Osthole Enhances the Therapeutic Efficiency of Stem Cell Transplantation in Neuroendoscopy Caused Traumatic Brain Injury

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Neuroendoscopy processes can cause severe traumatic brain injury. Existing therapeutic methods, such as neural stem cell transplantation and osthole have not been proven effective. Therefore, there is an emerging need on the development of new techniques for the treatment of brain injuries. In this study we propose to combine the above stem cell based methods and then evaluate the efficiency and accuracy of the new method. Mice were randomly divided into four groups: group 1 (brain injury alone); group 2 (osthode); group 3 (stem cell transplantation); and group 4 (osthode combined with stem cell transplantation). We carried out water maze task to examine spatial memory. Immunocytochemistry was used to test the inflammatory condition of each group, and the differentiation of stem cells. To evaluate the condition of the damaged blood brain barrier, we detect the Evans blue (EB) extravasation across the blood brain barrier. The result shows that osthode and stem cell transplantation combined therapeutic method has a potent effect on improving the spatial memory. This combined method was more effective on inhibiting inflammation and preventing neuronal degeneration than the single treated ones. In addition, there was a distinct decline of EB extravasation in the combined treatment groups, which was not observed in single treatment groups. Most importantly, the combined usage of osthode and stem cell transplantation provide a better treatment for the traumatic brain injury caused by neuroendoscopy. The collective evidence indicates osthode combined with neural stem cell transplantation is superior than either method alone for the treatment of traumatic brain injury caused by neuroendoscopy.

Key words osthode; neural stem cell; transplantation; apoptosis; blood brain barrier; traumatic brain injury

Traumatic brain injury (TBI) can cause severe brain damage and is accompanied with serious symptoms, such as seizures, chronic headache, and executive dysfunction. Now most research primarily focuses on traumatic brain injury caused by accidents, despite the fact that some modern clinical circumstances can also lead to TBI. For instance the process of applying neuroendoscopy to resect tumor and clean hydrocephalus can damage the central nervous system. Intraventricular neuroendoscopy is a technique used primarily in pediatric neurosurgery like endoscopic third ventriculostomy (ETA) a most widely utilized procedure for the treatment of hydrocephalus. In the procedure a metal endoscope is introduced into the lateral ventricle. However, these operations can result in Stab wound injury (SWI) which is a kind of TBI. All the patients studied in these research presented intracranial hypertension or other neurological Symptoms, some patients even lost the cognitive competence totally. Unfortunately, these repeated insults tend to be neglected. Thus, we aimed to set up an effective approach to treat traumatic brain damage caused by clinical interventions. The needle insertion was adopted because it is highly reproducible and quantifiable. To imitate the actual conditions of neuroendoscopy induced injury, we kept a 0.7 mm diameter metallic needle in the brain for 20min, and employed a digital force gauge to monitor force during model building process. Under the control of autostereotaxic frame, we can quantify the speed of the insertion and the deep of vulnus (Fig. 1B).

7-Methoxy-8-isopentenoxycoumarin (C15H16O3, MW244.39 Da) (Fig. 1A), also known as osthode is a natural derivative from coumarin. Osthode can be extracted from some medicinal plants such as Cnidium monnieri (L.), Angelica and Archangelica. In Campo Cnidium monnieri (L.) has been shown to be effective on improving intelligence. Osthode is known for its therapeutic effect on central nervous system diseases such as Alzheimer’s disease, Autoimmune encephalomyelitis, and Cognitive impairment. Previous studies demonstrated that osthode is an effective compound for the treatment of traumatic brain injury. Both in vivo and in vitro treatment of osthode exhibited a potent neuroprotective effect against neurodegeneration. These effective functions rely on the speciality of osthode in anti-inflammatory, anti-oxidative and anti-apoptotic. However, the effectiveness of osthode has some limitations. For example, damaged tissue restores itself slowly because dead neurons cannot renew and proliferate by themselves. Thus, we need to consider another method to support the therapeutic method.

Bone marrow-derived-neural stem cells (BM-NSCs) are self-renewable and can differentiate into neurons, astrocytes, and oligodendrocytes. The pluripotent characteristic of BM-NSCs make them good candidates for cell replacement therapy, not only limited to TBI but also other types of neurodegenerative diseases, including stroke, Alzheimer’s disease, Huntington’s disease and Parkinson’s disease. Stem cells can replace the apoptotic cells and release some neurotrophic factors like glial-cell-line-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and insulin-like growth factor-1 (IGF-1) to restore the damage tissue. Moreover, stem cells exhibit anti-inflammation function. Previous study demonstrated that stem cells suppress inflammation caused by some pro-inflammatory cytokines. Despite their versatile function, stem cells are not sufficient for the treatment of some nervous system diseases. For example, the stem cells...
methods to obtain a satisfactory outcome. We injected stem cells therapy always requires the aid of other methods. We injected BM-NSCs into the damaged region directly, a method which is considered to be the fastest way to allow them to reach the damaged region. On the other hand, osthole can inhibit the inflammatory reaction that hinders neural stem cell replication and differentiation.

Traumatic injury is considered to be a complex pathological process, involving brain damage causing inflammation. Cells in the damaged region released pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin (IL)-6 and IL-1, which then recruit peripheral leukocytes at the cerebral parenchyma, and activate resident immune cells such as neutrophils, monocytes, and lymphocytes. The entire process causes neuronal death and degeneration.

Fluoro-Jade B histo-fluorescence is a fluorescent dye that can stain both degenerate and dead neurons rapidly. In this study, we aimed to compare different therapeutic methods and their effects on neuronal degeneration. Astrocytes are abundant in the brain and have a variety of functions. Astrocytes respond to stroke, tumorigenesis, neurodegeneration, and epilepsy. Astrocytes can form a glial scar, prevent axonal sprouting, and disturb the circuits of the central nervous system that cause secondary tissue injury. However, too many astrocytes can result in ongoing damage, which is deleterious for tissues and can cause chronic inflammation and neuronal dysfunction.

Neutrophils contain toxic agents such as elastase, metalloproteinases, and other oxygen species. Neutrophil contributes to free radical formation, including superoxide, hydroxyl radicals, and nitric oxide radicals, which can damage cells and result in apoptosis. Thus, we chose to measure the number of myeloperoxidase (MPO)+ neutrophils and glial fibrillary acidic protein (GFAP)+ astrocytes as factors to test the curative effect of our combined treatment.

Mature filaments consist of five principal proteins and medium molecular mass neurofilament triplet proteins (NF-M). NF-M expresses during neuronal development and plays an important role in the stabilization of the stationary neural cytoskeleton network. Importantly, the reduced expression of NF-M can induce many neurodegenerative diseases like Alzheimer’s disease and Parkinson’s disease. So we decided to detect the expression of NF-M to evaluate the ability of each therapeutic method to promote neurogenesis.

In the present study, we show that osthole can reduce inflammation and prevent neuronal degeneration. Thus, osthole can increase the number of neurons differentiated by transplanted neural stem cells. As a result, the combined therapeutic method using osthole and neural stem cell transplantation together is much more effective for promote neuronal regeneration and the spatial memory recovery than other test methods.

METHODS

Animal Model We performed this experiment on 7–8 week old male C57BL/6J mice, weighing 30–35 g each (Cheney Chain). We were in complete compliance with the Institutional Guidelines for Animal Care and Use presented by the Animal Experimentation Ethics Committee. The method we used to make a stab wound injury model was modified based on previous studies. Mice were anesthetized by intraperitoneal injection of 4% chloral hydrate (1 mL/100 g). We use a needle diameter of 0.7 mm and the injury point was 2 mm left of the dura mater. Then, we controlled the speed of insertion to 100 μm/s. The depth of the insertion level was 5 mm. After the needle reached position we targeted, we keep it in the brain for 20 min. During the entire process, we used a warm pad to maintain the body temperature.

Mice were randomly divided into four groups: group 1 (brain injury alone); group 2 (osthole alone); group 3 (neural stem cell transplantation alone); and group 4 (combined osthole and neural stem cell transplantation).

Testing the Force of Insertion We anchored the needle on the digital force gauge to monitor force variation. Then we recorded the force tendency curve by the software. We tested ten groups to obtain the value of 0.02±0.0015N, the force of insertion.

Drug and the Method of Administration Osthole (7-methoxy-8-isopentenoxycoumarin, C_{15}H_{16}O_{3}, purity >99.2) was obtained from the Chinese Medicine Inspecting Institute and dissolved in dimethyl sulfoxides (DMSO, less than 0.1%, which would not have any neuronal effect). Mice were administered 10 or 30 mg/kg osthole by intraperitoneal (i.p.) injection 30 min after surgery. Osthole was administered once daily before harvesting the brain.

Cell Culture The BM-NSCs we used were generated from BM mesenchymal cells of adult (5–8 weeks old) GFP transgenic Mice (Jackson Laboratory). We harvested the whole
After that, we isolated stem cells antigen-1+/Sca-1+ cells using Microbeads to remove mature hematopoietic cells. U.S.A.). The lineage culture medium on poly-L-lysine coated plates and grown in BM form the mice and isolated cells. We purified the lineage Vol. 40, No. 7 (2017) 1045 Biol. Pharm. Bull.

53) to evaluate the effectified neurological severity score (NSS)
trol.

syringe. Other groups that did not undergo transplantation To avoid additional injury, we injected cells into the same

growth factor (b-FGF), 20 ng/mL of epidermal growth factor (EGF) (all from PeproTech, Rocky Hill, NJ, U.S.A.), and growth factor (b-FGF), 20 ng/mL of epidermal growth factor (EGF) (all from PeproTech, Rocky Hill, NJ, U.S.A.), and

200 mL of 0.1 M neutral PBS into the ascending

sections were then incubated with antibodies against GFAP (1 : 150, Stem-Cell Technologies, Vancouver, Canada), MPO (1 : 200, Abcam), Galactosylceramide (GALC, 1 : 200, Abcam), double cortin (DCX, 1 : 150, Abcam), overnight at 4°C. After washing again three times, sections were incubated by Cy3-conjugated secondary antibod-

tissues were fully weighed

Finally, the slides were rinsed three times with distilled water. Next, they were placed in a solution of 2 min in distilled water. Next, the slides were washed in distilled water for 2 min then dyed for 20 min.

Finally, the slides were rinsed three times with distilled water. The slides were then dried at 50°C until they were fully dry (5–10 min), and immersed in xylene for at least 1 min. The tissue was examined by an epifluorescent microscope with blue (450–490 nm) light.

Extravasation of Evans Blue (EB) to Test the Damage to the Blood–Brain Barrier (BBB) Because needle insertion can damage the BBB, we used EB extravasation to identify the level of damage in the BBB, and the therapeutic method to influence the damage. We injected 2% EB solution into the caudal vein 1 h before we excised the brain. Mice were transcardially perfused with PBS solution and placed into an air warmer at 50°C for 90 min. Then, the slides were soaked in solution containing 1% sodium hydroxide in 80% alcohol for 5 min, followed by 2 min in 70% alcohol and 2 min in distilled water. Next, they were placed in a solution of 0.06% potassium on a shaker table to ensure consistent background suppression between sections, for 10 min. The slides were washed in distilled water for 2 min then dyed for 20 min. Finally, the slides were rinsed three times with distilled water.

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Statistical Analysis Results were expressed as the mean±standard deviation (S.D.) from an appropriate number of experiments. Statistical evaluation was performed by one-way ANOVA with Bonferroni’s multiple comparison test. 56,57)
RESULTS

Stem Cells Differentiate into Oligodendrocytes, Astrocytes, and Neurons in Vitro and in Vivo  After 5 to 16 d in culture, BM-NSCs already proliferated and formed distinct neurospheres. We used specific markers (Nestin and Sox2) to identify it (Fig. 2A). The differential BM-NSCs also were detected after 10 d and the morphology of the BM-NSCs already changed. BM-NSCs developed into neurons (NF-M+), oligodendrocytes (NG2+), and astrocytes (GFAP+) (Fig. 2B).

At 14 dpi (days post-injury) after transplantation, we found survival stem cells at the margin of the injury site. To ensure the stem cells survived and differentiated, we used immunocytochemistry to test the three main types of stem cells formed (Fig. 2C). We found that at 14 dpi, stem cells already differentiated into oligodendrocytes (GALC+), astrocytes

![Image](image_url)

Fig. 2. A. The Immunocytochemistry Result Shows BM-NSCs Are Positive for Nestin and Sox2

Scale bar=100 µm. B. BM-NSCs have differentiated into neurons (NF-M+) oligodendrocyte precursors (NG2+) and astrocytes (GFAP+) in vitro as verified by immunostaining. GFP, DAPI and Specific Marker. Scale bar=50 µm. C. BM-NSCs have differentiated into neurons (NF-M+, arrows with solid lines), oligodendrocyte precursors (GALC+) and astrocytes (GFAP+) in vivo as verified by immunostaining (arrows with solid lines). GFP, DAPI, Specific Marker. Scale bar=25 µm.
Combined Treatment with Neural Stem Cell Transplantation and Osthole Can Promote Spatial Memory Recovery and Neurological Function  We tested the escape latency at 5, 7, and 14 dpi and used it to evaluate the spatial memory of mice in each group. In order to investigate whether the

(GFAP+), and neurons (NF-M+). The cells we transplanted carried a green fluorescent protein gene. The green fluorescence viewed on the microscope merged with other specific antibody dyed cells helped us to locate cells that had already differentiated.

Fig. 3.  A. Illustration Showed the Tracking Paths to Find the Platform beneath the Water at 7 dpi
B. Quantitative analysis of the escape latency of each group at 7 dpi. ***p<0.01. C. The escape latency to find the platform at 5, 7, and 14 dpi in each group. All data are presented as the mean±S.E.M. There was an obvious improvement on spatial memory recovery in the combined treatment group. ***p<0.01 vs. SW1 control group at 5, 7 and 14 dpi. However, at 5 or 7 dpi, there were no distinct differences in the osthole group (both osthole 30mg/kg group and osthole 10mg/kg group) or stem cell transplantation group, compared with the SW1 control group; Data are represented as the mean±S.D. (n=6 per group). D. Quantitative analysis of the NSS score in each group. ***p<0.01.
Fig. 4. Combined Treatment Has a Better Effect on Attenuating the Inflammatory Response in the Injured Brain

A. GFAP+ astrocytes detected by immunohistochemistry merged with nuclear DAPI staining. Scale bar, 20 µm. B. MPO+ neutrophils detected by immunohistochemistry merged with nuclear DAPI staining. Scale bar, 20 µm. C–D. Quantitative analysis of the number of the inflammatory cells by Image J. Combined treatment reduced the number of inflammatory cells at 3 dpi. **p<0.01, *p<0.05. Data are represented as the mean±S.D. (n=6 per group). E. The number of GFP-positive cells in SWI area merged with nuclear DAPI staining. Scale bar, 20 µm. F. Quantitative analysis of the number of the GFP-positive cells by Image J. ***p<0.01.
dose–response has an impact on improving its spatial memory capacity, we also set up two low-dose dosing groups (osthole 10 mg/kg group and osthole 10 mg/kg + stem cell group). We found that both low-dose osthole group and high-dose osthole group did not contribute to improved spatial memory compared with the SWI control group (p > 0.05 at 5 dpi). There are also no obvious differences between the stem cell transplantation treatment compared with the SWI control group (p > 0.05 at 5 dpi) at 5 dpi. Similarly, the same result appeared in osthole treatment group compared with the SWI control group (p > 0.05 at 5 dpi) at 5 dpi. However, at 7 dpi, there was an obvious improvement on spatial memory recovery in both high-dose administration plus stem cell transplantation group and low-dose administration plus stem cell transplantation group compared with the SWI control group (p = 0.05 at 7 dpi) at 7 dpi. However, at 7 dpi, there was an obvious improvement on spatial memory recovery in both high-dose administration plus stem cell transplantation group and low-dose administration plus stem cell transplantation group compared with the SWI control group (p < 0.01 at 7 dpi). It is worth mentioning that there is also a significant difference between the low-dose administration group and the high-dose administration group under the conditions of stem cell transplantation (p < 0.01 at 7 dpi) (Figs. 3A–C).

The final score was 4.83 ± 0.71 in the SWI group (N = 12) compared with the sham group (N = 12), in which the score was 0.75 ± 0.45. There was a distinct difference between groups (p < 0.01), illustrating that trauma had an influence on changing neurologic function in the mice. We also used the NSS score to examine the effect of each therapeutic method on neurological function at 7 dpi. The result shows that combined treatment can significantly improve neurological function (p < 0.01) (Fig. 3D).

The Combined Treatment with Neural Stem Cell Transplantation and Osthole Has a Better Effect on Attenuating the Inflammatory Response in the Injured Brain

In the pathologic process of neuroinflammation, neutrophil infiltration and microglia activation may be primary steps. Proinflammatory cytokines such as TNF-α and IL-6 released by the microglia can trigger astrogliosis. So, we quantified the number of MPO+ neutrophils and GFAP+ astrocytes to assess our therapeutic methods. We used immunohistochemistry to calculate the number of MPO+ neutrophils and GFAP+...
astrocytes at 3 dpi. Combined treatment has an improved ability to reduce the number of both GFAP+ astrocytes (Fig. 4A) and MPO+ neutrophils (Fig. 4B) at 3 dpi compared with osthole treatment group (GFAP+: 22.01±3.75 vs. 31.41±5.19 in osthole used group p<0.05 MPO+: 11.22±1.98 vs. 17.95±2.66 in osthole used group p<0.01) and stem cell transplantation treatment group (GFAP+: 22.01±3.75 vs. 45.94±4.56 in the neural stem cell transplantation group p<0.01 MPO+: 11.22±1.98 vs. 31±3.36 in neural stem cell transplantation group p<0.01). Similar to the effect on the degeneration of neurons, in the neural stem cell transplantation group has a tiny effect on the number of both MPO+ neutrophils and GFAP+ astrocytes compare with SWI control group. However, the osthole treatment group demonstrated a significant effect on alleviating the inflammatory response (GFAP+: 31.41±5.19 vs. 52.55±5.28 in the SWI control group, p<0.01 and MPO+: 30.99±3.63 vs. 36.45±3.60 in the SWI control group p<0.01) (Figs. 4C, D).

Because the strong anti-inflammatory effect of osthole will improve the survival of transplanted stem cells, we detected the number of GFP-positive cells in SWI areas at 14 dpi. The results showed that osthole can increase the survival of transplanted stem cells. There is also a significant difference between combined treatment group and osthole group. (p<0.01 at 14 dpi) (Figs. 4E, F).

Combined Treatment with Neural Stem Cell Transplantation and Osthole Significantly Reduces Neuronal Degeneration Compared with osthole or neural stem cell transplantation alone, combined treatment by neural stem cell transplantation and osthole has a more potent effect on neuronal degeneration (Fig. 5A). To investigate whether the dose–response has an impact on reducing neuronal degeneration, we also set up two low-dose dosing groups (osthole 10mg/kg group and osthole 10mg/kg+stem cell group). We found that administration of doses also affected neuronal degeneration, there was a difference between low dose group and high dose group under the conditions of stem cell transplantation (p<0.05) (Fig. 5B). At 3, 5, 7, and 9 dpi, we calculated the number of degenerated neurons (Fig. 5C). The significant decrease in neuronal degeneration was seen in the combined treatment (osthole 30mg/kg+stem cell group) group at 3 dpi (20.00±3.90 vs. 41.56±5.17 in the SWI control group p<0.01). Decreased neuronal degeneration was also observed in the osthole group at 3 dpi (29.70±6.37 vs. 41.56±5.17 in the SWI control group p<0.01), while there was no effect on neuronal degeneration in the neural stem cell transplantation group at 32 dpi. At 9 dpi, there was almost no neuronal degeneration detected in the combined treatment group or in the osthole group. There was also an improved effect on neuronal degeneration in the combined treatment group compares with the osthole group (20.00±3.90 vs. 29.70±6.37 in the SWI control group p<0.01) and neural stem cell transplantation group (20.00±3.90 vs. 37.93±4.72 in the neural stem cell transplantation group p<0.01) at 3 dpi (Fig. 5C).

Combined Treatment with Neural Stem Cell Transplantation and Osthole Can Protect against Breakdown of the BBB We used a standard curve to measure EB content in each group. We performed a comparison with each group at 7 dpi (Fig. 6A). The unit we used to depict EB content is mg/g. According the result, both osthole and stem cells have no effect on the repair of blood brain barrier (p>0.01). There was a distinct decline in EB extravasation in the combined treatment group compared with SWI group (0.16±0.01 vs. 0.24±0.20 in the SWI group p<0.01) (Fig. 6B).

Combined Treatment with Neural Stem Cell Transplantation and Osthole Can Promote Neuronal Regeneration We observed a sharp decrease in pixel intensity of NF-M in the injured site and we chose 14 dpi to detect neuronal regen-
eration (Fig. 7A). While distinct signs of neuronal regeneration were seen in the combined treatment group, the combined treatment group appeared to be most effective compared with osthole group (7385 ± 301 vs. 5967 ± 503 in the osthole group \( p < 0.01 \)) and neural stem cell transplantation group (7385 ± 301 vs. 6200 ± 407 in the neural stem cell transplantation group \( p < 0.01 \); Fig. 7C). However, neither stem cell transplantation nor osthole had any effect on nerve regeneration \( (p > 0.01) \).

We also detected the immature neurons by immunocytochemistry with antibodies against DCX at 14 dpi (Fig. 7B). The result shown osthole can increase the number of GFP+ immature neurons and obvious differences were observed between combined treatment group and neural stem cell transplantation group \( (p > 0.01) \) (Fig. 7B).

DISCUSSION

With the help of autostereotaxic frame, we built the needle insertion model and imitated endoscopic surgery successfully. In present endoscopic surgery instruments must avoid vital vessels and avert damage to tissue around the wound.\(^\text{58,59}\) Here we choose the 0.7 mm needle, which is close to the mainstream diameter endoscope adopt by clinical surgery\(^\text{60}\) and we did not observe any side-effect conditions, such as haemorrhage and death. Previous studies suggested that all kinds of trauma could influence neural function in a certain extent.\(^\text{61}\) In this study we found our trauma model could change the neurologic function and the spatial memory ability.

The primary damage caused by traumatic brain injury is irreversible and amenable, so only preventive measures can minimize the extent of damage.\(^\text{62}\) Therefore, the ensuing cascades of secondary injury mechanisms, including oxidative stress, edema, inflammation and apoptotic cascade, could affect our investigation significantly.\(^\text{63}\) It is well known that osthole can down-regulate IL-6, caspase-3, and TNF-\(\alpha\), which are inflammatory cytokines involved in inflammation.\(^\text{64,65}\) On the other hand, Bcl-2 overexpression inhibits neuronal degeneration and stimulates the recovery of neurological function. In contrast, overexpression of Bax induces apoptosis. According to our previous study, osthole can reduce Bax to Bcl-2 ratio in SWI.\(^\text{66}\) It is a proven fact that immune cells and inflammatory are able to protect neural system in TBI.\(^\text{67}\) Indeed, pro-inflammatory cytokines like IL-6, TNF-\(\alpha\) and IL-1 all have the ability to protect neural cells.\(^\text{68}\) Interestingly, in the secondary injury process these pro-inflammatory cytokines always overexpress and play a deleterious role in damaging tissue.\(^\text{69}\) In this study we mainly focused on the outcome of anti-inflammation changed by the three secondary injury therapeutic methods. It is noteworthy that osthole did not affect the number of GFAP in the similar model as our previous studies (the diameter of the needle is 1.1 mm and the speed of the insertion is faster than 100 \(\mu m/s\)), which may be
related to the size of the wound area and the degree of trauma. As we mentioned previously, stem cell therapy has a weak effect on anti-inflammation. On the other hand, the reduction of inflammation by the cell transplantation therapeutic method was almost not applicable at the early stage. Moreover, inflammation deteriorates the microenvironment that hinders stem cell replication and differentiation. Under combined treatment, osthole helps to inhibit acute inflammation at the early stages and neuronal stem cells exert the effect of reduced inflammation by themselves. Apparently combined therapeutic treatment provides a prominent better effect on the inhibition of inflammation when compared with other groups. The primary damage and secondary injury both can make cells undergo apoptosis, which represents a programmed process of cell death. Since inflammation plays an important role in induction of apoptosis, it is necessary to reduce cell apoptosis by inhibiting inflammation. Here we chose a range of 3–11 d to reflect notable results of FJB staining. We did not find a significant difference between the stem cell transplantation and osthole treated groups at the beginning. However, there was a slight decrease in neuronal degeneration in the drug treated groups, implying that osthole had the ability to prevent neuronal degeneration. We are not surprised that neural stem cell transplantation treatment had no effect on neuronal degeneration, since no inhibition of inflammation by stem cell transplantation has ever been observed in the early stages previously. In contrast, stem cell transplantation decreased neuronal degeneration at 5, 9 and 11 dpi, which may be due to the anti-inflammation ability of stem cells. It has been known that the neural precursor cells can exert the neuroprotective function even in the early stage of neurogenesis. However, the neurogenesis always initiated 5–7 d after stem cell graft and the inflammatory reactions have already occurred before that. Therefore, the best result on neuronal degeneration protection is obtained from combined treatment approach.

At distinct stages of stem cell differentiation, developing neurons express neuronal intermediate filament proteins, which correlate with axon outgrowth, guidance and conductivity. It has been known that osthole can activate the BDNF/TrkB signaling and affect the phosphorylation of cyclic AMP response element binding protein (CREB), which might be the underlying mechanisms of the enhancement of neurogenesis. As we observed in the drug treatment group, the expression level of NF-M had been elevated, which relied on the powerful anti-apoptosis function of osthole. So far whether increasing the number of neuronal cells or reducing the apoptosis of neuronal cells can increase the expression of neuronal intermediate filament proteins is still obscure. Here similar results also presented in stem cell transplantation group. Because the survival stem cell graft could be enhanced by anti-inflammatory therapy, the largest number of survival nerve cells was almost not applicable at the early stage. Moreover inflammation by the cell transplantation therapeutic method for dealing with the neural (opaque) floor. Therefore, the best result on neuronal degeneration protection is obtained from combined treatment approach.

In evaluating the combined therapeutic method, the recovery of the damaged BBB may provide a satisfying answer. Comparing the three therapeutic methods, EB extravasation was least in the combined therapeutic method group, strongly suggested that the combined therapeutic method can facilitate the self-recovery of damaged tissue better than other therapeutic methods. As a corresponding result the spatial memory also had an obvious improvement and this improvement in spatial memory under stem cell transplantation was correlated with drug dose.

The reason for this is that osthole inhibits the inflammatory reaction at the early stage and provides an ideal microenvironment for neural stem cell proliferation and differentiation. On the other hand, osthole can prevent neuronal degeneration while also increasing the number of cells at the injury site.

CONCLUSION

Osthole can reduce inflammation and prevent neuronal degeneration, providing a better microenvironment for neural stem cells to exert their function by themselves, to reduce inflammation and to prevent neuronal degeneration. Our results demonstrate that the combined therapeutic method using osthole and neural stem cell transplantation together is much more effective for the treatment of neuroendoscopy-induced traumatic brain injury than other test methods.

Conflict of Interest The authors declare no conflict of interest.

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