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Results with anti-adhesion gel used for simulated contusion spinal cord injury in rats

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Abstract

Objective was to investigate an effect of anti-adhesion hyaluronic-containing resorbable gel on the course of traumatic spinal cord disease caused by a simulated contusion spinal cord injury (SCI) in rats. **Material and methods** A moderate spinal cord injury was simulated at the Th_9 level of 40 female Wistar rats that were randomly divided into 2 groups. Group I received hyaluronic-containing absorbable antiadhesion gel Antiadgezin® that was intraoperatively applied to the dura mater of experimental rats; Group II of control rats received no gel. The BBB locomotor rating scale was used to evaluate the extent of recovered pelvic limb function. The animals were sacrificed on days 5, 15, 30, 60 and 90. Paraffin and epoxy semi-thin 1 μm sections were used for morphological examination of the spinal cord using light microscopy. **Results** The gel used for contusion SCI in rats caused neither significant changes during postoperative period nor local infectious and inflammatory complications, and showed no negative impact on functional results. Morphological examination revealed pathomorphological changes that were identical for both groups and characteristic of contusion SCI showing softening, necrosis of the nervous tissue, tissue and cell detritus of gray and white matter, small cysts in the early stages of the experiment; progression of syringomyelia and formation of large cysts primarily seen in the gray matter and the onset of regenerative processes in the white matter at 60 and 90 days of the experiment. **Conclusion** The absence of a negative effect of the anti-adhesion hyaluronic-containing resorbable gel on the functional results, and morphological characteristics of the spinal cord offer the opportunity of the further experimental use as a carrier of substance composition initiating beneficial metabolic and neuroprotective action for transdural delivery in simulated SCI.

Keywords: Antiadgezin gel, rat, contusion spinal cord injury, functional assessment of pelvic limb using BBB locomotor rating scale, morphological examination

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INTRODUCTION

The nervous system has a limited ability to regenerate that makes the consequences of neurotrauma, ischemia, hemorrhage or neurodegenerative disease destructive and irreversible [1–3]. Modern growth factor and stem cell technologies used to improve endogenous mechanisms of recovery of the central nervous system have been shown to be ineffective [3, 4]. Recent advances in neurology indicate the emergence of a promising strategy to enhance the therapeutic effect of cells and drugs in brain diseases through the use of natural and synthetic biomaterials. Hydrogels formed by hydrophilic polymers containing up to 90% water are considered a promising use [1, 3, 5–7]. Polymer materials can serve as a framework providing mechanical support and protection, a substrate for adhesion, metabolism and oxygen, act as local transport systems for the delivery of drugs and signaling molecules directly to the site of injury [3, 6, 8], and provide controlled release of substances in the pathologic area.

The use of hydrogels for spinal cord regeneration is widely discussed [9–14], since spinal cord injuries are complex due to the particular anatomy, and there are no effective treatment methods yet [11]. However, hydrogels used in laminectomies are physical barriers to post-operative epidural fibrosis [15]. Clinical studies have shown a positive effect of hyaluronic absorbable anti-adhesive hydrogel "Antiadhesin"® after decompressive operations for osteochondrosis of the lumbar spine decreasing the formation of scars and adhesions in the spinal canal by 35%, pain and the severity of complications [16]. There is no information about the effect of the gel on the preservation of spinal cord tissue and on anatomical and functional regeneration after contusion spinal cord injuries, which became the subject of this study.

The objective was to investigate an effect of anti-adhesion hyaluronic-containing resorbable gel on the course of traumatic spinal cord disease caused by a simulated contusion spinal cord injury (SCI) in rats.

Design Level of evidence 3b (Individual case-control study) UK Oxford, version 2009.

MATERIAL AND METHODS

The experiments were produced using 40 female Wistar rats aged 9–12 months with a body weight 270–320 g, randomly divided into 2 groups, experimental and control. Experimental rats (n = 20) underwent laminectomy at the Th_9 level under general anesthesia (Rometar 2% 1–2 mg/kg, Bioveta, Czech Republic; Zoletil 100 10–15 mg/kg, Virbac Sante Animale, France). The spine was rigidly fixed using the spinous processes of the Th_8 and Th_{10} vertebrae and a moderate contusion to the spinal cord performed [17–19] without injury to the dura mater. Pelvic limbs reflexively stretched after the contusion with subdural hematoma visualized and involuntary urination was observed in rare cases. With proper homeostasis of the operating wound 0.2 mL Antiadhesin® gel was applied to the dura mater. The exposure time of the drug in an open wound was 2 minutes to allow the gel over the spinal cord. Then the surgical wound was sutured layer by layer tightly. A similar injury was simulated for control rats (n = 20) without the use of anti-adhesive gel. Lateral radiography (Premium Vet, Sedecal, Spain) was performed to control the level of laminectomy.

The study received a favourable opinion from the relevant research ethics committee of the Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics Ministry of Health of the Russian Federation (Protocol No. 2 (57) of 17.05.2018). The manipulations were produced in accordance with The European Convention for the protection of vertebrate animals used for experimental and other scientific purposes and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Maintenance and care of laboratory animals was provided in accordance with GOST 33216–2014.

Methods and evaluation criteria The animals were kept in individual cages, access to food and water was not restricted throughout the experiment. Postoperative care consisted of additional heating for 1–2 days, relief of hematuria and prevention of infectious complications of the urinary system [20–21].

Locomotor activity of the pelvic limbs was evaluated in rodents of both groups from the first day after the operation using the open field test: daily for 30 days, twice a week from 31 to 60 days and once a week until the end of the experiment. The BBB locomotor rating scale [18] was used for function recovery and locomotor testing with 0 points meaning no hindlimb movement and 21 points used for forelimb and hindlimb coordination.

A modified assessment sheet was completed and the key of the score decryption of the assessment sheet data applied [22]. The Student's t-test was used to identify the significance of the intergroup differences of the variables during the experiment.

The animals were sacrificed on days 5, 15, 30, 60 and 90.

The spinal cord was taken as a single block for histological examination with the spine several segments above and below the injury and placed in 10 % neutral formalin. Bone structures were carefully removed after a week and a 1.5 cm fragment with a contusion zone resected. A portion of the material was embedded in paraffin according to the standard scheme, the sections were stained with Masson's trichrome stain, thionine Nissle stain, hematoxylin and eosin. The rest of the material was additionally fixed with a solution of osmium (IV) oxide, poured into Araldite. LKB Bromma Ultrotome Nova ultratome (Sweden) was used to make transverse semi-thin 1 μm sections stained with methylene blue and basic fuchsin. The Axioscope.A1 stereomicroscope and AxioCam digital camera (Carl Zeiss MicroImaging GmbH, Germany) were used for digitization and light microscopy of the preparations. Video Test Master-Morphology 4.0 soft (Russia) was employed to measure the cross–sectional area of the spinal cord (medulla spinalis, A_{ms}) and the total area of cystic cavities (syringomyelia, A_s) in the cross-section of the spinal cord. Ten intact rats were examined to get normal measurments. Statistical analysis included the arithmetic mean and standard error ($M \pm m$) calculated for the variables; the statistical significance of the differences was determined by a paired two-sample t-test.

RESULTS

Postoperative period OF the experimental animals of the group was uneventful. One rat developed fluctuating swelling at the site of surgical suture at 8 days of surgery. The cavity was exposed, emptied and drained. There was 3 mL of viscous hemorrhagic evacuation, drainage was placed for 3 days and added with antibiotic therapy. The neurological status of animals at the time of recovery from anesthesia showed no significant differences ($p = 0.33$) in both groups and corresponded to 0 points (Fig. 1). The first signs of spontaneous recovery of hind limb motor activity in controls were recorded after 2–5 days after surgery and after 2–7 days in experimental rats. Significant recovery of pelvic limb function was noted in rats of both groups within 4 weeks after surgery.

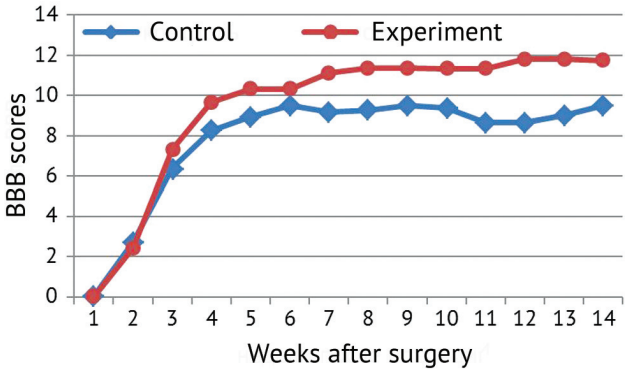

Fig. 1 Dynamics in locomotor recovery of rats with a moderate spinal cord contusion injury simulated at the Th_9 level

After that the recovery curve of the controls reached a maximum after 5 weeks of surgery (9.5 ± 0.28 points) and was steep with a plateau, and experimental animals showed further smooth regression of neurological deficit, reaching the maximum on the BBB scale after 11 weeks (11.8 ± 0.45 points). Later tests showed the recovery level achieved that remained stable in both groups until the end of the experiment. Motor activity recovery was different in rats of the groups. First signs of functional recovery at early stages were seen somewhat later in the experimental animals than in the controls. There were no statistically significant differences in the level of functional recovery of experimental and control rats at 1–2 weeks of surgery ($p > 0.05$) Functional recovery of the experimental rats was significantly higher ($p < 0.05$) than in controls at 3–4 weeks after surgery. Parameters of functional recovery of experimental animals were higher than those of controls at week 5 with no significant differences between the groups ($p = 0.15$). There was a statistically significant difference in the level of functional recovery of pelvic limb ($p < 0.05$) between the groups from the 6th week of observations and that persisted until the end of the experiment.

Histological cross-section examination showed deformed slightly flattened and edematous spinal cord at the level of injury in the control and experimental groups after 5 days of the experiment. The dura mater retained the integrity. Ruptured vascular wall were detected in some posterior spinal arteries and veins. Neurons were single (Fig. 2, a, d), the density of white matter fibers reduced and most axons appeared to be edematous partly surrounded by vacuole-like structures with myelin sheaths being thinned and weakly colored (Fig. 2, b, c, e, f).

Cysts with vague boundaries, tissue detritus, and gliosis were found in both groups at the level of contused spinal cord (Fig. 2, a, e) at 15 days. The central canal was preserved, an excessive number of microvessels observed, and pictures of diapedetic hemorrhages often seen. The lumens of the posterior and anterior spinal arteries and veins were dilated and filled with red blood cells in some animals. Destructive changes were seen in the gray and white matter of the spinal cord. The white matter contained mainly the decay products of myelinated fibers and a few preserved fibers with signs of demyelination, axonal and Wallerian degeneration, lymphocytes, macrophages, plasma cells and hyperplastic glia cells. A few neurons showed pictures of hyperchromatosis or chromatolysis, neuronophagy, some "shadows" in the gray matter.

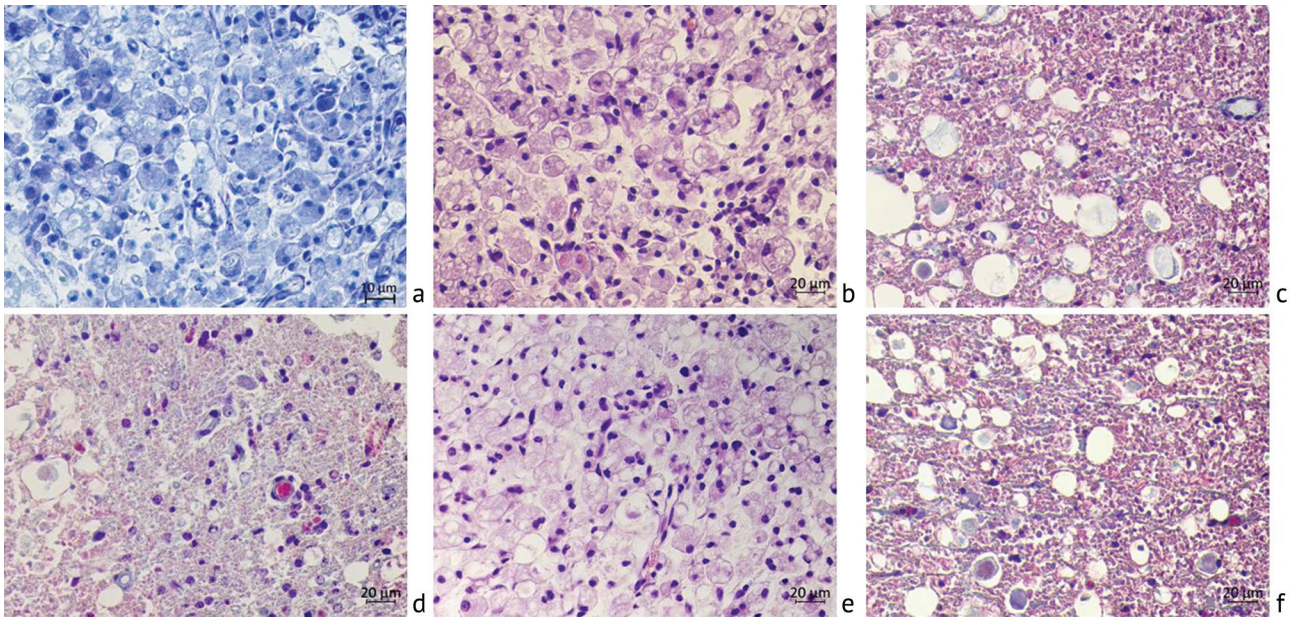

Fig. 2 Fragments of cross-section paraffin sections of the spinal cord of rats at the Th_9 level showing gray matter, posterior horns in (a) controls, (d) experimental animals; Burdach's wedge–shaped bundle in (b) controls, (e) experimental animals; side pillars in (c) in controls, (f) experimental animals. Stained with Nissle stain (a), hematoxylin and eosin (b, e), Masson's trichrome stain (c, d, f), vol. 40, approx. 10

The histological picture was also similar in both groups after 30 days of the experiment. The central canal was found in none of spinal cord samples of controls and was preserved in some experimental animals having an obliterated lumen. The spinal arteries and veins remained dilated and full-blooded, the vascular walls were hypertrophied. Total cross-sectional and longitudinal paraffin preparations showed larger and better visualized cystic-like cavities as compared to the pictures seen after15 days of the experiment (Fig. 3, b, f). Walls were seen to form in some cavities, separating them from the surrounding tissues. Necrobiotic changes in gray and white matter were progressing with single myelinated fibers and neurons observed.

A similar histological picture was observed in both groups after 60 and 90 days of the experiment. Signs of pronounced syringomyelia were revealed with clearly formed large one-, two- and multi-chamber cystic-like cavities (Fig. 4, c, d, g, h) with clear walls formed by ependym-like cells. Cysts were localized mainly in gray matter. The central channel was not seen in controls, preserved in some experimental animals after 60 days and was not detected in the majority of some animals after 90 days. Arterio-venous congestion persisted with lumens of the spinal arteries and veins dilated in the samples of both groups and most of them being full-blooded, the walls being hypertrophied. Regenerating, myelinating axons were found in the white matter with ongoing destruction (Fig. 4, a, b, d, e). There were single or no neurocytes in the gray matter at the level of contusion (Fig. 4, c, f).

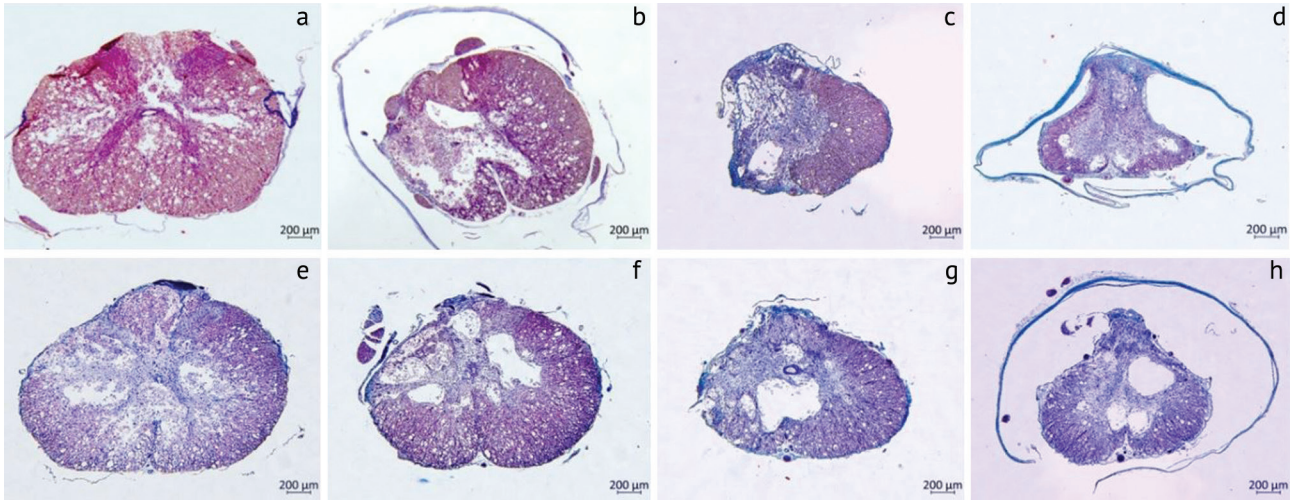

Fig. 3 Total cross-sectional paraffin sections of the spinal cord of rats made at the Th_9 level of controls after 15 (a), 30 (b), 60 (c), 90 (d) and of the experimental animals after 15 (e), 30 (f), 60 (g), 90 (h) days of the experiment. Stained with Masson's trichrome stain, vol. 4, approx. 10

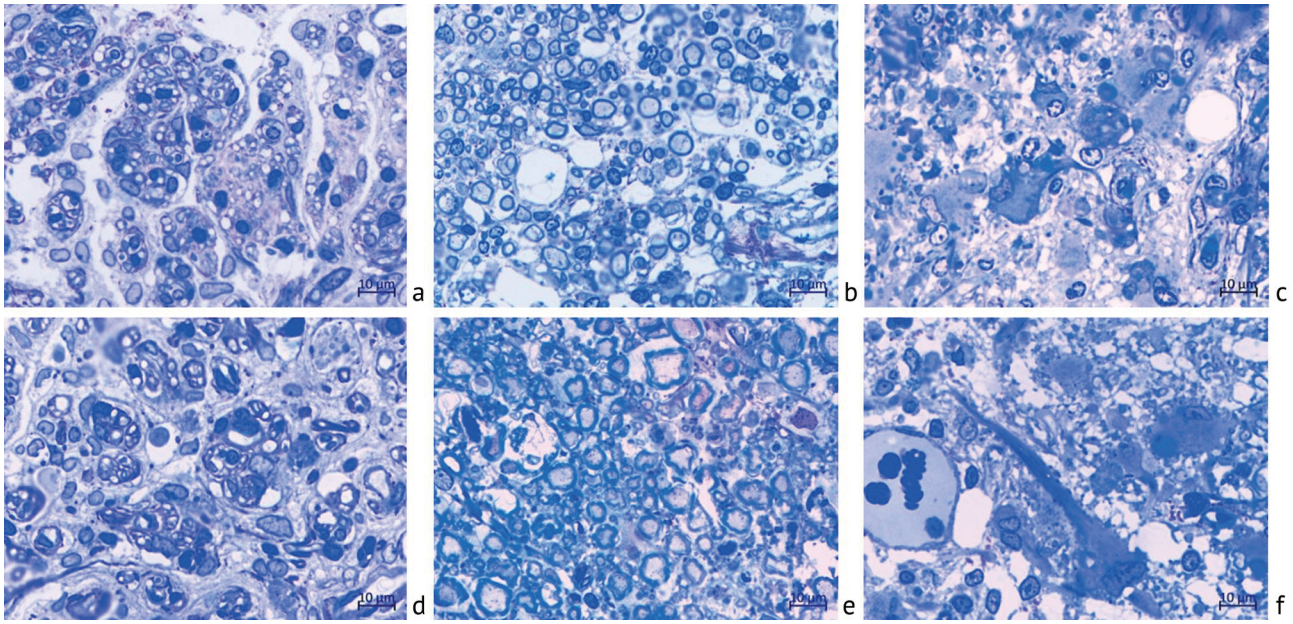

Fig. 4 Fragments of total semi-thin sections of the spinal cord of rats at the Th_9 level showing wedge–shaped Burdach bundle in (a) controls, in (e) experimental animals; white matter, side pillars in (b) controls, (e) experimental animals; gray matter, anterior horns in (b) controls, (f) experimental animals. Stained with methylene blue and basic fuchsin, vol. 100, approx. 10

Quantitative studies showed a gradual decrease in the cross–sectional area of the spinal cord that became more pronounced after 30 days ($p < 0.05$) in the controls (Table 1) due to contusion injury in both groups. The difference in the areas increased between the groups by almost a third after 90 days (Table 1). There was no differencea in total area of cystic cavities in the groups after 90 days but syringomyelia was more pronounced in the control group and occupied 16% of the area and 14 % in the experimental animals due the difference in the cross-sectional areas of the spinal cord (Table 2).

Table 1

The cross-sectional area of the spinal cord (medulla spinalis) – A_{ms}

Length of experiment (days)	Ams (10^3 mcm^2) (M ± σ)				
	Control group (k)	Experimental group (o)	ΔA_{ms}kn/ ΔA_{ms}n	ΔA_{ms}on/ ΔA_{ms}n	ΔA_{ms}ko/A_{ms}o
15	2891 ± 37	2895 ± 17	-22.18*	-22.07*	-0.14 %
30	2005 ± 16	2281 ± 24	-46.03*	-38.60*	-12.10 %*
60	1284 ± 79	1645 ± 17	-65.44*	-55.72*	-21.95 %*
90	1145 ± 35	1616 ± 215	-69.18*	-56.50*	-29.15 %*

Δa_{ms}kn/Δa_{ms}n – the difference (%) between the cross-sectional area of the spinal cord of control and intact rats (normal – n, A_{ms} measured 3715 ± 76 mcm^2); ΔA_{ms}on/ Δa_{ms}n – the difference between the area of the spinal cord of the experimental and intact rats, ΔA_{ms}ko/A_{ms}o – the difference between the area of the spinal cord of the control and experimental animals; * – the difference is significant according to the results of a paired two-sample t-test at $p < 0.05$

Table 2

The total area of cystic cavities (syringomyelia, A_s) in the cross section of the spinal cord (A_{ms})

Length of experiment (days)	A_s (10^3 mcm^2) (M ± σ)			
	Control group (k)	A_sk /A_{ms}k (%)	Experimental group (o)	A_so /A_{ms}o (%)
60	53 ± 86	19.39	315 ± 25	19.99
90	199 ± 87	16.27	200 ± 91	14.09

A_sk /A_{ms}k – the proportion of syringomyelia area in the cross-section of the spinal cord of control rats, A_so /A_{ms}o – experimental group (в %)

DISCUSSION

The findings seen during recovery of locomotor functions of the pelvic limbs in control rats were identical to the data observed by the designers of the BBB Mobility Assessment Scale in groups of animals with moderate spinal cord injury [18]. There were significant differences in the parameters of experimental animals. A comparative analysis of the recovery of motor activity in rats of both groups showed a higher level of behavioral characteristics in experimental animals who received Antiadhesin® gel.

Histological examination revealed pathomorphological changes in the spinal cord being characteristic of contusion injury [23, 24] and identical for both groups. Softening and necrosis of the nervous tissue with tissue and cellular detritus of gray and white matter, small cysts were observed in the paracontusion area due to mechanical trauma and microcirculatory disorder after 5–15 days of injury. Syringomyelia and large cystic-like cavities developed after 30 days with progressing destruction of gray and white matter due to impaired microcirculation, and liquor exchange due to the destruction of the central channel in controls. Hemodynamic disorders persisted after 60 and 90 days with impaired cerebrospinal fluid exchange in the controls that was also observed in some experimental animals; syringomyelia aggaravated with single neurocytes and regenerative processes initiated in the white matter with small regenerating, myelinating axons detected.

The absence of inflammatory reactions of the spinal cord at contacting area with the anti-adhesive hyaluronic-containing absorbable gel "Antiadhesin"®, a higher degree of recovery of locomotor activity in experimental rats due to less pronounced syringomyelia and a decreased cross-section of nerve trunks at the level of injury indicate the possibility of using this drug for contusion injuries of the spinal cord with the likelihood of improving the regression of neurological deficit. The results can be rewarding for future experiments to test the possibility of local delivery of drugs and cells into spinal cord with Antiadhesin® gel after concussion injury in order to optimize the use of the gel to facilitate a possible positive effect.

CONCLUSION

The absence of a negative effect of the anti-adhesion hyaluronic-containing resorbable gel on the functional results, and morphological characteristics of the spinal cord offer the opportunity of the further experimental use as a carrier of substance composition initiating beneficial metabolic and neuroprotective action for transdural delivery in simulated spinal cord injury.

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Authors declare no conflict of interest.