Brain Research 1672 (2017) 44–49

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Fractional anisotropy is a marker in early-stage spinal cord injury

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ARTICLE INFO

Article history:
Received 16 May 2017
Received in revised form 3 July 2017
Accepted 26 July 2017
Available online 29 July 2017

Keywords:
Diffusion tensor imaging
Spinal cord injury
Acute
Marker

ABSTRACT

This study was designed to investigate whether fractional anisotropy (FA) contributes to study pathologic changes in SCI. Here rats were divided into a control group and three injury groups. Those in the injury groups were administered a mild, moderate, and severe contusion injury at the T10 vertebral level, respectively. Three rats were randomly selected from each group at 6, 24 and 72 h after SCI for imaging examination. Magnetic resonance diffusion tensor imaging was FA and tractography. Once magnetic resonance was completed, blood was collected and serum levels of neuron specific enolase (NSE) and soluble protein-100β (S-100β) were determined. Then animals were sacrificed and histopathologic examination was conducted. The spinal cord in the SCI model rats produced prominent tissue damages characterized by neuronal injury in the affected regions. An obvious decrease in FA happened 24 h after SCI, and at 72 h, FA tended to be stable. There were significant differences in the serum levels of NSE and S-100β between 6 h and 24 h, respectively. FA significantly related with the serum testing results at 24 h. FA may be used as a marker for different severities of SCI. The optimal time for examination is at 24 h post-injury in rat.

1. Introduction

Spinal cord injury (SCI) is a type of severe neurologic condition with motor and sensory deficits. It is one of the most devastating accidents. Some prominent features in acute SCI, such as hematoma and axonotmesis, have high relevance for clinical outcome. For example, cord atrophy often occurs a few months following the acute phase in severe SCI. Therefore, it is pivotal to make management decisions in the early stage of the injury. Because of the spinal shock or concomitant injuries shortly after SCI, especially within 72 h, both neurological examinations and electrophysiological measures are considered unreliable, which are unable to predict the outcomes (Brown et al., 1991; Little et al., 1999). The initial severity of SCI is the best predictor of long-term neurologic outcomes (Lammertse et al., 2007). For the clinical evaluation of SCI, imaging has been a critical means because intrinsic microstructural changes in vivo cannot be examined in invasive ways. The technique of magnetic resonance imaging (MRI) thus plays an essential role in assessing the state of SCI.

In the early stage following SCI, neurological disabilities may involve spinal shock, axonal damage, or both. However, conventional MRI is not capable of differentiating these pathological entities. The imaging observation also has poor correlation with neurological and functional impairments (Martin et al., 2015). In addition, While T2 was suitable to localize SCI, it cannot accurately quantify the injured tissues. For these reasons, conventional MRI techniques may not be sufficient to discriminate the lesion types in SCI and consequently not effective to assess the post-injury functional status. Exhilaratingly, magnetic resonance diffusion tensor imaging (DTI) does a better job in detecting microstructure changes in injured tissues, which can potentially reveal the association between specific imaging parameters and SCI severity. In other words, DTI provides additional information that is not obtainable with conventional MR sequences.

In the last decade, DTI has been widely used to investigate microstructure changes in SCI (Herrera et al., 2008; Kim et al., 2007, 2009, 2010; Loy et al., 2007; Wang et al., 2014). However, the underlying association between the DTI findings and the pathological results has not been adequately determined. Our previous research (Li et al., 2015) suggests that DTI is sensitive in characterizing the microstructural changes at the early stage of SCI, and fractional anisotropy (FA), one of the DTI parameters, is very sensitive in identifying various levels of injury severity. Based on these results, we assume that FA can be used to evaluate the severity of SCI at the early stage.

To assess the performance of FA in observing early SCI, we need to see to what degree the FA findings are consistent with the pathological results. However, to the best of our knowledge, there

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http://dx.doi.org/10.1016/j.brainres.2017.07.024
0006-8993/

is no research concerning the association between the two. The present study was thus aimed to investigate the relation between FA and pathology in the early stage of SCI.

2. Results

2.1. DTI and DTT

The values of FA were recorded at the set time-points. In all the groups, FA values decreased dramatically 6 h after SCI and reached the minimum at 24 h, and then tended to be stable (Table 1). The values at 24 and 72 h were significantly lower than those at 6 h ($p < 0.05$). There were significant differences at all the time-points between the control group and each of the injury groups as well as between different injury groups ($p < 0.05$). Within each injury group, there was no statistically significant difference between 24 h and 72 h. These results indicated that the FA values derived from DTI decreased after SCI, and the decrease was proportional to the severity of injury.

Fig. 1 shows Diffusion tensor tractography (DTT) of the spinal cord which demonstrates the fiber tracking of spinal cord tracts. The tracked fiber tracts in the control group were uninterrupted and well-organized. Whereas, the diffusion signal from injured tissues differed from healthy tissues. Six hours after injury, DTT at the zone of moderate or severe injury showed changes in the color of fiber tracts. At 24 and 72 h post injury, DTT demonstrated conspicuous changes in the color at the location of injury.

Table 1
Fractional anisotropy values at designed time points.

Group	Time Point (h)		
	6	24	72
Control	0.677 ± 0.035	0.677 ± 0.042	0.673 ± 0.042
Mild	0.557 ± 0.015	0.467 ± 0.025	0.467 ± 0.015
Moderate	0.480 ± 0.026	0.410 ± 0.010	0.380 ± 0.020
Severe	0.397 ± 0.021	0.303 ± 0.031	0.303 ± 0.015

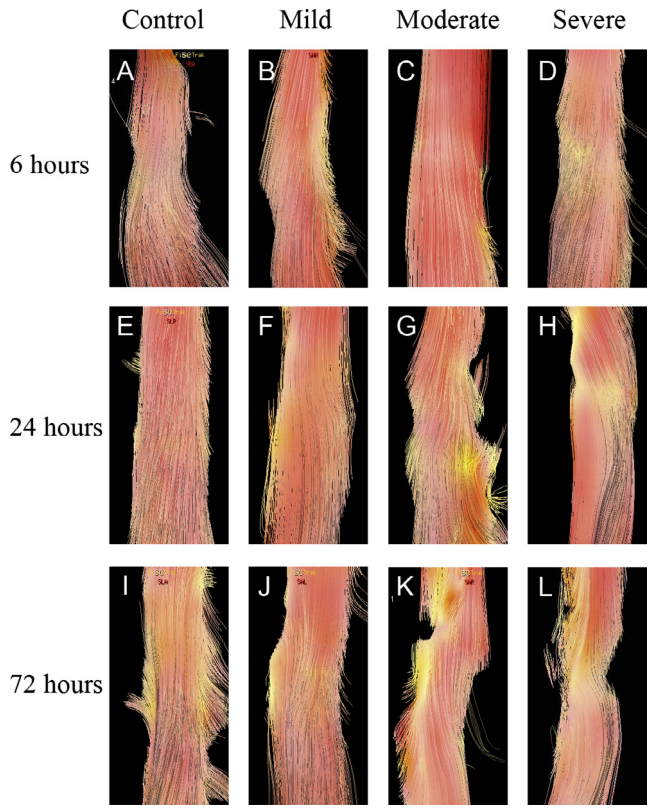

Fig. 1. Representative diffusion tensor tractography in spinal cord.

2.2. Serology

Significant increases in the serum levels of Neuron specific enolase (NSE) were observed at 6 h after SCI, as compared with the level in the control group ($p < 0.05$). The levels reached maximum at 24 h post injury, and statistically significant differences were demonstrable between 6 h and 24 h in all the injury groups ($p < 0.05$) (Table 2). At 24 h, the NSE levels in the moderate and severe SCI groups did not show notable difference ($p > 0.05$), but both were significantly higher than that in the mild SCI group ($p < 0.05$). The levels decreased with time and got lower at 72 h, but still remained elevated above the control level ($p < 0.05$).

Examination of soluble protein-100β (S-100β) elevation after SCI demonstrated considerable variations. No significant differences were observed among groups at 6 h after treatment ($p > 0.05$). Then, the S-100β levels in the injury groups were found increased significantly at 24 h as compared with that in the control group ($p < 0.05$) (Table 2), and decreased a little at 72 h but remained elevated above the control level ($p < 0.05$). At the time-points of 24 and 72 h, the S-100β levels differed significantly between the injury groups and the control group ($p < 0.05$), while no remarkable differences were demonstrated among the injury groups ($p > 0.05$).

2.3. H&E staining

The control animals revealed normal spinal cord morphology. There was no evidence of intramedullary pathology, which had a clear boundary between the gray matter and the white matter. In the gray matter, neurons were clearly defined and rich in Nissl bodies, nuclei were explicitly visible, and neural fibers were closely aligned.

In SCI animals, neurons swelled evidently at 6 h and focal hemorrhage was present. Nissl bodies in the severely injured animals were degenerated. At 24 h, hemorrhage became more severe and neurons degenerated remarkably. In the severe injury group, necrosis of neurons in the gray matter was significant. Axonal swelling could be seen, and some of the axons were disintegrated. In addition, a lot of holes were observed between the white and gray matter of the spinal cord. At 72 h, tissue structure was substantially disrupted in the severely injured animals. Most neurons were dead. Numerous neutrophil infiltrations were observed. The disruption of spared tissue was pronounced from the epicenter (Fig. 2). The histological changes that occurred after SCI were in concordance with the level of injury severity.

2.4. Immunohistochemistry

NSE. In the control animals, Brownish yellow staining distributed evenly within the cytoplasm of neurons in the spinal cord. Cellular boundary was clearly defined. In the injured animals, cytoplasm staining of neurons was weakened at 6 h post SCI. The boundary of the cells turned invisible at 24 h. In the severely injured group, NSE staining revealed that the spinal cord tissues were replaced by transparent, non-colored tissues (Fig. 3).

S-100β. Brownish yellow cells were seen as of a lower density and a scattering distribution in the control group. An increase in the cells was observed 6 h after SCI, and the morphology of cells became irregular at 24 h. In the severe injury group, the brownish yellow staining was also significantly increased (Fig. 4).