Glial scar and neuroregeneration: histological, functional, and magnetic resonance imaging analysis in chronic spinal cord injury

Laboratory investigation

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Object. A glial scar is thought to be responsible for halting neuroregeneration following spinal cord injury (SCI). However, little quantitative evidence has been provided to show the relationship of a glial scar and axonal regrowth after injury.

Methods. In this study performed in rats and dogs, a traumatic SCI model was made using a weight-drop injury device, and tissue sections were stained with H & E for immunohistochemical analysis. The function and behavior of model animals were tested using electrophysiological recording and the Basso-Beattie-Bresnahan Locomotor Rating Scale, respectively. The cavity in the spinal cord after SCI in dogs was observed using MR imaging.

Results. The morphological results showed that the formation of an astroglial scar was defined at 4 weeks after SCI. While regenerative axons reached the vicinity of the lesion site, the glial scar blocked the extension of regrown axons. In agreement with these findings, the electrophysiological, behavioral, and in vivo MR imaging tests showed that functional recovery reached a plateau at 4 weeks after SCI. The thickness of the glial scars in the injured rat spinal cords was also measured. The mean thickness of the glial scar rostral and caudal to the lesion cavity was 107.00 ± 20.12 μm; laterally it was 69.92 ± 15.12 μm.

Conclusions. These results provide comprehensive evidence indicating that the formation of a glial scar inhibits axonal regeneration at 4 weeks after SCI. This study reveals a critical time window of postinjury recovery and a detailed spatial orientation of glial scar, which would provide an important basis for the development of therapeutic strategy for glial scar ablation. (DOI: 10.3171/2010.3.SPINE09190)

KEY WORDS • chronic spinal cord injury • glial scar • axonal regeneration • magnetic resonance image

Spinal cord injury is a devastating clinical condition that can result in permanent disabilities. Worldwide, an estimated 2.5 million people live with an SCI, and more than 130,000 new injuries are reported each year. Following primary SCI, a progressive pathological process occurs during the acute and chronic stages. Although extensive studies have focused on SCI at the acute stage, little is known about the pathophysiology of SCI in the chronic period.

Glial scar and cavity formation is regarded as the prominent pathophysiological feature of chronic SCI. Reactive astrocytes, the major cellular component of scar...
tissue, exhibit a graded response to injury that includes changes in gene expression, hypertrophy, and process alteration, and, in some cases, cell division. The abortive regeneration after SCI has been attributed to a nonpermissive environment at the injury site, of which a glial scar has long been implicated as a major impediment to axon regeneration. Reducing or inhibiting the formation of a glial scar is thought to be a therapeutic strategy for SCI treatment. Yet, there are no proven methods for the ablation of a glial scar.

To develop glial scar ablation as an effective therapy, 2 vital questions need to be answered. How thick is the glial scar that needs to be ablated? What is the appropriate time window for the glial scar ablation? Effective ablation therapy requires an ablation range not larger or smaller than the true thickness of the glial scar. Recent studies have shown that reactive astrocytes may play complex roles in tissue repair at different stages after SCI. The optimal time window for glial scar ablation is therefore critical for the therapy. In the present study, based on our histological and MR imaging observations we found that the cavity and astroglial scar, which blocked the extension of axon growth, formed 4 weeks following SCI. Our quantitative data showed that the thickness of the glial scar in the rostral and caudal regions was different from that in the lateral part. In agreement with these findings, our results from electrophysiological and behavioral tests showed that functional recovery reached a plateau at 4 weeks after SCI. Thus, the process of spontaneous recovery halted at 4 weeks after SCI. Together, these results provide important evidence for the development of a therapeutic strategy in glial scar ablation in chronic SCI.

Methods

Spinal Cord Injury Model

Sprague-Dawley rats (180–220 g) and Beagle dogs (11 kg) were used for the experiments. Each animal was anesthetized with pentobarbital (60 mg/kg intraperitoneally). The animals’ body temperatures were maintained at 37°C throughout the procedure. Spinal contusion injury was produced at the T-9 segment using the weight-drop injury model. A 10-g rod with a diameter of 2.5 mm was released from a 25-mm height onto the exposed rat spinal cord, and a 30-g rod was released from a 50-mm height for the canine model. For sham surgery, animals underwent laminectomy without contusion. After surgery, animals were maintained under the same preoperative conditions.

All experiments were carried out in accordance with China’s animal welfare legislation and were approved by the Third Military Medical University Committee on Ethics in the Care and Use of Laboratory Animals. A total of 58 rats were used in our experiments, of which 37 were used for histological, immunohistochemical, and tracing observation. Among them, 4 rats were used for sham control, 3 rats were used for each time point (1 day, 1 week, 2 weeks, 4 weeks, 8 weeks, and 16 weeks after SCI) for histological staining, and 3 rats were used for each time point (1 day, 1 week, 2 weeks, 4 weeks, and 8 weeks after SCI) for immunostaining and tracing. A total of 21 rats were used for glial scar analysis in which 4 rats were used for sham control and 3 rats were used for each time point (1 day, 1 week, 2 weeks, and 8 weeks after SCI) for immunohistological observation. Five rats were used for glial scar quantitation at 4 weeks after SCI. All injured rats for the experiments described above were tested using the BBB Locomotor Rating Scale before they were killed. For the dog experiments, 4 dogs were used for sham control, and 12 dogs were used for MR imaging after SCI. Two dogs were killed for histological observation at each time point (1 day, 1 week, 2 weeks, 4 weeks, 8 weeks, and 16 weeks after SCI following the MR imaging test.

Locomotor Scoring

All behavioral tests were performed in a blinded fashion. Locomotor function was observed every 3 days after SCI and recorded using the BBB Locomotor Rating Scale. Briefly, the BBB is a 21-point ordinal scale ranging from 0 (no discernible hindlimb movement) to 21 (consistent and coordinated gait with parallel paw placement of the hindlimb and consistent trunk stability). Scores from 0 to 7 rank the early phase of recovery with return of isolated movements of 3 joints (hip, knee, and ankle); scores from 8 to 13 describe the intermediate recovery phase with return of paw placement, stepping, and forelimb-hindlimb coordination; and scores from 14 to 21 rank the late phase of recovery, measuring toe clearance during the step phase, paw positioning, truncal stability, and tail position.

Staining and Immunohistochemical Analysis

The animals were killed after induction of sodium pentobarbital anesthesia (60 mg/kg intraperitoneally), and their spinal cords were fixed by transcardiac perfusion with 0.1 M PBS followed by 4% paraformaldehyde in a 0.1 M phosphate buffer. The fixed spinal cords were equilibrated in 30% sucrose for 48 hours and then cut into 20-μm longitudinal sections on a cryostat. The sections of spinal cord were mounted on gelatinized slides and stained with H & E for histopathological and morphological observation. For immunohistochemical analysis, the sections were incubated overnight at 4°C with primary antibodies against NF-200 (dilution 1:400, Sigma) or GFAP (1:200, Sigma). After washing with PBS, the sections were incubated with biotinylated antibody for 1 hour followed by ABC (Vector Laboratories) for 1 hour. The final peroxidase reaction was visualized using a solution of DAB in 0.03% H2O2, in 0.01 M PBS with nickel ammonium sulfide. For immunofluorescent staining, the sections were incubated overnight at 4°C with primary antibodies against NF-200 and GFAP. After washing, the sections were incubated for 1 hour with secondary an-
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tibodies conjugated with isothiocyanate and Texas Red (1:200, Molecular Probes). The results were analyzed using a fluorescence microscope (Leica Microsystems) and confocal microscopy (Leica Microsystems). Immunohistochemical experiments were repeated at least 3 times.

Axonal Tract Tracing

For labeling axonal tract, animals were anesthetized with pentobarbital (60 mg/kg, intraperitoneally). Injections of 100 nl 10% BDA (Molecular Probes) were performed at each of 3 sites in the sensorimotor cortex using a Hamilton syringe (anteroposterior: −0.7 mm, −1.4 mm, −2.3/−2.5 mm; mediolateral: −2.3 mm; depth: −1.5 mm) on each side of the brain, using the bregma as a landmark for the anteroposterior and mediolateral directions, and the dura as a landmark for depth.34 The micropipette of the syringe was left in the tissue for 10 minutes before it was retracted. After the micropipette was removed, the craniotomy was covered with Gelfoam, and the scalp was closed.

Electrophysiological Recording

To record MEPs and SSEPs, animals were anesthetized and maintained with ketamine (100 mg/kg/hr intramuscularly). Ketamine anesthesia was reported to have a minimal suppressive effect on MEPs and the Hoffmann reflex.47 Motor evoked potentials were elicited by transcranial electrical stimulation (20 V, 200 μsec) of the motor cortex. Responses were recorded from the gastrocnemius muscle using 22-gauge silver needle electrodes (distance between recording electrodes 1 cm) connected to a preamplifier (HS4 fiber optic Bioamp Headstage, WPI) and a DB4 fiber optic amplifier (WPI). The MEPs were recorded at 7-day intervals and stored on a personal computer for analysis. Somatosensory evoked potentials produced by electrical stimulation of the shank were recorded using a monopolar silver-ball electrode placed on the surface of the sensorimotor cortex. Latencies and amplitudes of the SSEPs and MEPs were measured and analyzed.

Measurement of the Gliarial Scar

Animals were transcardially perfused with 4% paraformaldehyde followed by postfixation and 20% buffered sucrose. The spinal cord segments (1.2 cm in length) containing the site of experimental injury were obtained. The 35-μm-thick sections were serially cut in the longitudinal plane and were placed on a microscope slide that was covered with gelatin. The sections were double labeled with NF-200 and GFAP, and digital images of all the sections were obtained using a charge-coupled device camera mounted on a fluorescent stereomicroscope (Zeiss STE SV11) and monitored by a computer. Serial images were collected using a computer in which the region of interest was focused on the lesion site (changed into cavity at the chronic stage) and its surrounding tissues, where astrocytes with typical morphology were activated. The thickness of the glial scar (consisting of activated astrocytes surrounding the cavity) of each image was serially measured using Image-Pro Plus (Media Cybernetics, Inc.).

Magnetic Resonance Imaging

Magnetic resonance images were obtained from 1 day to 16 weeks after injury using a 1.5-T MR imaging/spectrometer. For imaging, the animal was placed in the left lateral decubitus position. The surface coil was located on the marked region of the injured spinal column. Images of the spinal region were acquired in the coronal, axial, and sagittal planes. The T1-weighted images (TR 600 msec, TE 13 msec, section thickness 4 mm with section gap of 0.4 mm to increase coverage) and T2-weighted images (TR 2227 msec, TE 85 msec, section thickness 4 mm with section gap of 0.4 mm to increase coverage) were obtained.

Statistical Analysis

All data are presented as the means ± SEMs from at least 3 independent experiments. The statistical significance of the difference between the groups was calculated using ANOVA with the post hoc Newman-Keuls test. A p value <0.05 was considered significant. All statistical analyses were performed using SPSS software (version 12.0, SPSS, Inc.).

Results

Histological Observation of SCI in Rats During the Chronic Stage

Using a well-established contusive SCI model in rats, we first characterized the histopathological changes in injured spinal cords at the chronic stage. We showed that the diameter of spinal cord at the impact site became smaller and smaller following SCI (Fig. 1a). The lesioned spinal cord appeared to be hemorrhagic and congestive within 1 week after SCI. At 2 weeks after SCI, the congestion and swelling disappeared and the spinal cord at the injury site became thinner, and at 4 weeks postinjury, the cord became thinner and the conglutination with surrounding tissue became tighter (Fig. 1a).

Under microscopic observation (Fig. 1b), the structure of the lesioned area became disorganized on the 1st day following SCI, with extensive parenchymal hemorrhage, necrosis, and edema. At 1 week after injury, in the lesion area with hemorrhage, the region of swelling and degeneration had enlarged. There was no clear border between normal and injured tissue. At 2 weeks postinjury, the pathological process was similar to that at 1 week except for hemorrhage absorption, liquefaction, and rudimentary cavity formation. At 4 weeks after the injury, a large cyst that filled with an amorphous substance and liquid formed surrounded by a glial scar. There were trabeculae inside the cavity. At 8 weeks and 16 weeks after SCI, the changes were similar to those at 4 weeks. These results indicate that the histopathological change is mitigative at 2 weeks and stops at 4 weeks after SCI (Fig. 1b).

Axon Growth in Chronic SCI in Rats

Using immunohistochemical and axonal tract tracing techniques, we examined the morphological changes of axons after SCI. In a healthy spinal cord, numerous NF-
200–labeled neurons and axons were in regular organization (Fig. 2a). However, this nonrandom structure was destroyed in the injured cord (Fig. 2b). The NF-200–labeled axons were interrupted and disproportionate, although there still were a few normally running axons in a rim of spared tissues at the lesion site. At 1 week postinjury, the axons at the lesion site became fewer and abnormal, with fewer nerve fibers in the spared tissues around the injury epicenter (Fig. 2c). However, larger numbers of randomly oriented axons in the region rostral to the lesion appeared to be growing. At 2 weeks after SCI, more axons seemed to regenerate, and some axons were seen to regrow into the vicinity of the lesion (Fig. 2d). The potential of axon regeneration remained even at 4 and 8 weeks after SCI (Fig. 2e and f). A number of axons could be observed in the trabeculae of the lesion cavity (Fig. 2e and f). By tracing the axonal tract, we found that some regenerative axons could grow into the vicinity of the lesion, and some of the axons even reached the wall of the lesion cavity (Fig. 2g–l). However, most of the axons could not penetrate the astroglial scar (Figs. 2j–l and 3i–m). Together, these observations suggest that axonal regeneration may occur at 1 week after SCI, and the regeneration potential could remain at the chronic stage. However, the glial scar restricts the axon extension.

Reactive Astrocytes in Chronic SCI in Rats

A glial scar is the major barrier to block axon extension at the chronic stage of SCI. Because glial scar is built up with reactive astrocytes, we examined the morphological changes of reactive astrocytes in the chronic stage of SCI. We showed that astrocytes in normal spinal cord were characterized by small bodies and slender processes (Fig. 3a–d). However, the astrocytes activated after SCI exhibited different morphology, and the morphological characteristics were distinct at different stages after injury (Fig. 3e–h). Compared with the astrocytes at 1 week postinjury, the number and diameter of astrocytes at 2 weeks became larger and the processes became thicker, with modest expression of GFAP (Fig. 3e and f). At 4 weeks after injury, the morphology of reactive astrocytes became more typical, characterized by a large somatic body, thick processes, and intensive GFAP labeling (Fig. 3g). Consistent with these changes, the glial scars and cavity appeared at 4 weeks after injury (Fig. 3i–m). The change of reactive astrogliosis at 8 weeks postinjury followed the same trend as that at 4 weeks (Fig. 3h). Double labeling with NF-200 and GFAP showed that activated astrocytes were cross-linked to form a barrier that obstructed the extension of regenerative axons (Fig. 3i and m). Although a few axons could be seen to regrow into the outer layer of the glial scar, the NF-200–positive axons showed little capability to penetrate the glial scar, especially the inner layer (Fig. 3i–n). These observations indicate that reactive astrogliosis turned into the component of glial scars at the chronic stage, and the glial scars became clearly defined at 4 weeks after injury.

Quantitative Examination of Glial Scar in Chronic Rat SCI

To provide experimental evidence for the development of a therapeutic strategy of glial scar ablation, we examined the thickness of the glial scar in the injured spinal cord during the chronic stage. The thickness of the glial scar was measured using immunofluorescent imaging with double staining of GFAP and NF-200. We showed that the mean thickness of the glial scar rostral and caudal to the cavity was 107.00 ± 20.12 μm and that the mean thickness of the lateral glial scar was 69.92 ±
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15.12 μm (Fig. 4, Table 1). In a detailed analysis, we found that the mean thickness of the glial scar without penetrated or spared axons (stained by NF-200) in the regions rostral and caudal to the cavity was 38.58 ± 11.07 μm, and in the lateral region it was 27.22 ± 10.63 μm (Table 1). The mean thickness of the glial scar with penetrated or spared axons was 68.42 ± 11.08 μm in the regions rostral and caudal to the cavity and 43.47 ± 22.42 μm in the lateral region (Table 1). Thus, the difference between the thickness of the glial scars rostral and caudal to the cavity and glial scars lateral to the cavity may be taken into account while developing glial scar ablation strategy.

Fig. 2. Axonal growth in an injured rat spinal cord. Panels a–f are stained by DAB intensified with nickel ammonium sulfide. a: In the control spinal cord, neurons stained by NF-200 are in regular organization, and NF-200–positive axons run parallel in the white matter. However, the nonrandom structure is destroyed following SCI. b: Axons labeled by NF-200 are interrupted and run disproportionately. c: At 1 week after SCI, the axons in the lesion site become fewer and are abnormal. d: At 2 weeks after SCI, axons begin to regenerate, and some approach the rim of the lesion site. At 4–8 weeks after SCI, the pathological change of axons becomes stable. e and f: Axons can be seen approaching the lesion site, and some axons even reach the cavity wall. Insets in panels a–f marked with capital letters are magnified images from each longitudinal section at low magnification. g: Biotin dextran amine–labeled nerve fibers (black-blue, also see arrow in h) observed in the region rostral to the lesion, approach the lesion site and sprout into the gray matter. h: The magnified image from inset in g (arrow showing BDA-labeled nerve fiber). i: A sample image showing BDA-labeled axons (arrow) approach activated glia (arrowhead indicating brown cells marked by GFAP). j–l: Fluorescent micrographs show that few BDA-stained axons can penetrate the glial scar (green, stained with GFAP).
The formation of a glial scar during the chronic stage after SCI would lead to functional and behavioral consequences. We therefore used electrophysiological and behavioral methods to examine the time course of functional and behavioral recovery in the injured animals. The SSEPs and MEPs were used to test the nervous transmission of the ascending and descending tracts along the

**Fig. 3.** Formation of glial scar in injured rat spinal cord (the images in d–n are immunofluorescent staining with NF-200 [red] and GFAP [green]). a–c: Images showing the morphology of astrocytes in the normal spinal cord (marked by GFAP and nickel-intensified immunostaining). Images showing typical morphology of astrocytes in the gray and white matter (b and c). d: Image showing astrocytes (arrow) and axons in control spinal cord double stained by NF-200 and GFAP. e: At 1 week after SCI, astrocytes (arrows) around the injury site are activated and display different morphology from control astrocytes. f: At 2 weeks following SCI, the morphological changes become more prominent, characterized by a large somatic body, high expression of GFAP, and thick processes (arrows). g: At 4 weeks postinjury, the reactive astrocytes display larger somata and thicker processes (arrows). h: The changes in astrocytes (arrows) at 8 weeks after SCI are similar to those at 4 weeks. i: Image showing reactive astrocytes with their processes (arrows) linked together to inhibit the extension of axon (arrowheads). j–l: Sample images showing the glial scar and cavity at 4 weeks after SCI. Few axons (red) exist in the glial scar around the cyst (green). m: High magnification image from inset in l. The dashed line indicates the border between the areas of the glial scar with and without penetrated axons. n: Image showing the entire view of longitudinal sections of an injured spinal cord with the cavity and glial scar at 4 weeks after SCI.
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spinal cord. Our results showed that the latency of SSEPs and MEPs was significantly delayed after SCI, with no response on the 1st day after primary impact. The latencies of SSEPs and MEPs recovered gradually and reached a plateau at 4 weeks after SCI (Fig. 5a and b). In conformity with the functional changes, the BBB scores showed that the hindlimb locomotion of injured animals dramatically decreased on the 1st day after SCI but recovered with time and reached a plateau at 4 weeks after SCI (Fig. 5c). These data indicate that functional and behavioral recovery reaches a plateau at 4 weeks after SCI, which is consistent with the histological change postinjury.

Magnetic Resonance Imaging in Chronic SCI in Dogs

Magnetic resonance imaging is a standard test for patients with SCI. To provide clinically relevant evidence of the pathological change in chronic SCI, we used a 1.5-T MR imaging unit, which is extensively used to assess patients with SCI in the clinic, for the experimental analysis of chronic SCI. Because it is not feasible to perform 1.5-T MR imaging in rats, an SCI model in dogs was used in these experiments. The in vivo MR imaging and H & E staining were performed in the injured spinal cords. As shown in Fig. 6, the pathological progression in SCI in canines was similar to that in rats and was characterized by necrosis, hemorrhage, and tissue destruction in the acute phase, followed by formation of an astrogial scar and cavity at the chronic stage. There were no obvious changes on MR images on the 1st day following SCI, although the histological section showed diffused hemorrhage and edema at the lesion site. At 1 week after injury, MR imaging showed that changes occurred, with hypointensity on T1-weighted images and hyperintensity on T2-weighted images. At 2 weeks after injury, lower intensity was seen on T1-weighted images and higher intensity on T2-weighted images, which was consistent with the histological changes including liquefaction and rudimentary cavity formation. The MR images obtained at 4 weeks postinjury showed typical pathological changes and were characterized by a fluid-filled cyst with lowest intensity on T1-weighted images and highest intensity on T2-weighted images. This is in accordance with the observed formation of a cavity and glial scar in the histological section assessed in the same animals. The pathological changes were even seen on MR images at 16 weeks after SCI. Thus, the changes observed on MR imaging are consistent with the histological alterations in chronic SCI.

Discussion

Although glial scars are thought to be responsible for halting neuroregeneration following SCI, little evidence has been provided to show the relationship of a glial scar and axonal growth. Using immunohistological, functional, behavioral, and MR imaging approaches, we studied in detail the formation of glial scars and the growth of axons in cases of SCI in rats and dogs at the chronic stage. We showed that the cavity and glial scars were formed at the chronic stage of SCI, which was clearly defined at 4 weeks after the initial injury. In conjunction with this observation, our data indicated that functional and behavioral recovery reached a plateau at 4 weeks after SCI. The in vivo MR images showed that the fluid-filled cavity was

| TABLE 1: Quantitative measurement of the glial scar* |
|-----------------|-----------------|-----------------|-----------------|
| Site | Glial Scar Thickness (µm) | Overall | w/ Penetrated Axons | w/o Penetrated Axons |
| rostral/caudal to cavity | 107.00 ± 20.12† | 68.42 ± 11.80* | 38.58 ± 11.07† |
| lat to cavity | 69.92 ± 15.12 | 43.47 ± 22.42 | 27.22 ± 10.63 |

* The means ± SEMs are calculated from measurements in 5 rats.
† p < 0.05 compared with thickness of the glial scar in the region lateral to cavity.
hypointense on T1-weighted images and hyperintense on T2-weighted images, the typical features of chronic SCI, which became prominent at 4 weeks following injury. Immunofluorescent labeling and tract tracing revealed that the injured axons showed potential to regrow, which might contribute to the spontaneous recovery after SCI. However, the glial scar–confined axonal extension and thereby blocked further recovery at 4 weeks after SCI. Together, our results provide comprehensive evidence suggesting that the formation of a glial scar may play a major role in blocking axon growth-mediated regeneration potential at the chronic stage of SCI. Thus, our study strongly supports the notion that it is critical to develop therapeutic strategies that eliminate and/or inhibit glial scar-induced blockade of axonal regeneration at the chronic stage after SCI.

Axons are severely damaged following SCI through primary and secondary injury mechanisms. However, injured axons are shown to have a regenerative capability that contributes to spontaneous recovery after SCI. In our study, spontaneous fiber sprouting was observed as early as 1–2 weeks. However, our evidence indicated that the astroglial scar obstructed the regeneration process. Although previous studies have shown that a glial scar is one of the main barriers inhibiting axonal regeneration, detailed studies of the relationship between a scar and the regenerative axons are lacking. As revealed in our study, astrocytes were activated after injury with an enlarged soma and intensive GFAP expression over time. At 4 weeks after SCI, the glial scar demarcation between the normal spared tissue and the epicenter cavity became clearly defined with only a few axons being seen to penetrate into the outer layer and few into the inner layer of the glial scar. These data offered direct evidence indicating that axons had regenerative potential. However, this was inhibited by glial scar. In addition to the mechanical blockade, the formation of glial scar was reported to inhibit axonal growth by producing growth inhibitory
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Fig. 6. Time course of MR imaging and histopathology in SCI model in dogs. The H & E–stained photomicrographs in the panels below each MR image are the corresponding histological section from the same animal. The MR images show that there is no obvious abnormal signal intensity on T1- and T2-weighted images at 1 day postinjury, but hemorrhage and necrosis are found in the histological section. At 1 week after injury, there is little signal intensity on the T1-weighted and some signal intensity on the T2-weighted images. In the photomicrographs, hemorrhage, necrosis, and exudation are observed. At 2 weeks postinjury, the change of MR image is similar to that at 1 week after injury. However, the histological sections show hemorrhage absorption, liquefaction, and rudiment formation of the cavity. At 4 weeks after SCI, evident abnormal signal intensity appeared with hypointensity on T1-weighted images and hyperintensity on T2-weighted images with clear border. Histological sections show that the cavity is formed with glial scar, in which a large amount of an amorphous substance and liquid is present. At 8 weeks postinjury, MR imaging changes followed the same trend as that at 4 weeks after SCI. Lower T1-weighted signal intensity and higher T2-weighted signal intensity continue to exist in the lesioned spinal cord, even at 16 weeks after SCI. Correspondingly, pathological change under light microscope exhibits a large cavity containing liquid with a clear border and surrounded by a thick glial scar. The arrows indicate the injury site of the cord. Original magnification of the photomicrographs × 200.
factors such as chondroitin sulfate proteoglycans, and keratan sulfate proteoglycans. Therefore, prevention or suppression of glial scar formation and growth inhibitory molecules is believed to promote axonal regeneration. It has been demonstrated that the intermediate filaments, such as GFAP and vimentin (a hallmark of reactive astrocyte) are upregulated after SCI, and knocking out or knocking down the expression of these filaments reduces glial scar formation and promotes axonal regeneration after CNS injury. Evidence also indicates that targeting growth inhibitory molecules markedly reduces the inhibitory effects of the extracellular matrix on axonal regeneration. Intrathecal application of chondroitinase ABC, which can digest chondroitin sulfate proteoglycans, enhances the regeneration of axons, restores postsynaptic plasticity, and increases functional recovery after SCI. In addition, inhibiting the activity of the growth inhibitors using specific antibodies, blocking the receptors of growth inhibitory factors, and suppressing intracellular signals coupled with the growth inhibitory components of the scar have been reported to enhance axonal regeneration. Furthermore, reducing astrogliosis by interrupting astrocyte proliferation enhances repair after SCI. These findings suggest that targeting glial scar formation and its inhibitory components may be a therapeutic strategy for patients who have sustained an SCI.

Although many methods have been proven useful for the prevention of scar formation in an acute SCI model, no effective strategy has been proven to eliminate or remove an existing glial scar at the chronic stage, which is often encountered clinically. Recently, several kinds of methods have been proposed to remove the existing glial scar after SCI, including x-ray irradiation, laser, the photochemical method with Rose Bengal, and resection, but the effects remain uncertain due to the unknown precise spatial distribution and thickness of glia scar.

It is interesting to note from our results that NF-200–positive axons were seen to penetrate into the glial scar and thus were mingled with the outer layer of scar tissue. It appears that preserving the outer layer of the glial scar, which contains the penetrated axons, might be a practical strategy that could at least avoid the loss of existing regeneration potential. Thus, when developing a strategy for the ablation of a glial scar, it is crucial to decide how the outer part of the glial scar mingled with axons could be preserved. As an important step toward development of the strategy, we quantified the thickness of the glial scar with and without penetrated axons (Table 1). These data may provide valuable experimental information for the treatment of patients with SCI.

If the dosage of radiation or the thickness of resection goes beyond the actual size of the scar, it will cause additional injury, or if the ablation of the glial scar is incomplete, it would result in no obvious effect. Therefore, making sure of the precise spatial distribution and thickness of the glial scar is crucial to use these therapeutic strategies. The scale of the glial scar obtained in the present study would be beneficial in establishing an appropriate radiation dosage, laser intensity, and resection range while ablating the glial scar in patients with chronic SCI. However, one must be careful when applying these therapeutic strategies to other types of SCI because the scar develops differently depending on the nature of injury. For example, gliosis commenced in the 3rd week in the transection injury model rather than in the 1st week in the contused SCI model.

The other important consideration for the development of a strategy for glial scar ablation is how to decide on an appropriate time window for the therapy. Recent transgenic experiments have demonstrated that reactive astrocytes provide essential activities that protect tissue and preserve function after SCI and traumatic brain injury at the early stage. Studies have also shown that an activated astrocyte exerts its protective functions through glutamate uptake, free radical scavenging, neurotrophin, and hormone synthesis. These findings indicate that reactive astrocytes may play a neuroprotective role in the early period of SCI. Thus, the time window for glial scar ablation should be the time when a mature glial scar has formed. Our results in experimental models indicate that the time beyond 4 weeks after SCI may be the time window for considering scar ablation.

Conclusions

We studied in detail the formation of glial scars and the growth of axons during the chronic stage of SCI in rats and dogs. We showed that the cavity and glial scar were clearly defined at 4 weeks following initial injury. We also showed that functional and behavioral recovery reached a plateau at 4 weeks after SCI. Furthermore, MR images showed the fluid-filled cavity as hypointense on T1-weighted images and hyperintense on T2-weighted images; these typical features of chronic SCI became prominent at 4 weeks after SCI. The mean thickness of the glial scar in the regions rostral and caudal to the cavity is different. This study provides critical spatiotemporal evidence that SCI evolved into steady state at 4 weeks after primary injury, which is the time when spontaneous functional and structural recovery hits a plateau. Our results offer an experimental basis for determining the optimal therapeutic time window and accurate spatial orientation for glial scar ablation in SCI treatment.

Disclosure

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