Original Article

Administration of LXW7 following transient cerebral ischemic stroke confers neuroprotection in rats

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Abstract: Certain integrins such as alpha v beta 3 and alpha 5 beta 1 perform very important functions in the pathophysiological processes involved in cerebral ischemia/reperfusion. The inhibition of alpha v beta 3 in the early stage of stroke may protect the brain against ischemic insult, possibly through the regulation of vascular endothelial growth factor (VEGF) and other mediators. We investigated the therapeutic effects of LXW7, a novel antagonist of alpha v beta 3, on the outcomes of middle cerebral artery occlusion (MCAO) in rats. Adult male Sprague-Dawley rats were subjected to 2 h of MCAO and then reperfusion. LXW7 was intravenously administered at doses of 100 and 200 μg/kg at 1 h after the induction of MCAO. Neurological deficit scores, infarct volume, brain edema, blood-brain barrier (BBB) disruption, and the levels of regulatory mediators such as VEGF, fibrinogen, and inflammatory cytokines were evaluated. At 24 h after ischemia, infarct volume, cerebral edema, BBB disruption, and the levels of regulatory mediators such as VEGF, P-FAK, P-Flk, fibrinogen, interleukin (IL)-1β, and tumor necrosis factor (TNF)-α, but not the neurological deficit scores, were significantly higher in the control group than in the two treatment groups. No remarkable difference was observed between the two treatment groups. These findings implicate LXW7, an antagonist of the integrin alpha v beta 3, exerts a neuroprotective effect after ischemic stroke, possibly via the down-regulation of VEGF and its associated pathways.

Keywords: LXW7, integrin alpha v beta 3, middle cerebral artery occlusion, vascular endothelial growth factor, cerebral edema

Introduction

Stroke, ischemic heart disease, and respiratory diseases constitute the top three killers around the world according to statistics published by the World Health Organization. In a Global Burden of Disease Study 2013, stroke was estimated to be the second leading cause of mortality around the world, and approximately half of all strokes were found to be ischemic in origin [1]. The high incidence of death and disability among stroke patients poses a significant burden on individuals and families as well as on the society. Therefore, effective and efficient therapeutic approaches are in need to alleviate and improve the neurological symptoms and other physiological impairments in stroke. Despite numerous experimental and clinical trials have been performed, none of the studied agents have been proved to be beneficial in clinical practice, and some of them still require further investigation. At present, only thrombolytic agents such as recombinant tissue plasminogen activator have been approved for clinical use in acute ischemic stroke cases [2]. However, the narrow therapeutic window, high possibility of intracerebral hemorrhage and reperfusion injury greatly limit their application. Therefore, the invention of alternative neuroprotective agents to ameliorate the damage following ischemia becomes an imminent issue.

Integrins are transmembrane receptors with two distinct subunits, namely, alpha and beta chains, and act as bridges for cell-extracellular matrix (ECM) and cell-cell interactions. By transmitting the signals sensed from changes in the external and internal environments of cells, integrins are capable of exhibiting numerous biological functions such as cell migration, proliferation, differentiation, and apoptosis [3, 4]. Integrin alpha v beta 3 is mainly expressed on activated endothelial cells, activated leukocytes, and macrophages, and participates in
various physiological and pathological processes, including angiogenesis, tumor invasion or metastasis, inflammation, bone resorption, blood clot formation, apoptotic events, and wound repair [5-9]. During ischemic stroke, the level of alpha v beta 3 is significantly elevated [10, 11], and inhibitors of alpha v beta 3 have been scattered investigated in some cerebral ischemic models. Research has demonstrated that the inhibition of this integrin alleviates the outcomes of cerebral ischemia in middle cerebral artery occlusion (MCAO) models, possibly via the downregulation of vascular endothelial growth factor (VEGF), fibrin, inflammatory factors, and other molecules. A considerable amount of angiogenic factors such as VEGF, integrin alpha v beta 3, and fibrinogen are increased as early as 1-2 h following ischemia [10, 12]. During the early stages of post-ischemia, the inhibition of VEGF might decrease blood-brain barrier (BBB) permeability, alleviate the extent of cerebral edema, and lower the risk of hemorrhagic transformation [13]. Fibrin deposition can block the microvasculature and further potentiate ischemic stroke; as fibrinogen is one of the ligands of alpha v beta 3, the antagonists of this integrin may theoretically help ameliorate the injuries resulting from stroke [14-16].

Cyclic peptide LXW7, which is synthesized using one-bead one-compound combinatorial library technology, exhibits excellent binding affinity and specificity to the arginine-glycine-aspartic acid (RGD) sequence containing alpha v beta 3; high tumor uptake and low liver uptake of this peptide antagonist of alpha v beta 3 have been observed both in vivo and ex vivo [7]. In the present study, we aimed to determine whether LXW7 had any neuroprotective effect and if so, elucidate the possible mechanisms through which it exerts this effect. We utilized the MCAO model to analyze infarct lesions, neurological status, BBB permeability, extent of edema, as well as changes in fibrinogen, VEGF and its associated receptors, and some other inflammatory mediators.

Materials and methods

Adult male Sprague-Dawley (SD) rats weighing 250-280 g were chosen for this study. The rats were raised in identical specific pathogen-free (SPF) environments with an alternating 12-h light/dark cycle, and enough food and water. The animals were divided into following four groups in random: (A) control group; (B) sham-operated group; (C) LXW7-treated group (100 μg/kg); and (D) LXW7-treated group (200 μg/kg).

The rats for study were anesthetized with 10% chlorate hydrate and fixed on an operating table. After disinfection, a cervical vertical incision was made, followed by careful exposure of the common carotid artery, external carotid artery, and internal carotid artery. A 4-0 monofilament nylon suture coated with silicone was inserted into the external carotid artery and gently pushed forward into the internal carotid artery until the origin of the middle cerebral artery (18-20 mm). At 2 h after the occlusion, the suture was carefully withdrawn to allow for cerebral reperfusion. A heating pad and lamp were used to stabilize the rectal temperature at around 37°C from the beginning of the surgery until the recovery of the rats from anesthesia. At 22 h after reperfusion, all the rats were anesthetized and euthanized.

In the control group, phosphate-buffered saline (PBS; pH, 9.0), was intravenously injected at 1 h after the induction of cerebral ischemia. The sham-operated rats received the same treatment as the control rats, except that no suture was inserted into the artery. In the low- and high-dose LXW7 groups, LXW7 dissolved in PBS was intravenously administered at doses of 100 μg/kg and 200 μg/kg, respectively, at the same time as the PBS injections in other two groups.

All animal welfare and experimental procedures were in agreement with the National Institutes of Health guidelines on the use of laboratory animals, and were approved by the Animal Experimentation Ethics Committee of Peking University Shenzhen Hospital. All efforts were made to the best for the sake of minimizing the pain and suffering of the animals.

Neurological assessment

The rats were subjected to neurological deficit evaluations, described by Longa et al. [17], before as well as 2 and 24 h after the induction of ischemia. The neurological findings were scored based on a five-tiered grading system as follows: 0, no observable deficit; 1, failure to extend the contralateral forelimb; 2, circling to
the contralateral side, especially, if the animal was pulled by its tail; 3, falling down to the contralateral side; and 4, lack of spontaneous walking and decreased level of consciousness. The neurological evaluations were carried out by an investigator who was blinded to the grouping.

**Measurement of cerebral infarct volume**

The rats were anesthetized with 10% chlorate hydrate administered intraperitoneally and euthanized by decapitation. Their brains were removed immediately and stored at -20°C for 20 min. The brains were then cut into six 2-mm-wide slices coronally and immersed into 2% 2,3,5-triphenyltetrazolium (TTC) solution at 37°C for 15 min. Then, 4% paraformaldehyde was used for fixation. The stained slices were photographed and analyzed using an imaging software system (ImageJ 1.33u, NIH, USA). The infarct volume was calculated using a previously described formula as follows: infarct volume = (volume of non-infarct hemisphere - intact volume of infarct hemisphere)/volume of non-infarct hemisphere.

**Determination of BBB permeability**

BBB permeability was quantitatively measured by the detection of extravasated Evans blue dye. Approximately 2 ml/kg of 2% Evans blue dye in cold PBS was administered intravenously at 22 h after the induction of ischemia. Two hours later, at the time of euthanasia, the rats were anesthetized with chlorate hydrate and transcardially perfused with saline until colorless fluid was observed. After decapitation, the brains were quickly removed, divided into hemispheres, and weighed separately. The brain samples were homogenized in 500 µl of 50% trichloroacetic acid solution and then centrifuged for 15 min at 12,000 rpm at 4°C. Ethanol (1:3) was added to the extracted supernatants to precipitate proteins. Fluorescence was measured using a spectrophotometer (excitation: 620 nm; emission: 680 nm). The amount of extravasated Evans blue dye was expressed as Evans blue dye (µg)/hemisphere (g).

**Water content**

The rats were decapitated under anesthesia with 10% chlorate hydrate at 22 h after cerebral reperfusion. The brain was immediately removed and separated into two hemispheres along the middle longitudinal fissures. Each of the hemispheres was weighed to determine the wet weight and then placed into a drying oven at 120°C for 24 h to obtain the dry weight. The water content of each hemisphere was calculated using the following formula: water content (%) = (wet weight - dry weight)/wet weight × 100%.

**Western blot analysis**

At 22 h after reperfusion, the rats were anesthetized with 10% chlorate hydrate and then sacrificed by decapitation. Brain slices were quickly extracted, and the corresponding peri-ischemic areas were obtained and stored at -80°C. The frozen samples were homogenized in ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (50 mM Tris buffer, pH 8.0; 150 mM NaCl; 1% NP-40; 0.5% deoxycholate; and 0.1% sodium dodecyl sulfate) with phenylmethylsulfonyl fluoride (Beyotime Biotech, China). The homogenate was extracted and centrifuged at 4°C at 12,000 g for 10 min. Equivalent amounts of protein were subjected to 8%-12% SDS-PAGE and transferred to polyvinylidene fluoride membranes (PVDF) (0.22 µm, Millipore, MA, USA). The membranes were blocked in 5% non-fat milk (Sangon Biotech, China) for 2 h at room temperature and then incubated overnight with the primary antibodies at 4°C. The primary antibodies used included polyclonal anti-VEGF (1:1000; Abcam, Cambridge, UK), anti-phosphorylated FAK (1:500; Santa Cruz, Calif, USA), anti-FAK (1:1000; CST, MA, USA), anti-phosphorylated VEGF receptor 2 (1:1500; Abcam, Cambridge, UK), anti-VEGF receptor 2 (1:1000; Abcam, Cambridge, UK), anti-fibrinogen (1:4000; Proteintech, Chicago, USA), anti-β-actin (1:3000; Bioworld, Georgia, USA), anti-interleukin (IL)-1β (1:500; Proteintech, Chicago, USA), and anti-tumor necrosis factor (TNF)-α (1:1000; Proteintech, Chicago, USA). After being washed 3 times with Tris-buffered saline supplemented with Tween 20 (TBST), the membranes were incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:10,000; Bioworld, Georgia, USA) and washed 5 times with TBST. An ECL system (Bio-Rad, USA) was used to visualize specific immunoreactive bands. A Bio-Image Analysis system (Bio-Rad, USA) was used to quantify the results of corresponding densitometry.
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**Immunohistochemistry**

The rats were transcardially perfused with saline solution and then with 4% paraformaldehyde under deep anesthesia at 24 h after the induction of ischemia. Brain samples were harvested and postfixed in 4% paraformaldehyde overnight. The samples were then paraffin-embedded and cut into 5-μm-thick slices. The slices were incubated with 3% hydrogen peroxide for 20 min to quench endogenous peroxidase. After being rinsed 3 times (for 5 min each) with PBS, the slides were blocked with 10% normal goat serum for 40 min and incubated overnight with the primary antibodies at 4°C. This was followed by incubation with the secondary antibody for 30 min at room temperature. The primary antibodies included polyclonal anti-VEGF (1:1000; Abcam, Cambridge, UK), anti-phosphorylated FAK (1:500; Santa Cruz, Calif, USA), anti-phosphorylated VEGF receptor 2 (1:400; Abcam, Cambridge, UK), and anti-fibrinogen (1:800; Proteintech, Chicago, USA). The secondary antibody was purchased from MaxVision (KIT-5004). The stained reaction products were visualized after being immersed into PBS with 3,3-diaminobenzidine (DAB) and hydrogen peroxide.

**Enzyme-linked immunosorbent assay**

At 22 h after cerebral reperfusion, the rats were sacrificed, and their brain tissues (approximately 100 mg) were quickly removed and stored at -80°C. After homogenization and centrifugation, the obtained supernatants were adjusted for the determination of protein levels. The levels of IL-1β and TNF-α were determined using ELISA kits (Bioworld, USA) in accordance with the manufacturer’s instructions.

**Statistical analysis**

All quantitative data were presented as means ± standard deviations (SD). Statistical significance was evaluated by one-way Analysis of variance (ANOVA) if the data were homogeneity of variance; otherwise, they were analyzed by Welch ANOVA. Multiple comparisons were made between the least significant difference (LSD) if the data were homogeneity of variance; or they were analyzed by Dunnett’s method. Values of $P < 0.05$ were regarded as significant.

**Results**

**Measurement of neurological function and infarct size**

Neurological function and infarct size were measured 22 h after cerebral reperfusion. There were no neurological deficits or cerebral infarcts in the sham-operated group. Rats treated with LXW7 (both high and low doses) at
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1 h after the induction of ischemia had slightly better neurological evaluation scores compared with the control rats, but the difference was indistinct. The infarct size, however, was significantly smaller in the LXW7 groups than in the control group (Figure 1A, 1B). Neither the neurological scores nor the infarct volume significantly differed between the two LXW7 groups (Figure 1A, 1B). Figure 1C demonstrated representative photographs of coronal slices of individual group.

**Brain water content and Evans blue leakage**

The brain water content was used to determine the extent of cerebral edema after the induction of cerebral ischemia and reperfusion. The water content of the ischemic hemisphere was obviously greater in the control group than in the sham-operated group (Figure 2A). Brain edema was distinctly ameliorated in the two LXW7 groups as compared to the control group, but no significant difference in the extent of edema was found between the two LXW7 groups (Figure 2A).

The extravasation of Evans blue was utilized to determine the degree of BBB disruption. The amount of extravasated dye was much lower in the LXW7 groups than in the control group (Figure 2B), and was lower in the low-dose LXW7 group than in the high-dose LXW7 group, although the difference was not statistically significant (Figure 2B).

**Determination of IL-1β and TNF-α levels**

IL-1β and TNF-α, two of the most well-established pro-inflammatory cytokines, are regarded as useful markers of the brain damage process. The levels of these markers were detected using ELISA on day 1 after the induction of ischemia. Among all the groups, the sham-operated group had lowest values of both IL-1β and TNF-α, while the control-group rats exhibited significantly higher levels of both markers than those in the two treatment groups (Figure 3A, 3B). As shown in Figure 3A and 3B, the LXW7 200 μg/kg group presented improving trend of IL-1β in comparison with LXW7 100 μg/kg group; on the other hand, in terms of TNF-α, the outcome was opposite, but no remarkable significance was obtained considering the differences of trends at two doses. Consistent results were obtained on western blot analysis (Figure 3C, 3D).

**Quantification of fibrinogen, VEGF, and associated ligands**

To investigate the effect of LXW7 on the levels of fibrinogen, VEGF, and its associated ligands, we used western blot analysis to measure protein expression. The expression levels of fibrinogen and VEGF at 24 h after MCAO were significantly lower in both treatment groups than in the control group (Figure 4A, 4B). However, there was no significant difference in these levels between the two treatment groups. To confirm the results of western blot analysis, we performed immunohistochemical analysis to estimate the changes in the levels of these two markers. Consistent results were obtained on immunohistochemistry, indicating the relatively strong inhibitory effect of LXW7 on fibrinogen and VEGF (Figure 5A, 5B).
The expression of P-Flk-1, or VEGF receptor 2, in the ischemic hemisphere was significantly elevated in the control group while no significant change in expression was detected in the sham-operated group (Figure 4D). Moreover, the treatment groups exhibited an apparent suppression in the relative content of p-FLK-1, the degree of which did not differ between the two groups (Figure 4D).

Western blot analyses showed that MCAO elicited a significant increase in P-FAK, the phosphorylated form of intercellular presence of VEGF, whereas treatment with LXW7 reduced the elevation of P-FAK expression (Figure 4D). Interestingly, unlike the previous trend of outcomes, the high-dose LXW7 group revealed more plain inhibition in the reduction of P-FAK than that in the low-dose LXW7 group (Figure 4C). Immunohistochemistry confirmed that the treatment groups had lower levels of P-FAK than the control group, but showed that the P-FAK levels did not differ between the two treatment groups (Figure 5C).

Discussion

In the present study, LXW7 was administered to rat models of MCAO at 1 h after the induction of ischemia. Our findings revealed that LXW7 was associated with amelioration of cerebral edema and BBB disruption, reduced brain infarction, and decreased the expressions of fibrinogen, VEGF and its associated receptors, IL-1β and TNF-α.
and inflammatory cytokines, including IL-1β and TNF-α.

Cerebral edema, one of the most common complications of cerebral stroke or ischemia, accounts for the majority of the lethal consequences of stroke. The underlying mechanisms are far more sophisticated than previously thought, and have only been partially elucidated. One of the well-known explanations is the breakdown of the BBB, leading to the so-called vasogenic edema. The BBB is composed of capillary endothelial cells, which are stitched together by tight junctions with high selectivity; the endothelial cells are surrounded by astrocytes, which provide biochemical support, as well as by microglia [18]. Numerous biochemical cytokines or mediators have been proposed to play pivotal roles in BBB function, including VEGF, some inflammatory cells, free radicals, endothelin, aquaporin, nitric oxide, and high mobility group box-1 (HMGB1) [19-24]. It has
been proposed that VEGF possesses strong vascular permeability, and the early administration of VEGF antagonists to ischemic animals is quite effective in reducing hypoxia/reperfusion-induced vascular permeability and thereby ameliorating cerebral edema [25, 26]. Possible insights into how VEGF induces vascular leakage during ischemia have been provided in several research studies, mainly focusing on the leakage of small solutes through the formation of fenestrations or caveolae in endothelial cells as well as on the transport of larger proteins or other circulating cells via the tight junctions between endothelial cells [27]. Among the mediators involved in BBB disruption, inflammatory factors such as IL-1β and TNF-α have garnered much research attention owing to their pathophysiological effects. On the other hand, another proposed mechanism is that ion pump/energy failure without BBB damage is related to ischemia/reperfusion-induced edema [28]. In our present study, both brain water content and the amount of extravasated Evans blue dye were significantly lower in the LXW7 groups than in the control group, revealing that LXW7 ameliorates BBB damage as well as cerebral edema after MCAO.

Fibrinogen is a well-known ligand of the integrin alpha v beta 3; therefore, we hypothesized that the inhibition of this integrin was likely to result in the downregulation of fibrinogen. In our study, western blot analysis of the tissue samples obtained from the ischemic penumbra indicated that compared to the control treatment, LXW7 treatment (both high and low doses) reduced fibrinogen levels. The overproduction of fibrinogen and deposition of fibrin following ischemia/reperfusion have been confirmed in previous studies [16, 29]. Fibrin deposition may either block cerebral blood perfusion alone or help activate platelets and thus attenuate ischemic injury; fibrinogen has also been reported to be potentially capable of inter-
rupting neurological recovery via the suppression of neurite outgrowth and axon regeneration [30, 31]. Furthermore, published reports suggest that the extravasation of fibrinogen is prone to increase following ischemia/reperfusion and reach a peak on the third day, coinciding with the peak in BBB permeability; thus, fibrinogen is a relatively trustworthy marker of BBB disruption [13, 32]. The inhibitory outcome we observed was quite likely attributable to the direct suppression of the integrin alpha v beta 3 by LXW7 or to an indirect consequence of the partial restoration of the BBB or both.

Our observation that the elevated levels of VEGF, P-Flk-1, and P-FAK induced by ischemia/reperfusion were markedly attenuated in the treatment groups demonstrates that VEGF and its receptors might be involved in the mediation of alpha v beta 3-associated changes. A growing body of evidence suggests that integrins and VEGF have mutual impacts on each other. The upregulation of alpha v beta 3 has been found as early as 1 h after ischemia, and its expression, together with that of some other integrins such as α5β1 and α2β1, contributes to various complex pathophysiological processes that occur following cerebral stroke [33-35]. Numerous research studies have confirmed that VEGF potentiates the expression of alpha v beta 3, and vice versa [36-38]. In experimental stroke models, the rapid expression of VEGF and its associated receptors, especially Flk-1, occurs within several hours after the ischemia-related insult, and remains for days to weeks, primarily in the ischemic penumbra [27, 39]. Although VEGF was previously considered solely as a beneficial molecule owing to its effects of mediating angiogenesis and permeability, it has been documented in recent studies to be highly relevant to ischemia/reperfusion insults via participating in various processes involved in stroke, including collateral circulation formation, neuroprotection, neurogenesis, atherosclerosis, cerebral edema, and post-ischemic restoration, of which the most prominent in the acute phase is the development of brain edema [39-42]. A bunch of in vivo and in vitro experiments have indicated that VEGF aggravates ischemic injury; for example, the early administration of a VEGF analogue to animals with MCAO remarkably increased BBB disruption and infarct size [13]. Notwithstanding the above finding, the induction of VGEF 2 days after ischemia has been well documented to significantly ameliorate stroke outcomes [41]. Flk-1, which is mainly expressed in the endothelial cells and astrocytes, along with its phosphorylated form P-Flk as an indicator of activation, could interact with alpha v beta 3 to transmit diverse specific signals, thereby coordinating biochemical responses such as permeability, angiogenesis, as well as cell migration, proliferation, and differentiation [43]. FAK is a protein tyrosine kinase located in integrin-abundant sites in which focal contacts are formed; FAK can transmit multiple extracellular stimuli by directly binding with integrins or by indirectly associating with integrins via integrin-associated proteins, and thus, modulates signaling pathways associated with cell adhesion, shape, and migration [44]. The phosphorylation of FAK in response to particular stimuli is a rapid event that could possibly be regarded as a reliable approach for perceiving or detecting integrin activation. Therefore, the diminished P-FAK levels in the LXW7 treatment groups suggested the inhibition of intracellular signaling by the successful suppression of integrin alpha v beta 3.

It is well recognized that inflammatory genes are up-regulated several hours following ischemic or hypoxic insults; hence, inflammatory cytokines such as IL-1β, IL-10, and TNF-α are significantly increased and detectable even 2 weeks after an ischemic insult [45-47]. Multiple factors, including inflammatory cytokines and leukocytes are reported to be involved in ischemia/reperfusion-related inflammatory responses, the early occurrence of which usually worsens the injury, whereas their late occurrence seems to aid in neurological recovery [48]. The elevation of IL-1β and TNF-α levels in the control group in our study is in accordance with the findings of previous reports, while the inhibitory effects on these markers observed in the two LXW7 groups appear to indicate a mechanism for neuroprotection. This finding is likely attributable to the amelioration of BBB disruption or cerebral edema, suppression of VEGF or the integrin alpha v beta 3, or a combination of all of them. According to documented evidences in published literature, blockage of the integrin alpha v beta 3 contributes to the decrease in the number of macrophages and some other inflammatory cells in peri-infarct regions [49, 50]. However, in contrast to our expectation,
the evaluation of neurological deficits 24 h after ischemia in the LXW7 groups did not show a significant improvement. This discrepancy might be attributable to the differences in the animals used in different experiments, inaccuracies in examination, or other unidentified variables. More importantly, we should also take into consideration the possibility that a significant improvement in neurological outcomes may only become apparent in the late phase, and therefore, future studies with longer observation periods are required.

Compared with traditional RGD ligands, the cyclic RGD peptide cGRGdvc (LXW7) used in our experiment has been demonstrated to exhibit better binding affinity and specificity towards the integrin alpha v beta 3; furthermore, this integrin showed less undesirable liver accumulation as well [7].

In summary, LXW7, an antagonist of the integrin alpha v beta 3, might confer protection to cerebral tissues from ischemia/reperfusion injury in animals models of MCAO by attenuating cerebral edema, infarct size, and BBB disruption, and reducing the levels of fibrinogen, inflammatory cytokines such as IL-1β and TNF-α, and VEGF and its associated receptors such as P-FAK and P-Flk. One of the limitations in our study is that we only tested two doses of LXW7, and no notable difference was revealed between these two doses. It should be interesting and of vital importance to measure the effects of more doses to determine the most desirable therapeutic dose and to investigate whether any deleterious consequence exists. Additionally, we administered the treatment at 1 h after ischemia to demonstrate the feasibility of our proposal, since the therapeutic time window plays a pivotal role in offering neuroprotection following ischemic stroke. Future studies should be performed by setting more time points for LXW7 administration to identify the optimal time of administration. The data obtained in our research may lead to a novel approach for the management of cerebral ischemic stroke, and LXW7 may hopefully be used alone or in conjunction with other therapeutic agents in the future, although more detailed and sounded investigations are required.

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Disclosure of conflict of interest

None.

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