



International Journal of Pharmacology

ISSN 1811-7775



Research Article

γ -Oryzanol Loaded Hyaluronic Acid Hydrogel Alleviates The Loss of Motor Function After Spinal Cord Injury in Mice by Inhibiting Endoplasmic Reticulum Stress

¹Zhiyi Fan, ¹Wanda Zhan, ²Xing Su, ¹Qiqiang Xing, ³Xuan Yao and ¹Jun Cai

¹Clinical Medical College, Yangzhou University, 225000 Yangzhou, Jiangsu, China

²State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, 300020 Tianjin, China

³Department of Clinical Hematology Faculty of Laboratory Medicine, Army Medical University (Third Military Medical University), 400038 Chongqing, China

Abstract

Background and Objective: Spinal cord injury (SCI) is typically the result of compressive forces on spinal cord tissue, leading to loss of motor or sensory function. As a cheap and biocompatible substance, γ -Oryzanol has been shown to cross the blood-brain barrier and exert beneficial effects on neural tissue. Our Previous studies have demonstrated the potential of a hydrogel based on the cross-linking of oxidized hyaluronic acid (OHA) and Hyaluronic Acid-Adipic Dihydrazide (HA-ADH) for treating osteoarthritis by loading Selenium Nanoparticles (SeNPs) through a Schiff base reaction. This study investigated the use of this hydrogel loaded with γ -Oryzanol to treat SCI in mice. **Materials and Methods:** Synthesize the hydrogel according to the previous method and load the appropriate concentration of γ -Oryzanol. Mice were randomly divided into groups and treated accordingly. Within 6 weeks after surgery, we used multiple methods including Basso mouse scale (BMS) score to conduct behavioral tests and analyzed the lesion area by Hematoxylin and Eosin (H&E) staining and immunofluorescence staining. **Results:** The study showed that mice treated with γ -Oryzanol showed better motor function. Observation of the lesion area showed that the inflammatory and demyelinating conditions of the injury site in the mice treated with γ -Oryzanol improved and more axons related to motor function were preserved with less scar formation. Endoplasmic reticulum stress-related proteins in the spinal cord tissue of γ -Oryzanol-treated mice decrease. **Conclusion:** Although further research is warranted, we propose that γ -Oryzanol, as a relatively inexpensive dietary phytochemical, has potential clinical value.

Key words: γ -Oryzanol, hyaluronic acid, spinal cord injury, endoplasmic reticulum stress, hydrogel

Citation: Fan, Z., W. Zhan, X. Su, Q. Xing, X. Yao and J. Cai, 2024. γ -Oryzanol loaded hyaluronic acid hydrogel alleviates the loss of motor function after spinal cord injury in mice by inhibiting endoplasmic reticulum stress. *Int. J. Pharmacol.*, 20: 257-268.

Corresponding Author: Jun Cai, Clinical Medical College, Yangzhou University, 225000 Yangzhou, Jiangsu Province, China

Copyright: © 2024 Zhiyi Fan *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Spinal cord injury (SCI) is damage to the structure and function of the spinal cord resulting from direct or indirect external factors, often resulting in loss of sensory, motor and autonomic nervous function in the injured individual. This causes immense suffering for the patient and their families, posing an urgent concern for the entire society¹⁻³. The injury mechanisms leading to SCI involve loss of neurons, the generation of reactive oxygen species (ROS), aggregation of microglia and macrophages at the injury site, activation of astrocytes, formation of glial scar tissue and cavities, secretion of growth-inhibitory molecules and deficits in various neurotrophic and neurogrowth factors. These factors create a hostile microenvironment that hinders neural regeneration and contributes to persistent functional impairments after SCI⁴.

The pathophysiological typically unfolds over three stages: Acute, subacute and chronic⁵. During the acute stage, vascular damage occurs in the affected area, leading to edema and nerve cells undergo disintegration, apoptosis and necrosis⁶. In the subacute stage, an influx of macrophages, microglia and neutrophils into the injured area leads to a series of inflammatory reactions, resulting in damage to the blood-spinal cord barrier. This infiltration also induces the proliferation and stress response of astrocytes, which secretes chondroitin Sulfate Proteoglycan (CSPG) and aggregates to form glial scar. While this barrier limits further invasion of inflammation into surrounding tissues to some extent, it also becomes a physical barrier to neural regeneration^{7,8}. In the chronic stage, the scar stabilizes and forms a cavity, which hinders the formation of new synapses and the reconstruction of neural circuits⁹.

Reconstructing functional neural circuits through tissue engineering grafts and delivering various drugs or neurotrophic factors to injured spinal cords in a sustained and effective manner represents a promising strategy. In biomedical applications, natural polymer biomaterials exhibit excellent biocompatibility advantages¹⁰. Hyaluronic acid (HA), a glycosaminoglycan with a disaccharide structural unit, is ubiquitous in the extracellular matrix of natural nerve cells. Its unique linear macromolecular structure ensures high water content and elasticity, playing a crucial role in the growth and development of the central nervous system¹¹. Different molecular weights of HA have distinct biological functions. Studies have shown that low molecular weight HA can promote angiogenesis, while high molecular weight HA can inhibit astrocytes proliferation, reduce CSPG production and microglial infiltration and inflammatory damage, all of which are beneficial for neurological function recovery¹²⁻¹⁵. However,

pure natural HA possesses poor mechanical properties, lacks cell adhesion sites and exhibit a high degradation rate, highlighting the limitations of its practical applications. Modified HA can serve as a delivery carrier for biomolecules and cells, with broad application prospects in SCI repair¹⁶.

The γ -Oryzanol is a significant component of cereal plant extracts, comprising 1.8-3.0% of the total content. It exists as a white to light yellow powder with no distinct odor or taste, although it can produce a fragrance upon oxidation¹⁷. The γ -Oryzanol exhibits various biological activities, including inducing cancer cell apoptosis, inhibiting inflammatory factors, scavenging free radicals and modulating human physiological mechanisms. Its versatility makes it a widely used component in the food industry, functional materials, pharmaceutical field and healthcare sector. Additionally, γ -Oryzanol has been shown to act on the autonomic nervous system and endocrine center, providing effective relief for endocrine disorders and neurological conditions. It also demonstrates good efficacy in reducing blood lipids, resisting cholesterol absorption and preventing lipid oxidation^{17,18}.

In the previous study, a novel injectable hydrogel was developed for delivering Selenium Nanoparticles (SeNPs), which can interfere with the expression of selenoprotein to treat osteoarthritis¹⁹. In this investigation, hydrogel system leveraged to load γ -Oryzanol and then utilized the hydrogel to evaluate its therapeutic efficacy in promoting axonal regeneration and recovery of hindlimb motor function in a mouse SCI model.

MATERIALS AND METHODS

Study area: All the experiments of formulation and development were performed in the medical college of Yangzhou University. Animal experiments were conducted in Clinical Hematology Faculty of Laboratory Medicine, Army Medical University.

Materials: All materials were purchased from China. Hyaluronic acid (HA, Mw: 10-50kDa), sodium periodate (SP) and morpholineethanesulfonic acid buffer (MES) buffer (pH = 6.5), N-(3-dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride (EDC); Hydroxybenzotriazole (Hobt, 99%), were purchased from Beyotime, Focus Biotechnology (Shanghai, China). Cell Counting Kit-8(CCK-8), oxalyl dihydrazide (ADH), penicillin-streptomycin, trypsin-EDTA and fetal bovine serum (FBS), modified Eagle medium were purchased from Sigma (Shanghai, China). The γ -Oryzanol were purchased from MedChemExpress (Shanghai, China). The N2A cells (CL-0168) were provided by Procell Life Science and Technology.

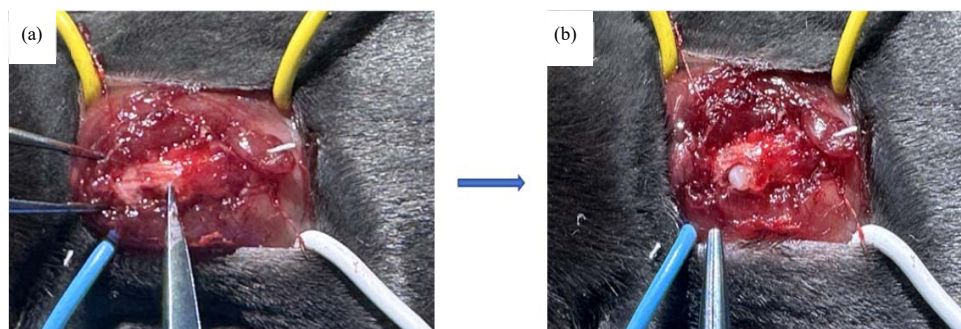


Fig. 1(a-b): Surgical procedure of mice and the placement of hydrogel

Synthesis of OHA and HA-ADH hydrogel: Briefly, HA was dissolved in water and added SP. The mixture was magnetic stirred at room temperature in the dark for 24 hrs. An equal molar amount of diethylene glycol was added. The mixture was dialyzed thoroughly for 3 days. Then, at room temperature, ADH was added. After the reaction was complete, the mixture was dialyzed thoroughly for 4 days.

Cell culture: The N2a cells were cultured in modified Eagle's medium supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum at 37°C under a humidified atmosphere of 5% carbon dioxide, with media replacement every two days.

Cellular viability: Cytotoxicity of γ -Oryzanol was evaluated using the Cell Counting Kit-8 (CCK-8). Briefly, 100 μ L of N2A cells with a density of 10×10^5 cells/mL were seeded in a 96-well plate and incubated for 24 hrs. Then, different concentrations of γ -Oryzanol (0, 20, 40 and 80 μ M) were added and incubated for 24 and 48 hrs. After adding 10 μ L of CCK-8 solution for 1 hr, the absorbance at 450 nm was measured using an enzyme marker.

Preparation of OHA/HA-ADH@ γ -Oryzanol hydrogel: As previously reported by Hu *et al.*¹⁹, hydrogels were prepared with 2% (w/w) OHA and 2% (w/w) HA-ADH of equal volume. The concentration of γ -Oryzanol in the hydrogel is 40 μ L/mL. The obtained hydrogel was lyophilized three times with demineralized water, then frozen at -80°C and stored at 4°C.

Determination of γ -Oryzanol release rate: Soak the hydrogel in 40 mL of PBS solution with a pH of 7.4. Then, place the hydrogel in a 37°C constant temperature shaker at 100 rpm/min and collect 1 mL of release solution from the hydrogel at predetermined time points. Replace the

collected release solution with fresh PBS solution in equal amounts and measure the absorbance at the characteristic peak of 279 nm using an ultraviolet spectrometer. Calculate the release amount of γ -Oryzanol by creating a standard curve.

Animals: Female C57BL/6 mice were procured from institutional animal house, 9 weeks old, $n=40$, average weight 20 g. According to laboratory regulations, mice were kept in a 12 hrs light/dark cycle and maintained at 20-22°C with a relative humidity of 50%. This study was approved by the Animal Experiment Ethics Committee of Yangzhou University and animal breeding and welfare trials were conducted in accordance with Yangzhou Laboratory standards.

Grouping and surgical procedures of mice: Mice were randomly divided into four groups. The mice were anesthetized with 2% isoflurane in oxygen and the T10 vertebra was exposed to expose the spinal cord. The posterior midline of the spinal cord served as the boundary. The control group did not undergo further treatment after exposure, while the remaining three groups had a 2 mm rectangular defect created in the left spinal cord (Fig. 1a). The injury group (SCI) did not undergo further treatment, while the intervention group was implanted with OHA/HA-ADH hydrogel (Hy) and OHA/HA-ADH at the defect site, respectively, @ γ -Oryzanol hydrogel (Hy-ory) (Fig. 1b). To prevent adhesion between connective tissue and the dura mater after surgery, a piece medical gelatin sponge was placed on both sides of the paraspinal muscle space to absorb bleeding. The muscles, subcutaneous tissues and skin were then sutured layer by layer and the mice received injections of ampicillin (150 mg/kg) once a day for 7 days after surgery to prevent infection. To help the rats urinate again, the bladder was manually squeezed twice a day until urination function was restored.

Assessment of mouse hind-limbs motor function: The hindlimb motor function was evaluated using the open field motor test of the Basso mouse scale (BMS)²⁰. The BMS scores were recorded at days 1, 3, 5, 7 and 14 after injury, followed by weekly assessments for 6 weeks, as previously described in the literature (plus one literature).

After 6 weeks of spinal cord injury, gait analysis was performed. Using non-toxic ink to paint the hindlimbs of mice black and placing them on a white track. Measuring the footprints left by the mice when they move on the track (the measurement results include step length and sway distance). An average of three steps used for each case.

All mice were pre-trained on the treadmill for 3 days before surgery. To promote adaptability, the mice were first placed on a stationary treadmill for 180 sec and if they did not fall off by themselves, they were continuously trained three times a day with an interval of 300 sec.

Mice with an average fall-off time of more than 390 sec were selected for further modeling. During the subsequent 390 sec of testing, the time was recorded for the mouse to fall off from the treadmill for the first time. Each experimental mouse was continuously measured for 7 days, with three tests per measurement and an interval of 5 min. The average value was taken.

Through observing the mice's curiosity in exploring new places and their aversion to open spaces, as well as detecting their spontaneous activity behavior and depression status, the open field box test was performed. The open field box was a 50 × 50 cm square, white-bottomed open box. The mice were acclimated to the room environment about 3 hrs before the experiment and then placed in the middle area of the open field box. The Smart v 3.0 software at the top recorded the activity of the mice within 5 min and the stationary time of the mice in the middle area was counted. After the test, the open field box was cleaned with 75% alcohol and dried to avoid residual odors from previous mice interfering with subsequent experiments.

Disease areas and demyelination: Spinal cord tissues were analyzed using H&E and Luxol fast blue (LFB) staining 6 weeks after surgery. Mice were administered intraperitoneally with 2% pentobarbital (50 mg/kg). Following cardiac perfusion with 4% paraformaldehyde, the spinal cord was quickly dissected and fixed overnight with 4% (w/v) paraformaldehyde. The specimens were dehydrated with ethanol and embedded in paraffin. The paraffin-embedded tissues were cut into 4 µm thick sections (Leica CM1900) for staining.

Immunofluorescence analysis: The 20 µm horizontal slices in the cryostat were incubated with primary antibodies at 4°C for 2 hrs. The slices were washed with 0.01 M PBS for 3 times and then incubated with secondary antibodies in the dark at room temperature for 1 hr. The primary antibodies used in this study include mouse monoclonal anti-Glial Fibrillary Acidic Protein (GFAP) (Sigma, 1:200 dilution) for staining astrocytes, mouse monoclonal anti-neuron III class beta-tubulin antibody (Tuj1) (Abclone, 1:200 dilution), Rabbit anti-rat 5-HT (ImmunoStar, 1:200 dilution) and rabbit anti-rat CHAT (Millipore, 1:200 dilution) for staining immature and mature motor neurons. Macrophages were identified using the CD68 antibody and M2 macrophages were identified using the CD206 antibody (Abcam, 1:50 dilution). After primary antibody staining, the sections were washed with PBS three times and stained with DAPI for 3 min in the dark. Finally, fluorescence microscopy (BX-51; Olympus) was used for random image acquisition.

Endoplasmic reticulum stress (ERS) protein assay: To determine whether treatment with γ -Oryzanol reduced the occurrence of ERS, the spinal cord tissue of SCI group and Hy-oryan group mice was extracted for total protein after killing. The expression of ERS-related indicator proteins (GRP78, CHOP) in these samples was detected by western blotting.

Statistical analysis: All data were analyzed using SPSS software (version 22.0) and graphs were drawn using GraphPad Prism 8 software. Quantitative data were expressed as Mean $\pm$ Standard Deviation. For comparisons between two groups, independent sample t-tests were used, while one-way analysis of variance was used for comparisons between multiple groups. Statistical significance was defined as $p < 0.05$, $p < 0.01$, $p < 0.01$ or $p < 0.001$.

RESULTS

Optimal concentration of γ -Oryzanol: Figure 2a shows the molecular structure of γ -Oryzanol. To investigate the effect of γ -Oryzanol on cells, different concentrations of γ -Oryzanol (0, 20, 40, 80 µM) were added to N2a cells and cultured for 24 and 48 hrs, respectively. Cell viability was determined by CCK-8 assay. The γ -Oryzanol did not show significant toxicity on N2a cells and at a concentration of 40 µM, it showed the best cell viability (Fig. 2b-c).

γ -Oryzanol release experiment: The release curve of the OHA/HA-ADH hydrogel loaded with γ -Oryzanol was tested. As shown in Fig. 3, the release rate of OHA/HA-ADH hydrogel was

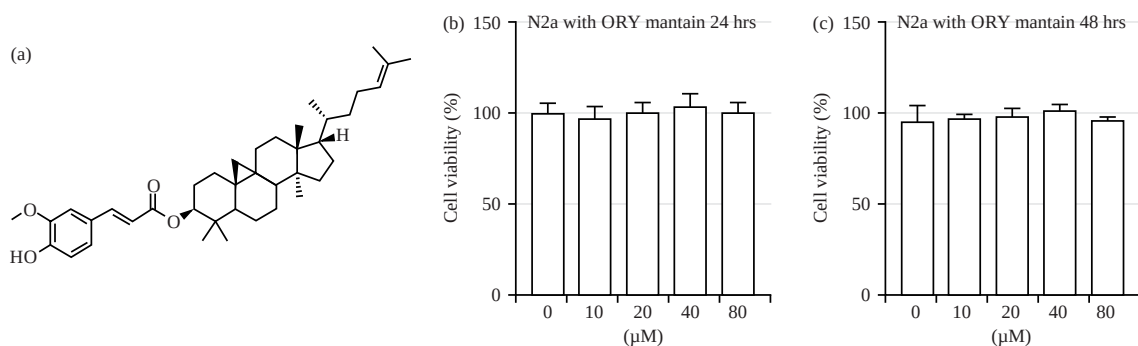


Fig. 2(a-c): Chemical structure of γ -Oryzanol and its effect on the viability of N2a cells

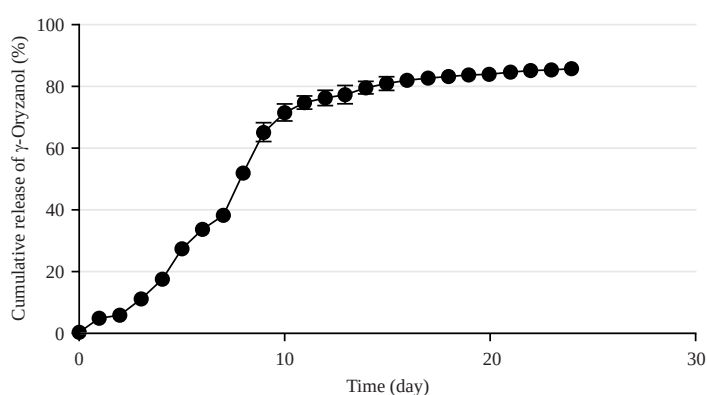


Fig. 3: *In vitro* release rate of OHA/HA-ADH hydrogel loaded with γ -Oryzanol

85% after 24 days of *in vitro* testing. The release trend showed a rapid release in the first ten days, followed by a stable release trend from 10 days to 24 days (Fig. 3). Overall, the OHA/HA-ADH hydrogel showed a good release trend for γ -Oryzanol.

γ -Oryzanol treatment improved motor performance of mice:

Recovery of motor function is an important measure for repairing SCI. At 6 weeks after surgery, the sham group showed persistent symptoms and signs of inflammation. The gait analysis showed stable gait in the sham group, while the SCI group could not operate their toes during exercise, resulting in reduced stride length and increased swing distance. The stride length of the sham group was (5.10 ± 0.08) cm, while the simple Hy group and the Hy-ory group had a stride length of (3.06 ± 0.21) cm and (3.36 ± 0.62) cm, respectively (Fig. 4a-b). Additionally, in terms of swing distance, the Hy-ory group had a value of (3.52 ± 0.30) cm, which was also closer to the sham group's value of (1.86 ± 0.17) cm (Fig. 4a and c). The BMS motor score scale was used to assess the motor behavior of mice on a regular basis and the results showed that all groups exhibited significant hind limb paralysis from day 1 to day 7 after injury.

The scores of each group increased over time. The study showed that after 6 weeks, the BMS scores of the Hy and Hy-ory groups were significantly improved and the recovery degree of the Hy-ory group was greater (5.4 ± 0.54) (Fig. 4d).

From the overall change trend, the balance rotor retention time of mice in each group is relatively short within 1-5 days after surgery and slightly rises within 5 days later. On the 35th day, the Hy group (54.4 ± 9.41) and the Hy-ory group (55 ± 7.42) were higher than the SCI group (34.2 ± 8.05) (Fig. 4e-f). This suggests that γ -Oryzanol has advantages in promoting balance ability in mice after SCI.

At 5 weeks after surgery, the open field test was conducted to observe the duration of the mice's stay in the central area of the open field and analyze their behavioral and depression-like behavior. According to the trajectory map of the mice, the motion trajectory in the central region was improved in the Hy-ory group compared to the SCI group (Fig. 4g).

Pathological structure and demyelination of the injury area:

At 6 weeks after SCI, Hematoxylin and Eosin (H&E)

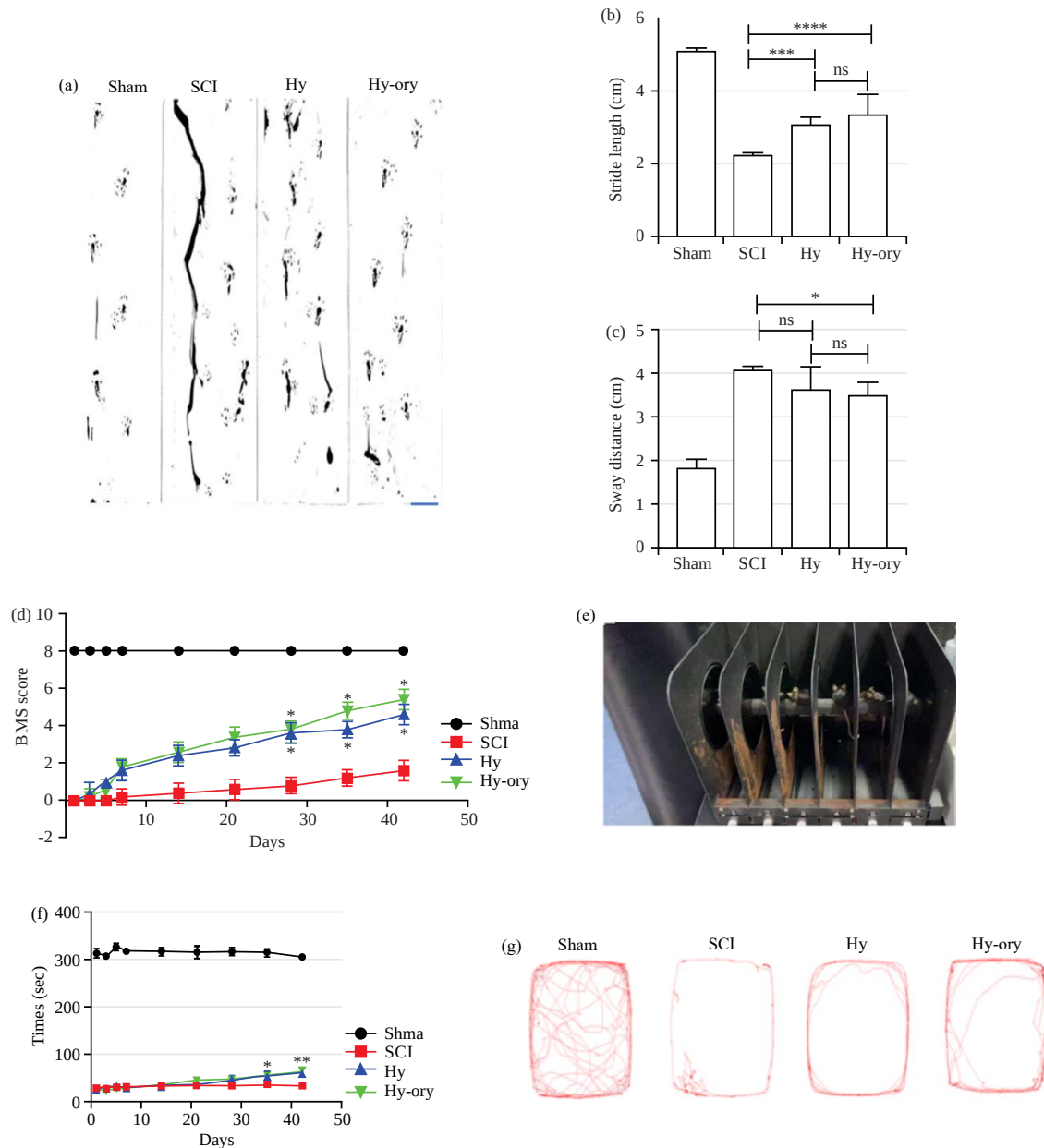


Fig. 4(a-g): BMS score, gait analysis, balancing rod experiment and open field box test of mice

**** $p < 0.0001$, *** $p < 0.001$ and * $p < 0.05$

staining was used to evaluate the pathological condition of the injury area in each group. The Hy-ory significantly improved the pathological structure of the spinal cord after injury (Fig. 5a). The LFB staining is specific to myelin; the blue area shows positive expression of myelin, while the pale area represents demyelination. Compared to the sham group, the demyelination in the SCI group was significant. In contrast, compared to the SCI group, Hy-ory had a better protective effect on myelin tissue (Fig. 5b-c).

Immunofluorescence staining of the injury area: The spinal cord immunofluorescence staining method was used to detect M2 macrophages in the injury area. The proportion of M2 macrophages in the SCI group, Hy group and Hy-ory group was 31.4, 34.2 and 41.6%, respectively (Fig. 6a-b). The formation of glial scar after SCI is not conducive to the regeneration and repair of the spinal cord. To understand the growth of neural scars, we performed double staining for GFAP and Tuj1 (Fig. 7a). The expression level of GFAP in the Hy group and Hy-ory group was lower than that in the SCI

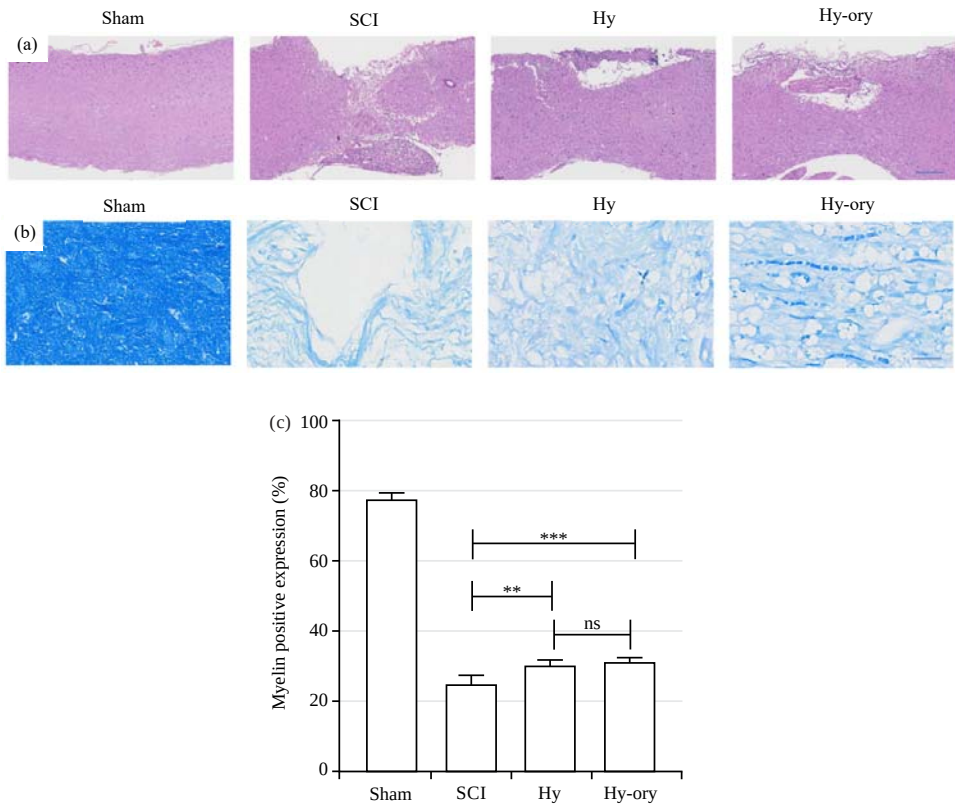


Fig. 5(a-c): Spinal cord tissue and demyelination
***p<0.001 and **p<0.01

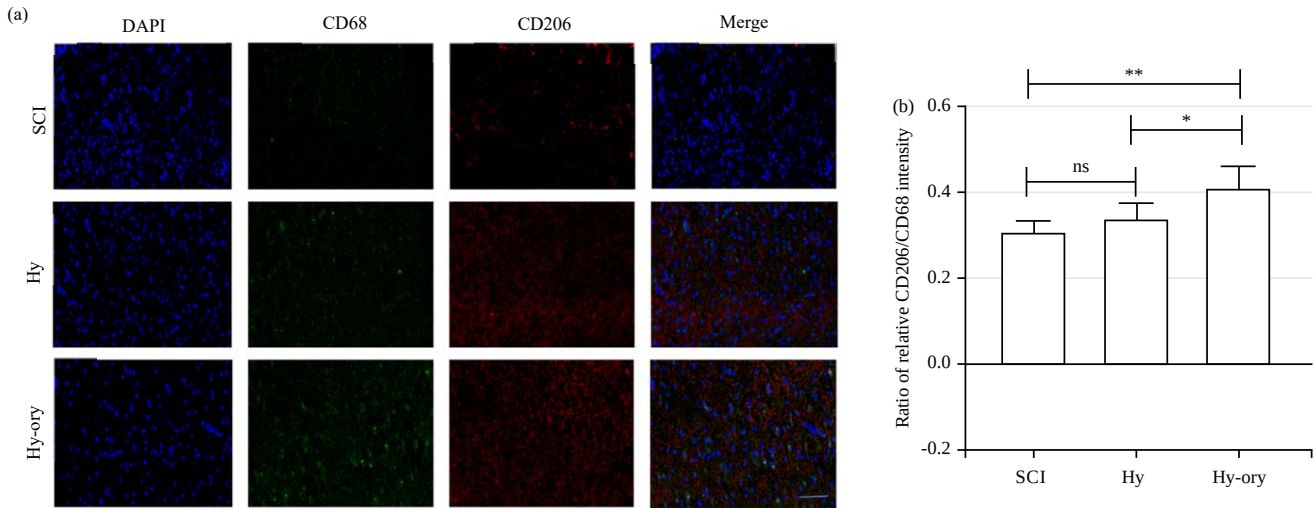


Fig. 6(a-b): Immunofluorescence staining of M2-phenotype macrophages labeled with CD206
**p<0.01 and *p<0.05

group (4.18 ± 0.20) (4.12 ± 0.27), respectively. There was no significant difference within the Hy group and Hy-ory group. The Tuj1 is present in the cell bodies of immature neurons. As

shown in Fig. 7, the intensity of Tuj1 (3.0 ± 0.23) (3.32 ± 0.11) in the Hy group and Hy-ory group was higher than that in the SCI group (2.66 ± 0.13). The difference in the intensity of Hy-ory

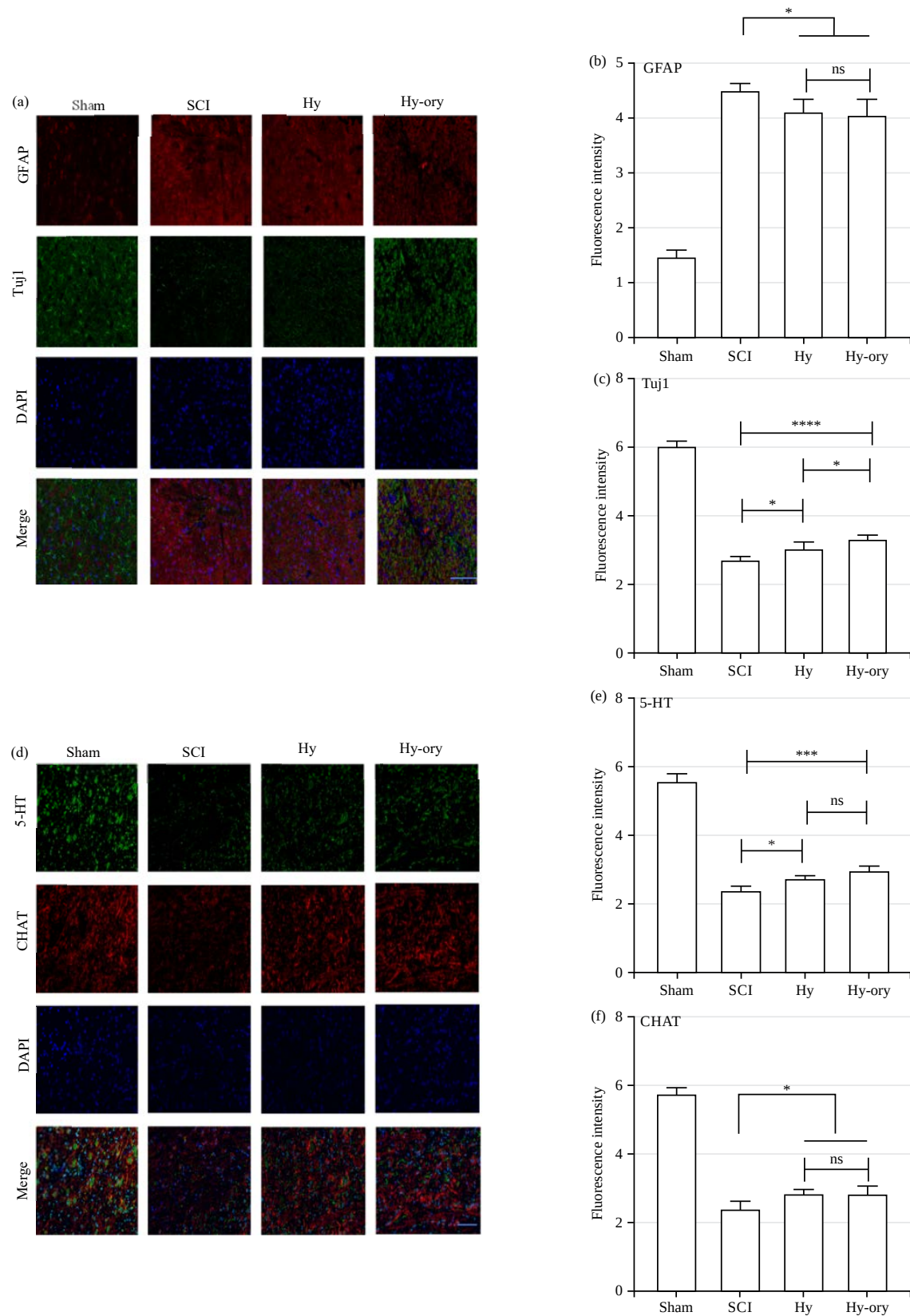


Fig. 7(a-f): Axon growth and inhibition of glial scar formation

**** $p < 0.0001$, *** $p < 0.001$ and * $p < 0.05$

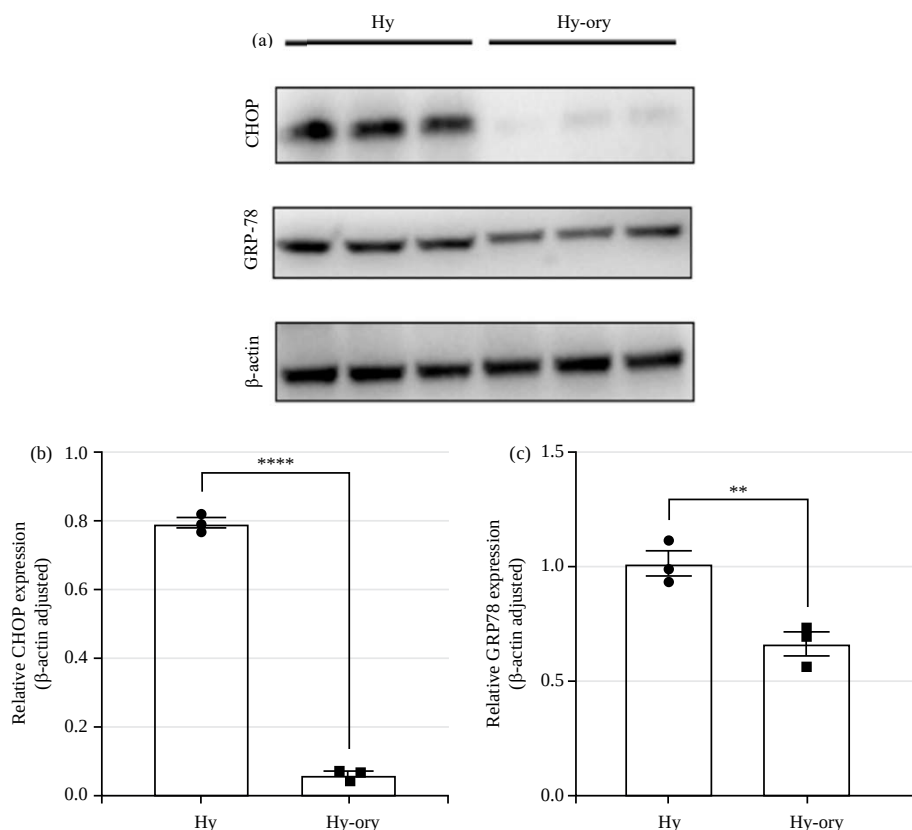


Fig. 8(a-c): Detection of endoplasmic reticulum stress marker protein expression

**** $p < 0.0001$ and ** $p < 0.01$

group was more pronounced (Fig. 7b-c). The intensity of 5HT- and CHAT-positive axons is an important indicator of the degree of recovery of motor function (Fig. 7d). The results showed that compared with the SCI group (2.36 ± 0.13), the strength of 5-HT axons was significantly restored by Hy (2.72 ± 0.09) and Hy-ory group (2.94 ± 0.13). The recovery effect of Hy-ory group was more significant (Fig. 7e). The staining results of CHAT-positive axons also showed a similar trend (Fig. 7f).

ERS protein: The expression level of GRP78/Pip and CHOP, the main ERS markers in the spinal cord tissue of the Hy group mice, was higher than that of the Hy-ory group (Fig. 8). This suggests that the hydrogel loaded with γ -Oryzanol may improve the prognosis of SCI by inhibiting the ERS pathway.

DISCUSSION

The SCI poses significant economic burdens on individuals and society due to high treatment costs and long-term disability of patients. Therefore, it is crucial to

reduce treatment costs while promoting patient rehabilitation. The γ -Oryzanol has a wide range of sources, is highly safe and exhibits various beneficial effects, including antioxidant, anti-tumor, anti-diabetic and immunomodulatory properties. Its ability to cross the blood-brain barrier in complete form and distribute extensively throughout the brain suggests potential neuroprotective effects^{21,22}. Given these advantages, γ -Oryzanol's role in SCI treatment was investigated.

Several factors after SCI create a harsh microenvironment that hinders neural regeneration. Therefore, when selecting therapeutic agents, it is crucial to consider the creation of a conducive microenvironment for repair. In the field of tissue engineering, carrier scaffolds, bioactive molecules and seed cells play essential roles in promoting functional synapse formation between regenerating axons and target cells, thereby achieving comprehensive repair of SCI²³. Biological scaffolds provide support for cell adhesion, proliferation and directed differentiation, while appropriate biomolecules enhance axonal regeneration and inhibit secondary injury caused by SCI²⁴.

The HA is a fundamental component of the natural extracellular matrix of central nervous system cells. The HA-based hydrogels have been shown to improve the survival rate of human embryonic stem cell-derived neural stem cells and increase their ability to differentiate into oligodendrocytes, thereby promoting recovery of motor function. However, pure natural HA has limitations that preclude direct use²⁵. In previous studies, a hydrogel based on selenium rebalancing was developed, which exhibits self-healing ability, excellent mechanical performance and sustained SeNPs delivery through the mixture of OHA and HA-ADH via dynamic equilibrium of Schiff base linkages¹⁹. Importantly, OHA/HA-ADH@SeNPs hydrogels maintain cartilage homeostasis through a synergistic effect of ROS scavenging and apoptosis inhibition *in vitro*. To further enhance the potential of these hydrogels, we loaded γ -Oryzanol into the hydrogel, leveraging its ability to provide a beneficial microenvironment for nerve regeneration while exerting its therapeutic effects.

After SCI, microcirculation disorders appear in the injury area²⁶, which promotes further death of neurons²⁷. After stimulation by inflammatory cells and other factors, astrocytes secrete a large amount of nerve growth inhibitors, leading to the formation of glial scars, which is an important reason for hindering the regeneration of neurons and axons²⁸. In this study, the implantation of hydrogel significantly inhibited demyelination and improved the pathological environment, which may be related to tissue regeneration mediated by γ -Oryzanol and the integration of hydrogel with the host spinal cord.

At the same time, macrophages can differentiate into different phenotypes²⁹. The M1 phenotype produces pro-inflammatory molecules, which exacerbate the process of secondary injury. The M2 phenotype is beneficial for axon and nerve regeneration^{30,31}. The immunofluorescence results reveal that transplanting γ -Oryzanol scaffolds significantly increases the number of CD206-positive cells in the central area of the injury, while also reducing the related inflammatory reaction in the adjacent area of the injury transplantation. This may be attributed to the good biocompatibility of the composite gel scaffold *in vivo* and the continuous release of γ -Oryzanol, which regulates the immune response.

Astrocytes are the predominant glial cell type in the Central Nervous System (CNS). Ongoing functional activity after SCI can give rise to the formation of detrimental glial scars, hindering the repair and regeneration of the spinal cord¹⁷. In this study, we discovered that the implantation of the scaffold inhibited astrocyte expression, significantly reduced glial scar formation and promoted nerve

regeneration and myelin sheath formation in both the damaged area and adjacent regions. The combination of local delivery mode and composite gel scaffold further augmented this outcome.

The ER is a crucial eukaryotic cell organelle that functions through a complex network of membranes, playing a central role in protein synthesis and folding. The ER is responsible for post-translational modification, oligomerization and calcium ion release from the ER lumen in response to environmental signals³². This study found that SCI can induce significant ERS, leading to cellular imbalances. Interestingly, the Hy-ory compound was able to inhibit the expression of related proteins, potentially offering a new approach for treating SCI-induced ERS.

CONCLUSION

Overall, γ -Oryzanol and hyaluronic acid are both natural compounds that are easy to extract and inexpensive. This study is the first to use them together in the repair of SCI and shows good therapeutic effects. We will conduct further research on their mechanisms. This discovery may provide new ideas for the treatment of SCI. This study shows the effect of γ -Oryzanol on SCI and accurately delivers the drug to the injured site by combining it with hyaluronic acid hydrogel. Oral or injection delivery methods often cause a decrease in drug effect. Therefore, the local delivery method of combining drugs with hydrogels may be a superior alternative to oral and injection delivery. As γ -Oryzanol and hyaluronic acid are both inexpensive and easy to obtain, this safe, efficient and low-cost method may provide SCI researchers with some opportunities.

SIGNIFICANCE STATEMENT

Spinal cord injury (SCI) has a huge impact on human health and there is currently no ideal treatment. As a cheap and biocompatible substance, γ -Oryzanol has been shown to cross the blood-brain barrier and exert beneficial effects on neural tissue. Previous studies have demonstrated the potential of a hydrogel based on the cross-linking of oxidized hyaluronic acid (OHA) and hyaluronic acid-adipic dihydrazide (HA-ADH). In this study, we investigated the use of this hydrogel loaded with γ -Oryzanol to treat SCI in mice. The research results indicate that the water-based hydrogel loaded with γ -Oryzanol can alleviate the loss of motor function in mice after SCI. Although further research is warranted, we propose that γ -Oryzanol, as a relatively inexpensive dietary phytochemical, has potential clinical value.

REFERENCES

- Norenberg, M.D., J. Smith and A. Marcillo, 2004. The pathology of human spinal cord injury: Defining the problems. *J. Neurotrauma*, 21: 429-440.
- Ahuja, C.S., J.R. Wilson, S. Nori, M.R.N. Kotter, C. Druschel, A. Curt and M.G. Fehlings, 2017. Traumatic spinal cord injury. *Nat. Rev. Dis. Primers*, Vol. 3. 10.1038/nrdp.2017.18.
- Eckert, M.J. and M.J. Martin, 2017. Trauma: Spinal cord injury. *Surg. Clin. North Am.*, 97: 1031-1045.
- Lee, B.B., R.A. Cripps, M. Fitzharris and P.C. Wing, 2014. The global map for traumatic spinal cord injury epidemiology: Update 2011, global incidence rate. *Spinal Cord*, 52: 110-116.
- Dumont, R.J., D.O. Okonkwo, S. Verma, R.J. Hurlbert, P.T. Boulos, D.B. Ellegala and A.S. Dumont, 2001. Acute spinal cord injury, part I: Pathophysiologic mechanisms. *Clin. Neuropharmacol.*, 24: 254-264.
- Ahuja, C.S., A.R. Martin and M.G. Fehlings, 2016. Recent advances in managing a spinal cord injury secondary to trauma. *F1000Research*, Vol. 5. 10.12688/f1000research.7586.1.
- Tetzlaff, W., E.B. Okon, S. Karimi-Abdolrezaee, C.E. Hill and J.S. Sparling *et al.*, 2011. A systematic review of cellular transplantation therapies for spinal cord injury. *J. Neurotrauma*, 28: 1611-1682.
- Uldreaj, A., J.C.T. Chio, C.S. Ahuja and M.G. Fehlings, 2016. Modulating the immune response in spinal cord injury. *Expert Rev. Neurother.*, 16: 1127-1129.
- All, A.H., P. Gharibani, S. Gupta, F.A. Bazley and N. Pashai *et al.*, 2015. Early intervention for spinal cord injury with human induced pluripotent stem cells oligodendrocyte progenitors. *PLoS ONE*, Vol. 10. 10.1371/journal.pone.0116933.
- Costăchescu, B., A.G. Niculescu, M.G. Dabija, R.I. Teleanu, A.M. Grumezescu and L. Eva, 2022. Novel strategies for spinal cord regeneration. *Int. J. Mol. Sci.*, Vol. 23. 10.3390/ijms23094552.
- Mothe, A.J., R.Y. Tam, T. Zahir, C.H. Tator and M.S. Shoichet, 2013. Repair of the injured spinal cord by transplantation of neural stem cells in a hyaluronan-based hydrogel. *Biomaterials*, 34: 3775-3783.
- Khaing, Z.Z. and S.K. Seidlits, 2015. Hyaluronic acid and neural stem cells: Implications for biomaterial design. *J. Mater. Chem. B*, 3: 7850-7866.
- Khaing, Z.Z., B.D. Milman, J.E. Vanscoy, S.K. Seidlits, R.J. Grill and C.E. Schmidt, 2011. High molecular weight hyaluronic acid limits astrocyte activation and scar formation after spinal cord injury. *J. Neural Eng.*, Vol. 8. 10.1088/1741-2560/8/4/046033.
- Austin, J.W., C.E. Kang, M.D. Baumann, L. DiDiodato and K. Satkunendrarajah *et al.*, 2012. The effects of intrathecal injection of a hyaluronan-based hydrogel on inflammation, scarring and neurobehavioural outcomes in a rat model of severe spinal cord injury associated with arachnoiditis. *Biomaterials*, 33: 4555-4564.
- Chistyakov, D.V., A.A. Astakhova, N.V. Azbukina, S.V. Goriainov, V.V. Chistyakov and M.G. Sergeeva, 2019. High and low molecular weight hyaluronic acid differentially influences oxylipins synthesis in course of neuroinflammation. *Int. J. Mol. Sci.*, Vol. 20. 10.3390/ijms20163894.
- Thompson, R.E., J. Pardieck, L. Smith, P. Kenny, L. Crawford, M. Shoichet and S. Sakiyama-Elbert, 2018. Effect of hyaluronic acid hydrogels containing astrocyte-derived extracellular matrix and/or V2a interneurons on histologic outcomes following spinal cord injury. *Biomaterials*, 162: 208-223.
- Minatel, I.O., F.V. Francisqueti, C.R. Corrêa and G.P.P. Lima, 2016. Antioxidant activity of γ -oryzanol: A complex network of interactions. *Int. J. Mol. Sci.*, Vol. 17. 10.3390/ijms17081107.
- Szczęśniak, K.A., P. Ostaszewski, A. Ciecierska and T. Sadkowski, 2016. Investigation of nutritive phytochemical- γ -oryzanol in experimental animal models. *Anim. Physiol. Anim. Nutr.*, 100: 601-617.
- Hu, W., X. Yao, Y. Li, J. Li and J. Zhang *et al.*, 2023. Injectable hydrogel with selenium nanoparticles delivery for sustained glutathione peroxidase activation and enhanced osteoarthritis therapeutics. *Mater. Today Bio*, Vol. 23. 10.1016/j.mtbio.2023.100864.
- Basso, D.M., L.C. Fisher, A.J. Anderson, L.B. Jakeman, D.M. Mctigue and P.G. Popovich, 2006. Basso Mouse Scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. *J. Neurotrauma*, 23: 635-659.
- Fujiwara, S., S. Sakurai, I. Sugimoto and N. Awata, 1983. Absorption and metabolism of γ -oryzanol in rats. *Chem. Pharm. Bull.*, 31: 645-652.
- Fujiwara, S., K. Noumi, I. Sugimoto and N. Awata, 1982. Mass fragmentographic determination of ferulic acid in plasma after oral administration of γ -oryzanol. *Chem. Pharm. Bull.*, 30: 973-979.
- McCreedy, D.A. and S.E. Sakiyama-Elbert, 2012. Combination therapies in the CNS: Engineering the environment. *Neurosci. Lett.*, 519: 115-121.
- Yang, K., J.S. Lee, J. Kim, Y.B. Lee and H. Shin *et al.*, 2012. Polydopamine-mediated surface modification of scaffold materials for human neural stem cell engineering. *Biomaterials*, 33: 6952-6964.
- Fu, Y., Q. Wu, W. Yang and S. Liu, 2022. Synthesis and properties of hydrogels on medical titanium alloy surface by modified dopamine adhesion. *Gels*, Vol. 8. 10.3390/gels8080458.
- Tator, C.H., 1995. Update on the pathophysiology and pathology of acute spinal cord injury. *Brain Pathol.*, 5: 407-413.
- Alizadeh, A. and S. Karimi-Abdolrezaee, 2016. Microenvironmental regulation of oligodendrocyte replacement and remyelination in spinal cord injury. *J. Physiol.*, 594: 3539-3552.

28. García-Álías, G. and J.W. Fawcett, 2012. Training and anti-CSPG combination therapy for spinal cord injury. *Exp. Neurol.*, 235: 26-32.
29. Rezvan, M., S. Meknatkhah, Z. Hassannejad, M. Sharif-Alhoseini and S.A. Zadegan *et al.*, 2020. Time-dependent microglia and macrophages response after traumatic spinal cord injury in rat: A systematic review. *Injury*, 51: 2390-2401.
30. Devanney, N.A., A.N. Stewart and J.C. Gensel, 2020. Microglia and macrophage metabolism in CNS injury and disease: The role of immunometabolism in neurodegeneration and neurotrauma. *Exp. Neurol.*, Vol. 329. 10.1016/j.expneurol.2020.113310.
31. Xiong, X.Y., L. Liu and Q.W. Yang, 2016. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. *Prog. Neurobiol.*, 142: 23-44.
32. Chapman, R., C. Sidrauski and P. Walter, 1998. Intracellular signaling from the endoplasmic reticulum to the nucleus. *Annu. Rev. Cell Dev. Biol.*, 14: 459-485.