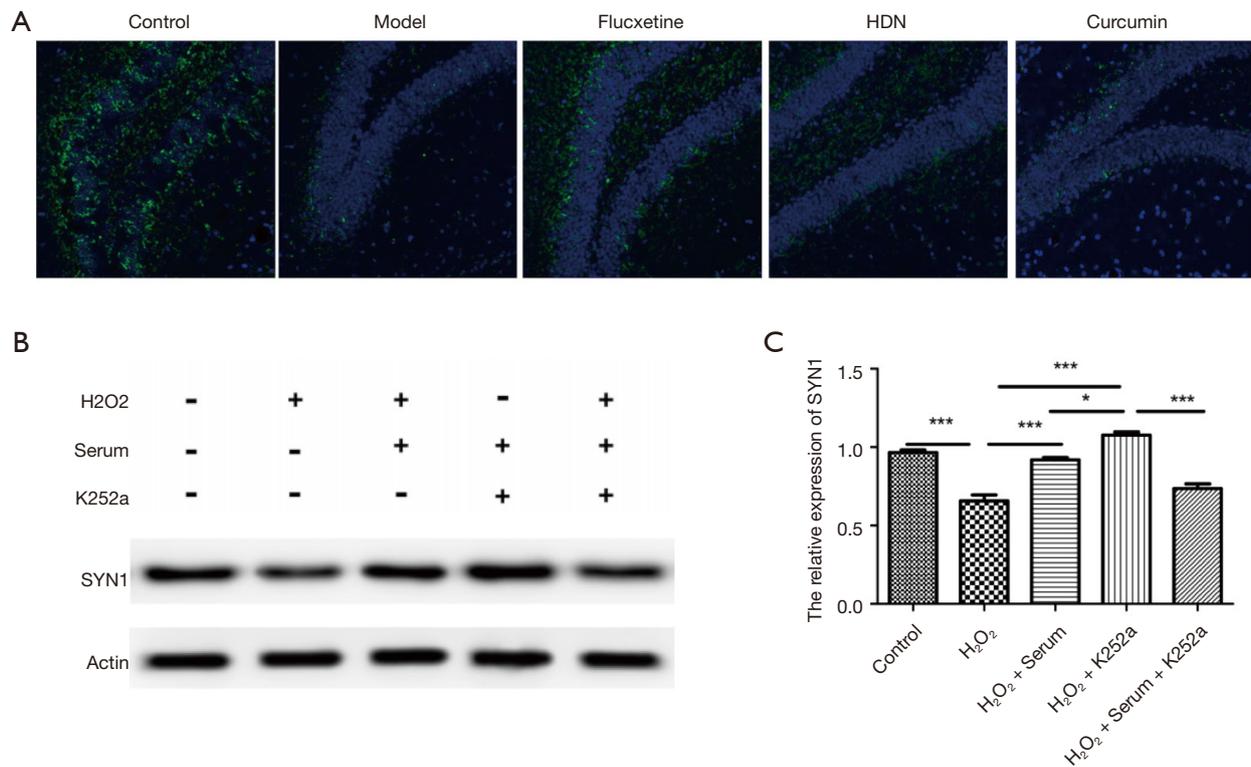
Figure 3 The expression of SYN1 in the hippocampus and culture neurons. (A) Immunofluorescence result of SYN1 shown at the DG area in control rat, PSD rat, Fluoxetine treated rat, high dose NFF treated rat and Curcumin treated rat (4 rat per group), ×100. (B) The expression of SYN1 in the culture neurons treated with H_2O_2, serum of high dose NFF and K252a and H_2O_2. (C) The relative expression of SYN1 (4 samples per group). *, P<0.05; ***, P<0.001. SYN1, Synapsin-1; DG, dentate gyrus; PSD, post-stroke depression; NFF, Neural Fuyuan Formula.
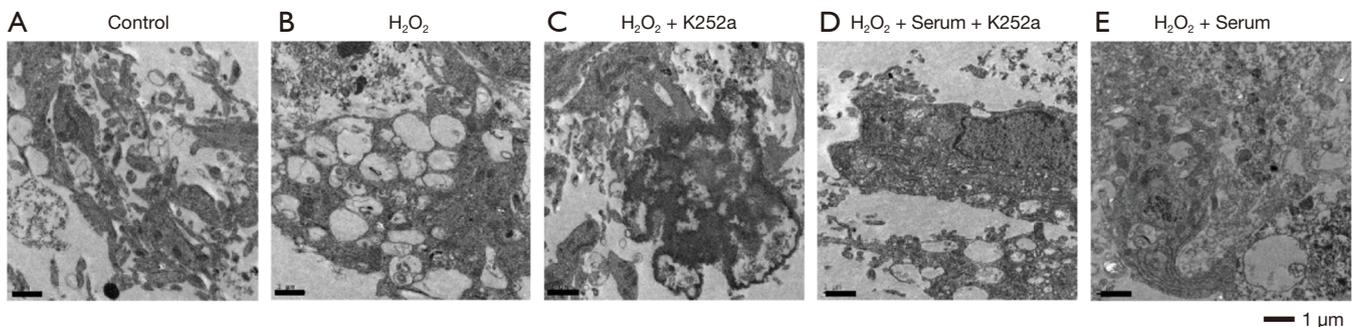

Figure 4 The structure of the culture neurons. Primary culture neurons at DIV9 (9 days *in vitro*) treated with H_2O_2. (A,B) Compared with the control group, the synaptic density of H_2O_2 group was decreased, indicating that that H_2O_2 can induce oxidative stress and damage hippocampal neurons (C,D,E) The synaptic density increased and the thickness of postsynaptic densities increased in the serum containing H_2O_2. When drug-containing serum and K252a were added, the synaptic density of neurons decreased, the thickness of postsynaptic densities became thinner, indicating that that NFF can effectively repair the injury of neurons by H_2O_2, through the inhibition of BDNF/TrkB signal pathway, the effect could be attenuated (4 rat per group).

strategies include antidepressant therapy, psychotherapy, surgical therapy, electroconvulsive therapy, drug therapy and natural products. Currently, the treatment on PSD mainly included four kinds of antidepressants in combination with psychotherapy (14). Though these drugs showed efficiency, the toxic was still a big problem in the long-term use (15,16). It has been shown that antidepressants could improve the hippocampal neurogenesis, which is thought to be an etiological factor of depression (17).

Some traditional Chinese medicines has also been shown to have potential antidepressant effect in animal studies (18,19). Traditional Chinese medicines displayed anti-depressant-like effects after repeated treatment for at least 2 weeks, while it is agreed that the delay in the antidepressant effect of synthetic drugs is required in the neuroadaptive process that may enhance neuroplasticity and cellular resilience (20).

Neurotrophins, particularly brain-derived neurotrophic factor (BDNF), are expressed in both central and peripheral nervous systems, particularly in the cortex and hippocampus (21). Perturbations in BDNF signaling are associated with depression (22). Serum BDNF are decreased in PSD patients (23). Depression animal models also showed that BDNF deficiency was related to depression, while antidepressants can reverse this change (24). Our results showed that NFF significantly increased the levels of BDNF and phosphorylation of TrkB in the brain of *APP/PS1* mice. It suggested that BDNF-TrkB signaling was associated with the neuro-protective effect of NFF. To our best knowledge, this is the first study reporting the molecular mechanism of the neuro-protective effect of NFF. TrkB receptor agonists have also been reported to improve cognition in 5XFAD mice by down-regulating beta-secretase 1 precursor levels (25).

The effect of herbal medicine to elicit neuroadaptive processes may imply its antidepressant efficacy. Current study on herbal medicines is mainly focused on the mechanisms involving increased monoamines concentrations, particularly serotonin, rather than neuroplasticity (18,26). As it has already been showed that serotonergic receptors could initiate neuroplasticity related pathways via CREB activation (27), and BDNF could enhance the survival of serotonergic neurons (28), we consider that additional medicines which increase serotonergictrans mission could also impact neuroplasticity.

NFF, a Chinese formula used for treating depressive disorders, is composed of 8 ingredients: Habitat,

Anemarrhena asphodeloides, Codonopsis pilosula, Tulip, Scorpion, Bombyx mori, Acorus tatarinowii, and Angelica dahurica. In the present study, a reduction of the synapse density and postsynaptic density length were found in the PSD model rat, whereas high dose NFF could attenuate the change, just like Fluoxetine. Furthermore, *in vitro* culture neuron experiment, data revealed that NFF also play a positive role in regulating the neuron complexity. Taken together, these results revealed that NFF, the traditional Chinese medicine, plays an effective role in treatment on PSD. Furthermore, in the mechanism way of the present study, it was found that BDNF/TrkB signaling pathway played a significant role in the NFF therapeutic effect. The NFF promoted to the recovery of neurological function, regulated synaptic proteins and corresponding BDNF signaling pathways, and further improved neuron complexity in the hippocampal dentate gyrus region. There could be also other mechanism of the effect of NFF, therefore, further study on the mechanism of the drugs and PSD was still needed.

In conclusion, NFF showed effect on the recovery of neurological function through BDNF signaling pathways, which further affirm the curative effect of NFF for treatment of post-stroke depression.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the ARRIVE Reporting Checklist. Available at http://dx.doi.org/10.21037/apm-19-533

Data Sharing Statement: Available at http://dx.doi.org/10.21037/apm-19-533. We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-19-533). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (NO. 2017SHL-KY20) granted by institutional ethical committee board of Shanghai Municipal Hospital, in compliance with Animal Care and Use Committee of Shanghai Municipal Hospital guidelines for the care and use of animals.

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References

1. Robinson RG, Jorge RE. Post-Stroke Depression: A Review. Am J Psychiatry 2016;173:221-31.
2. Cai W, Mueller C, Li YJ, et al. Post stroke depression and risk of stroke recurrence and mortality: A systematic review and meta-analysis. Ageing Res Rev 2019;50:102-9.
3. Chemerinski E, Robinson RG, Kosier JT. Improved recovery in activities of daily living associated with remission of poststroke depression. Stroke 2001;32:113-7.
4. Wang C, Wu C, Yan Z, et al. Ameliorative effect of Xiaoyao-jieyu-san on post-stroke depression and its potential mechanisms. J Nat Med 2019;73:76-84.
5. Villa RF, Ferrari F, Moretti A. Post-stroke depression: Mechanisms and pharmacological treatment. Pharmacol Ther 2018;184:131-44.
6. Butler L, Pilkington K. Chinese herbal medicine and depression: the research evidence. Evid Based Complement Alternat Med 2013;2013:739716.
7. Yan Y, Fan W, Liu L, et al. The effects of Xingnao Jieyu capsules on post-stroke depression are similar to those of fluoxetine. Neural Regen Res 2013;8:1765-72.
8. Peng L, Zhang X, Kang DY, et al. Effectiveness and safety of Wuling capsule for post stroke depression: a systematic review. Complement Ther Med 2014;22:549-66.
9. Barbacid M. Structural and functional properties of the TRK family of neurotrophin receptors. Ann N Y Acad Sci 1995;766:442-58.
10. Li Y, Xia B, Li R, et al. Expression of brain-derived neurotrophic factors, neurotrophin-3, and neurotrophin-4 in the nucleus accumbens during heroin dependency and withdrawal. Neuroreport 2017;28:654-60.
11. Guo W, Ji Y, Wang S, et al. Neuronal activity alters BDNF-TrkB signaling kinetics and downstream functions. J Cell Sci 2014;127:2249-60.
12. Andero R, Choi DC, Ressler KJ. BDNF-TrkB receptor regulation of distributed adult neural plasticity, memory formation, and psychiatric disorders. Prog Mol Biol Transl Sci 2014;122:169-92.
13. Fornasiero EF, Bonanomi D, Benfenati F, et al. The role of synapsins in neuronal development. Cell Mol Life Sci 2010;67:1383-96.
14. Nabavi SF, Turner A, Dean O, et al. Post-stroke depression therapy: where are we now? Curr Neurovasc Res 2014;11:279-89.
15. Fox S, Azman A, Timmons S. Palliative care needs in Parkinson's disease: focus on anticipatory grief in family carers. Ann Palliat Med 2020;9:S34-43.
16. Saarelainen T, Hendolin P, Lucas G, et al. Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. J Neurosci 2003;23:349-57.
17. Wang SH, Zhang ZJ, Guo YJ, et al. Hippocampal neurogenesis and behavioural studies on adult ischemic rat response to chronic mild stress. Behav Brain Res 2008;189:9-16.
18. Farahani MS, Bahramsoltani R, Farzaei MH, et al. Plant-derived natural medicines for the management of depression: an overview of mechanisms of action. Rev Neurosci 2015;26:305-21.
19. Sarris J, Panossian A, Schweitzer I, et al. Herbal medicine for depression, anxiety and insomnia: a review of psychopharmacology and clinical evidence. Eur Neuropsychopharmacol 2011;21:841-60.
20. Calabrese F, Molteni R, Racagni G, et al. Neuronal plasticity: a link between stress and mood disorders. Psychoneuroendocrinology 2009;34 Suppl 1:S208-16.
21. Chen HH, Zhang N, Li WY, et al. Overexpression of brain-derived neurotrophic factor in the hippocampus protects against post-stroke depression. Neural Regen Res 2015;10:1427-32.
22. von Bohlen und Halbach O. Involvement of BDNF in age-dependent alterations in the hippocampus. Front Aging Neurosci 2010;2:36.
23. Zhou Z, Lu T, Xu G, et al. Decreased serum brain-derived neurotrophic factor (BDNF) is associated

with post-stroke depression but not with BDNF gene Val66Met polymorphism. Clin Chem Lab Med 2011;49:185-9.

24. Zhang Y, Pardridge WM. Blood-brain barrier targeting of BDNF improves motor function in rats with middle cerebral artery occlusion. Brain Res 2006;1111:227-9.

25. Devi L, Ohno M. 7,8-dihydroxyflavone, a small-molecule TrkB agonist, reverses memory deficits and BACE1 elevation in a mouse model of Alzheimer's disease. Neuropsychopharmacology 2012;37:434-44.

26. Pathak L, Agrawal Y, Dhir A. Natural polyphenols in the management of major depression. Expert Opin Investig Drugs 2013;22:863-80.

27. Gardier AM. Mutant mouse models and antidepressant drug research: focus on serotonin and brain-derived neurotrophic factor. Behav Pharmacol 2009;20:18-32.

28. Mamounas LA, Blue ME, Siuciak JA, et al. Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci 1995;15:7929-39.