Original Paper

Geniposide Attenuates Post-Ischaemic Neurovascular Damage via GluN2A/AKT/ERK-Dependent Mechanism

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Key Words

Geniposide • tMCAO • NMDAR • GluN2A • AKT • ERK

Abstract

Background/Aims: Calcium-permeable ionotropic NMDAR-mediated hyperactivity is regarded as the critical factor in modulating the development of ischaemic stroke. Recently, there has been increasing interest in preventing post-stroke neuronal death by focusing on intervening in the function of subpopulations of NMDARs and their downstream signalling. Geniposide, an iridoid glycoside, has been found to have cytoprotective functions in various conditions. However, it is still unclear whether and how geniposide affects neuronal insult under experimental stroke. **Methods:** We demonstrate that dose-dependent geniposide significantly decreased the infarct volume in tMCAO models. **Results:** A medium level of geniposide improved anti-apoptotic functions and inhibited BBB leakage/haemorrhage via elevating GluN2A-containing NMDAR expression in tMCAO rats. Importantly, these effects could be eliminated by co-treatment of geniposide with the GluN2A antagonist NVP but not the GluN2B inhibitor ifenprodil. Moreover, geniposide's protection was due to the enhancement of GluN2A-dependent survival signals, including pAKT, pERK and PSD-95. **Conclusion:** The results suggest that geniposide protects neurons against post-ischaemic neurovascular injury through the activation of GluN2A/AKT/ERK pathways. As a very promising natural agent, geniposide may be a future therapeutic for stroke patients.

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Published by S. Karger AG, Basel

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Introduction

The N-methyl-D-aspartate (NMDA) receptor, which mediates glutamatergic overexcitation and the Ca²⁺ overload that induces excitotoxicity, is believed to be an overwhelming factor in stroke-triggered cell death [1]. Given that NMDA receptor overexcitation could break the balance between excitation and inhibition in ischaemia and reperfusion, restoring this equilibrium by intervening in neural transmission in the post-ischaemic brain seems to be a useful and selective strategy [2-4]. GluN2A and GluN2B, which are the main subtypes of NMDA receptors, are prominently expressed in the hippocampus and confer distinct pharmacological and electrophysiological properties [5, 6]. It has been implicated that GluN2A and GluN2B differently modulate neuronal survival and death following ischaemia and reperfusion, respectively [3, 4, 7, 8]. However, little benefit of NMDA receptor antagonists has been shown in clinical trials [9]. Therefore, a growing number of studies have focused on novel natural compounds that target NMDA receptor subunits as having a positive effect on ischaemia.

Geniposide, as an iridoid glycoside, was initially isolated from the herb *Gardenia jasminoides* [10], which has been noted for its variable pharmacological effects, including antioxidation [11], anti-inflammation [12, 13], anti-asthma [14], and anti-diabetes [15] effects, among others. However, researchers are most interested in the therapeutic potential of geniposide in the central nervous system (CNS). Early studies showed that geniposide induced neuronal differentiation [16] and attenuated cell injury [17, 18]. By using western blotting techniques in cell lines, geniposide was demonstrated to be involved in a GLP-1R-dependent mechanism [19, 20]. Furthermore, the cytoprotective effects of geniposide were demonstrated in the hippocampus [21, 22]. Studies also showed that geniposide-induced protection could be attributed to PI3K/AKT activation [17, 22] and Nrf2 upregulation [22], whereas geniposide's protection from microglia could be owed to the downregulation of ERK/p38 phosphorylation, interleukin/TNF α secretion and NF κ B activation [23].

Although the cytoprotective effects of geniposide were discovered in an OGD model [21, 23], to date, it has remained unclear whether and how geniposide may be neuroprotective in the brain in conditions of neurovascular diseases, such as cerebral ischaemic stroke. In the present study, we investigated the role of geniposide in cerebro-vascular ischaemic insult and probed its mechanism in a rat model of experimental stroke. We hypothesized that geniposide would contribute to survival against neuronal insult induced by transient middle cerebral artery occlusion (tMCAO) in rats and that this neuroprotection would occur through activation of the NR2A/pAKT/pERK pathway.

Materials and Methods

Animals and tMCAO model

Male Sprague-Dawley rats (280-320 g for tMCAO) were purchased from Shanghai Super-B&K laboratory animal Corp. Ltd. The transient middle cerebral artery occlusion (tMCAO) surgery was conducted as previously described [4, 24]. The animals were anaesthetized with chloral hydrate (0.3 mg/kg) intraperitoneally. Middle cerebral artery occlusion was achieved with a monofilament suture (4-0 silicone-coated nylon suture) for 1 h. At 4 h after the tMCAO induction, the rats were injected intraperitoneally with drugs and then sacrificed at 25 h after tMCAO onset. The sham and I/R (vehicle) groups were administered Normal Saline intraperitoneally. All surgical procedures and experimental protocols were approved by the ethical committee of Nanjing Medical University and were performed in accordance with approved guidelines and regulations.

TTC staining

The tMCAO rats were anaesthetized with chloral hydrate and perfused with ice-cold saline. The brains were rapidly removed and then chilled at -20°C for 10 min. Each brain was sliced into coronal sections (2 mm thickness). Cerebral infarct size was analysed by incubating these sections for 30 minutes at 37°C with 2% 2, 3,5-triphenyltetrazolium chloride (TTC) solution [4, 24].

NSS behavioural tests

Neurological severity scores (NSSs) were tested using a six-grade scale to assess post-stroke behavioural deficits [4, 24].

Western blot Analysis

At 25 hours after tMCAO onset, proteins were extracted from the infarct hemisphere of the rats. The brain samples were homogenized and centrifuged. Proteins were separated on sodium dodecyl sulphate (SDS) polyacrylamide gels (10%) and transferred onto polyvinylidene fluoride membranes. The membranes were then incubated with pAKT, pERK, AKT, ERK, Bcl-2, Bax, GAPDH (Cell Signalling Technology, USA), GluN2A, GluN2B and PSD-95 (Santa Cruz Biotechnology, USA) antibodies at 4°C overnight. After incubating the membranes with secondary antibodies (horseradish peroxidase-conjugated IgG), the immunoreactive bands were visualized [24, 25, 26]. The images were scanned and quantified with Quantity One software (Bio-Rad, USA).

Immunohistochemistry and Nissl staining

The brain samples were coronally sectioned (10 µm thickness) with a paraffin slicer (CM1900, Leica, Germany). The hippocampal and cortical sections were divided into three groups. The first group was Nissl stained for histological assessment of neuronal death, the second one was stained with haematoxylin and eosin (HE) to visualize haemorrhages, and the third one was assayed by immunohistochemistry for histological assessment of GluN2A/GluN2B expression. For the immunohistochemistry, an ABC kit (Zhongshan Biotechnology Company, Beijing, China) was used for primary antibody (GluN2A/GluN2B, Santa Cruz Biotechnology) reactions. Then, a DAB kit (Zhongshan Biotechnology Company, Beijing, China) was used for visualization. Finally, the sections were mounted on slides and analysed using a digital microscope (Leica Microsystems, Germany). The GluN2A-/GluN2B-positive and Nissl-positive cells in the hippocampal DG region were quantified (at approximately -3 and -4.5 mm posterior to bregma) using light microscopy [24].

Blood-Brain Barrier (BBB) Permeability

BBB permeability in tMCAO rats was measured by intravenous injection of 1 ml extravasated Evans blue (EB, 1% EB, Tocris, USA) dye after tMCAO completion [24, 27]. The rats were sacrificed 25 hours after tMCAO onset by transcardial perfusion of heparinized saline. The fluorescence intensity of EB in the cerebral hemispheres was measured at 630 nm by spectrofluorophotometry.

Haemorrhage Measurement

Cortical haemorrhage were assessed based on haematoxylin and eosin (HE) staining under light microscopy [27]. The percentage and size of petechial and gross haemorrhages were quantified in each histological section.

Drugs

Geniposide was purchased from Abcam and Yuanye Shanghai Biotechnology Co., Ltd.

All drugs were dissolved in Normal Saline.

Statistical Analysis

All data are expressed as the mean ± SEM. Statistics were analysed with one-way ANOVA, followed by the Newman-Keuls multiple range test. Statistical significance was deemed when $P < 0.05$.

Results

Geniposide attenuates infarct volume and improves neurologic behaviours in ischaemia

Following the 1 h ischaemic episode and 2 h reperfusion episode of the I/R protocol (Table 1), rats were intraperitoneally injected with exogenous Geni-L (low dose of geniposide, 25 mg/kg), Geni-M (medium dose of geniposide, 75 mg/kg) or Geni-H (high dose of geniposide, 150 mg/kg). The neurological severity scores (NSSs) and TTC staining were

Table 1. Overview of the experimental design

Fig. 1. Geniposide exerts protective effects against infarct volume and behavioural deficits in tMCAO ischaemia. A, B: The changes in infarct volumes were obtained 25 h after the treatment with geniposide in stroke. C: Alterations were observed in the 6-point neurological severity score (NSS) tests. Geni-M reduced the infarct sizes (n=8) and increased the NSSs (n=8) after tMCAO, whereas Geni-L and Geni-H failed to show the same effects (n=8). I/R: rats subjected to 60 min of transient distal MCA occlusion followed by reperfusion; Geni-L, Geni-M, Geni-H: rats treated with an intraperitoneal injection of 25 mg/kg, 75 mg/kg and 150 mg/kg geniposide, respectively, 3 h after ischaemia. Bars represent the mean $\pm$ SEM. **p<0.01 vs I/R.

assessed 25 h after the tMCAO onset. It was found that Geni-M significantly reduced the total infarct volume (n=8, $P<0.01$; Fig. 1A, B, C) compared with that observed in the I/R subjects. However, Geni-L and Geni-H failed to exert the same effects under the same conditions (n=8, Fig. 1A, B, C), inconsistent with the TTC data. NSS showed that Geni-M remarkably attenuated post-ischaemic deficits in behavioural outcomes (n=8, $P<0.01$; Fig. 1D), whereas effects of Geni-L and Geni-H were not evident (n=8, Fig. 1D). The overall effect of geniposide in the tMCAO rats was dose dependent: Geni-M was apparently neuroprotective, whereas Geni-L and Geni-H were invalid. Accordingly, it was suggested that geniposide at the medium level (75 mg/kg) has the potential for neuroprotective effects against post-ischaemic neuronal injury.

Fig. 2. Geniposide prevents cellular and mitochondrial apoptosis by enhancing the number of intact neurons and the Bcl-2/Bax ratio after stroke. A, B: Morphological changes consistent with neuronal death were observed in the hippocampal DG region by Nissl staining (n=4). Bars represent the mean $\pm$ SEM. *p<0.05 vs I/R. C, D: Compared with that in the I/R group, the expression of Bcl-2/Bax was elevated in the rats that received geniposide administration (n=4). Bars represent the mean $\pm$ SEM. *p<0.05 vs I/R.

The protective effects of geniposide were attributed to the prevention of neuronal death/apoptosis and vascular deterioration in tMCAO rats

Geniposide has the capacity for neuroprotection. Therefore, Nissl staining of hippocampal slices (Fig. 2A, B) and western blotting of Bcl-2/Bax (Fig. 2C, D) were used to assess the protective effect of treatment with the medium level geniposide (75 mg/kg) on cellular injury and mitochondrial apoptosis. The results showed that geniposide significantly enhanced the number of intact neurons in the hippocampal DG region compared with the corresponding value in I/R-operated subjects (n=4, p<0.05, Fig. 2A,B). Meanwhile, as shown in Fig. 2C,D by the analysis of alterations in apoptosis-related proteins, geniposide dramatically increased the Bcl-2/Bax ratio (n=4, p<0.05) after H-NFPS administration in ischaemia *in vivo*. Thus, the overall effect of geniposide in ischaemic stroke is achieved through both elevating anti-apoptotic signalling and suppressing neural cellular death pathways.

Having measured that geniposide inhibits neuronal death and apoptosis after ischaemia and reperfusion, we further investigated whether geniposide affects blood-brain-barrier (BBB) integrity and the tendency for haemorrhage. The quantitative data showed that geniposide significantly decreased extravasated Evans blue (EB) leakage (n=4, p<0.05, Fig. 3A,B) in the ipsilateral cortex, indicating that geniposide reduced the BBB permeability after ischaemia and reperfusion. In line with the EB results, geniposide also markedly reduced the cerebral haemorrhage volume (n=4, p<0.05, Fig. 3C,D), as revealed by HE staining of the post-stroke brain. These results indicate that the geniposide treatment protected the tMCAO animals against vascular remodelling, a multifactorial phenomenon in which ischaemic tissue results in BBB disruption and controlled haemorrhagic lesion, thereby exacerbating vascular injury.

Geniposide enhances expression of the NMDA receptor subtype GluN2A, instead of GluN2B, after stroke

The NMDAR subtypes GluN2A and GluN2B are believed to be critical factors in ischaemia-dependent glutamatergic overexcitation [4, 28], which determines the direction

Fig. 3. Geniposide protects against the deterioration of BBB damage and haemorrhage after stroke. A, B: Leakage of EB (blue area) indicative of BBB breakdown after tMCAO (n=4). Bars represent the mean $\pm$ SEM. *p<0.05 vs I/R. C, D: Red area indicates cerebral haemorrhage volume (n=4). Bars represent the mean $\pm$ SEM. *p<0.05 vs I/R.

Fig. 4. Enhanced GluN2A instead of GluN2B expression by geniposide. A, B: GluN2A/GluN2B expression was examined in the hippocampal DG region by immunohistochemistry. C, D: GluN2A/GluN2B protein levels were analysed by western blotting. Treatment with geniposide markedly enhanced the GluN2A expression level (n=4) compared with that in the I/R group; however, GluN2B expression did not show the same effect (n=4). Bars represent the mean $\pm$ SEM. *p<0.05 vs I/R.

Fig. 5. The effects of geniposide in ischaemic insult differ across NMDAR subtypes. A, B: Changes in infarct volume were observed after different treatments of ischaemia and reperfusion. C: Alterations were observed in the 6-point neurological severity score (NSS) tests. Geniposide significantly attenuated the infarct sizes and elevated NSSs after tMCAO (n=4). Administration of geniposide together with the GluN2A inhibitor NVP worsened the infarct sizes and NSSs compared with those in the geniposide group (n=6); however, these effects were not evident in the group that received co-treatment of geniposide with the GluN2B blocker ifenprodil. Bars represent the mean $\pm$ SEM. * $p < 0.05$ vs I/R.

of neuronal survival or death [3, 4, 7, 8]. To explore whether different NMDAR subunits are associated with M-Geni, we examined GluN2A/GluN2B expression levels by applying both immunohistochemistry (Fig. 4A) and western blot analysis (Fig. 4B,C,D) to the hippocampus of the tMCAO rats. The downregulation of GluN2A after ischaemia and reperfusion was less severe (n=5, $p < 0.05$; Fig. 4A,B,C) with geniposide than without. However, the differences in GluN2B protein levels and immunoreactivity between the groups were weak (n=5, Fig. 4A,B,D). These results provide evidence that GluN2A-containing NMDARs are involved in the neuroprotective effects of geniposide.

The neuroprotective effects of geniposide against post-ischaemic injury are GluN2A dependent

Given that GluN2A is an effective and novel target of geniposide, it is of great importance to ascertain whether post-ischaemic inhibition of GluN2 subunits would be effective against stroke in tMCAO models. Co-applying geniposide with the GluN2B-specific antagonist ifenprodil (3 mg/kg) failed to produce any evident effects (n=6; Fig. 5A,B,C) relative to the corresponding measures in I/R animals. In contrast, treatment with geniposide along with the GluN2A inhibitor NVP-AAM077 (NVP, 2.4 mg/kg) significantly reversed the amelioration of infarct size (n=6, $p < 0.05$; Fig. 5A,B) and behavioural deficits (n=6, $p < 0.05$; Fig. 5C) achieved by geniposide, indicating that geniposide protected against post-ischaemic injury via selective enhancement of GluN2A-containing NMDARs. The reversal of geniposide's effects by pharmacological inhibition of GluN2A suggest that GluN2A-containing NMDARs play a critical role in geniposide's neuroprotective effects.

Fig. 6. Geniposide protects neurons by activating GluN2A/AKT/ERK pathways. A: Samples of AKT, p-AKT, ERK, p-ERK and PSD-95 were significantly altered by geniposide, as shown by western blotting. B, C, D: The expression levels are quantified and represented as follows: p-AKT (n=4); p-ERK (n=4); PSD-95 (n=4). However, the effects were eliminated when geniposide was co-injected with the GluN2A antagonist (p-AKT and p-ERK). Bars represent the mean $\pm$ SEM. * $p < 0.05$ vs I/R.

GluN2A/Akt/ERK-dependent synaptic protection is the fundamental mechanism of geniposide

Previous studies showed that geniposide is cytoprotective due to its involvement in the signalling of kinases, such as PI3K/AKT [17, 22] and ERK [23]. To determine whether these *in vitro* findings could also be demonstrated *in vivo*, we performed western blot analysis in geniposide-treated tMCAO rats. The results showed that geniposide significantly elevated the expression of phosphorylated AKT (n=4, $P < 0.05$, Fig. 6A,B), phosphorylated ERK (n=4, $P < 0.05$, Fig. 6A,C) and PSD-95 (n=4, $P < 0.05$, Fig. 6A,D) compared to the corresponding expression levels in the I/R group. However, the elevation of pAKT (n=4, $P < 0.05$, Fig. 6A,B) and pERK (n=4, $P < 0.05$, Fig. 6A,C) was successfully eliminated when geniposide was treated simultaneously with the GluN2A blocker. Thus, these results suggest that geniposide's effects against neurovascular damage in this model of cerebral ischaemia and reperfusion occur via its influence on GluN2A/pAKT/pERK survival signalling.

Discussion

Quantitative studies have suggested the therapeutic potential of geniposide under variable conditions, especially in cellular death [17-23]. However, the underlying mechanisms that account for the neuroprotective effects of geniposide against post-ischaemic injury remain unclear, and the ischaemic model needs improvement. We report here that a medium level of geniposide applied *in vivo* could be an effective treatment to attenuate the deterioration of post-stroke neuronal injury.

The four major findings of our investigation are listed below. First, dose-dependent geniposide is neuroprotective because it decreases infarct volume in tMCAO rats. Second, a

medium level of geniposide protects against post-stroke injury by modulating neuronal loss/apoptosis and BBB leakage/haemorrhage, particularly in tMCAO. Third, GluN2A, instead of GluN2B, is significantly enhanced by geniposide treatment. However, the treatment effects could be reversed only by co-applying geniposide with the GluN2A antagonist NVP and not the GluN2B inhibitor ifenprodil. We infer that GluN2A enhancement accounts for the neurovascular protective effects of geniposide in MCAO rats. Activation of GluN2A-containing NMDARs results in remarkable elevation of network excitation, which, in turn, activates pro-survival signals in ischaemia [3, 29]. Fourth, geniposide's protection can be attributed to considerable elevation of GluN2A-dependent survival signals, including pAKT, pERK and PSD-95. Taken together, we show that geniposide is a potent neuroprotective agent in ischaemic stroke, particularly by targeting GluN2A NMDA receptors and its downstream molecular signals.

Previous studies showed that geniposide was involved in cytoprotection in various models, including PC-12 cells [17, 18], INS-1 cells [19], microglia [23], spinal neurons [20] and hippocampal neurons [22]. Recently, an interesting study reported that geniposide could be protective in the rat MCAO model [23]. However, the underlying mechanism of this *in vivo* neuroprotection has not been fully studied. Therefore, we explored geniposide's effects *in vivo* in tMCAO rats. Based on the neuroprotection observed in different ischaemic models, our results provide further evidence that geniposide has the ability to not only inhibit neuronal loss/BBB leakage/haemorrhage but also increase the Bcl-2/Bax ratio. Nevertheless, it remains an open question which component of the protection, the inhibition of cerebrovascular deterioration or the elevation of neurovascular survival, should be considered the core mechanism of geniposide in stroke. Given that geniposide protects neurons in the OGD model [17, 22, 23], it is possible that modulation of glutamate-mediated excitotoxicity may be a clue to the balance between the death decreasing and survival increasing effects of geniposide treatment in ischaemia.

Under pathological conditions such as stroke, epilepsy and TBI, excessive glutamate stimulation results in NMDAR hyperactivity, which induces excitotoxic insult [1]. Subsequent to ischaemic stroke, the resulting excitotoxicity induces the expression of GluN2A- and GluN2B-containing NMDARs, which are regarded as crucial factors in neuronal survival and death [2-4]. Extrasynaptic GluN2B-containing NMDAR activation contributes to neuronal damage, whereas synaptic/extrasynaptic GluN2A-containing NMDAR stimulation results in protection of the brain [3, 4, 7, 8]. Based on these observations, decreasing GluN2B expression could provide an effective strategy for the resistance of excitotoxic injury [28, 29]. Alternatively, GluN2A enhancement could protect the brain against ischaemia [7, 8, 30, 31]. To distinguish between these competing hypotheses, we performed western blot analyses of GluN2A and GluN2B expression, which indicated they played different roles in the neurovascular protection of geniposide. The present study showed that the upregulation of GluN2A is separately induced by geniposide after stroke, indicating that the neuroprotective effects of geniposide are probably due to the elevation of GluN2A rather than to the inhibition of GluN2B. Furthermore, we observed that the previous pro-neuronal survival effect of geniposide in tMCAO was abolished after co-applying geniposide with the GluN2A inhibitor NVP. In contrast, application of the GluN2B antagonist ifenprodil did not affect geniposide-related ischaemic protection. Thus, we confirmed that the protective role of geniposide is GluN2A elevation dependent. These results are also in line with previous work supporting the hypothesis that direct enhancement of GluN2A promotes cerebral recovery in ischaemia [4, 7, 24].

Although elevating the expression of GluN2A by targeting the receptor itself may reflect the survival pattern in cerebral death and repair, clinical limitations are still evident and may restrict the practical use of antagonists of NMDARs and its subtypes. In an effort to overcome these limitations, scientists broadened their focus on therapeutic agents targeting NMDARs, GluN2B and GluN2A to include those that target their downstream molecular sites. There is considerable interest in the PI3K/AKT [32, 33] and ERK [34-36] signals related to the pro-survival effects exerted by synaptic, instead of extrasynaptic, NMDARs in ischaemic

stroke. Activation of AKT and ERK has also been shown to participate in the regulation of not only neuronal survival but also BBB impairment. Importantly, previous studies have shown that the protective effects of geniposide can be attributed to PI3K/AKT activation [17, 22] and ERK/p38 phosphorylation [23] in OGD ischaemia. Actually, we observed that the phosphorylated forms of AKT and ERK were unregulated by geniposide after stroke. Thus, it is understandable that geniposide can be neuroprotective in tMCAO ischaemia due to pAKT/pERK upregulation. However, considering the hypothesis that geniposide is a possible GLP-1 analogue [19, 20], it is not clear whether GluN2A stimulation is the sole factor accounting for geniposide-induced AKT/ERK phosphorylation in ischaemia. We further evaluated the alteration of pAKT/pERK by co-injecting geniposide with the GluN2A antagonist NVP in tMCAO rats. Unsurprisingly, the geniposide-induced increase in pAKT/pERK was significantly reversed under blockade of GluN2A. Above all, we successfully showed that pAKT/pERK, as downstream proteins, are involved in the modulatory effects of geniposide treatment on GluN2A-containing NMDARs after stroke. According to the well-established link between the molecular mechanisms and geniposide's neuroprotective function, our findings related to the GluN2A/AKT/ERK pathway may resolve the discrepancy in previous investigations.

Numerous studies have repeatedly reported that potential therapeutic compounds reduce post-ischaemic damage through deactivation of NMDARs in rodent stroke models [29, 37]. Until now, however, the clinical application of NMDAR antagonists in human stroke participants has failed [9, 38] due to several reasons, as follows: (a) non-selective triggering of the broad intracellular cascades related to NMDARs, (b) the narrow time window for treatment within the acute phase of stroke, and (c) cognitive and emotional side effects. Importantly, growing evidence has shown that some of the positive effects of herbal extracts on cerebral ischaemia occur via natural stimulation of NMDARs; such extracts include resveratrol [39], 1-methoxyoctadecan-1-ol [40] and MQA [41]. Based on our results, geniposide seems to be a highly promising bioactive compound against ischaemic stroke *in vivo*. Considering it specifically targets GluN2A-containing NMDAR and the downstream AKT/ERK proteins, geniposide may be as efficient as traditional NMDAR blockers, with both simpler practical uses and fewer side effects.

Consequently, we conclude that geniposide treatment improves neurovascular recovery after stroke through upregulation of GluN2A and phosphorylation of AKT/ERK. Our data are consistent with quantitative studies showing that geniposide is generally beneficial, with a cytoprotective effect. However, we built a new model that not only proves the neuroprotection of geniposide *in vivo* but also demonstrates a link between the protective function and molecular pathways. Therefore, we suggest a new strategy of the application of geniposide as a clinical therapy for brain diseases associated with modulation of the GluN2A/AKT/ERK pathway.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China- Young Scientists (81501059), the Research Foundation of Jiangsu Provincial Medical Youth Talent, the Project of Invigorating Health Care through Science, Technology and Education (QNRC2016858) and the Science and Technique Development Foundation of Nanjing Medical University (2016NJMUZD035).

Disclosure Statement

Competing financial interests: The authors declare no competing financial interests.

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