Diffusion Anisotropy MRI for Quantitative Assessment of Recovery in Injured Rat Spinal Cord

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Spinal cord injury and its devastating consequences are the subject of intensive research aimed at reversing or at least minimizing functional loss. Research efforts focus on either attenuating the post-injury spread of damage (secondary degeneration) or inducing some regeneration. In most of these studies, as well as in clinical situations, evaluation of the state of the injured spinal cord poses a serious difficulty. To address this problem, we carried out a diffusion-weighted MRI experiment and developed an objective routine for quantifying anisotropy in injured rat spinal cords. Rats were subjected to a conus injury of the spinal cord caused by a controlled weight drop. Untreated control rats were compared with rats treated with T cells specific to the central nervous system self-antigen myelin basic protein, a form of therapy recently shown to be neuroprotective. After the rats were killed their excised spinal cords were fixed in formalin and imaged by multislice spin echo MRI, using two orthogonal diffusion gradients. Apparent diffusion coefficient (ADC) values and anisotropy ratio (AI) maps were extracted on a pixel-by-pixel basis. The calculated sum of AI values (SAI) for each slice was defined as a parameter representing the total amount of anisotropy. The mean-AI and SAI values increased gradually with the distance from the site of the lesion. At the site itself, the mean-AI and SAI values were significantly higher in the spinal cords of the treated animals than in the controls (P = 0.047, P = 0.028, respectively). These values were consistent with the score of functional locomotion. The difference was also manifested in the AI maps, which revealed well-organized neural structure in the treated rats but not in the controls. The SAI values, AI histograms, and AI maps proved to be useful parameters for quantifying injury and recovery in an injured spinal cord. These results encourage the development of diffusion anisotropy MRI as a helpful approach for quantifying the extent of secondary degeneration and measuring recovery after spinal cord injury. Magn Reson Med 45:1–9, 2001. © 2001 Wiley-Liss, Inc.

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The long-term goal of this study is to develop a tool for noninvasive follow-up of the state of the spinal cord after injury. With the aid of such a tool, it should be possible to quantify the extent of the primary injury during the first critical hours, as well as the resulting later processes. These processes include secondary degeneration, which is expressed within a few days and up to a few weeks after injury, and a possible therapy-induced regeneration, which is assumed to appear much later. The experiment described here, in which the rat spinal cord was subjected to a “weight drop” injury and was imaged ex vivo, serves as a first stage towards this goal.

By modifying the usual method of analysis of diffusion-weighted magnetic resonance imaging (MRI) scans, we obtained a set of simple parameters that can document and quantify the state of the neural tissue. Using these parameters, we compared the final outcome of spinal cord injury in two groups of rats that differed in the treatment they received. This work summarizes the MRI experiment, data analysis procedure, and results. The biological basis for the therapeutic approach was recently published (1).

CONSEQUENCES OF INSULT IN THE MAMMALIAN SPINAL CORD

Injury to the mammalian central nervous system (CNS) usually results in an irreversible functional deficit (2). Two major factors contributing to this deficit. (a) The lack of effective regeneration, and (b) the inevitable secondary degeneration. Both have been ascribed mainly to the nature of the surrounding neural tissue rather than exclusively to the neurons (2). Lack of regeneration refers to the extremely limited neurogenesis (formation of new cell bodies) and regrowth of injured axons. Secondary degeneration refers to the destructive series of events that leads to a longitudinal and lateral spread of damage to neurons that initially escaped the direct injury (3–5) (Fig. 1). This latter process results in an additional functional deficit, i.e., a delayed loss of residual motor and sensory abilities several weeks after injury. The extent of the final functional recovery is thus spread of the functional motor deficit to muscles that are operated via ganglia located above the site, and is determined by the amount of undamaged tissue minus the loss due to secondary degeneration.

Attempts to promote CNS recovery have focused on two goals: 1) stimulation of regeneration (6–10), and 2) arrest of secondary degeneration, or neuroprotection (11–13). The present diffusion MRI analysis is part of a study in which induction of neuroprotective conditions was attempted through cell therapy involving systemic administration of autologous T cells specific to the CNS self-antigen, myelin basic protein (MBP) (1).
The rationale behind the use of anti-MBP T cells was the initial finding that the response of T cells to CNS injury is restricted relative to the T cell response to injuries in the peripheral nervous system (PNS) (13). Subsequent studies demonstrated that injection of anti-MBP T cells following a partial lesion of the optic nerve reduced the postinjury loss of neurons (11). The mechanism by which T cells induce neuroprotection is still not fully understood. One possible mechanism is an antigen-dependent production of neurotrophic factors by the T cells encountering their specific antigens at the site of lesion (12).

**PROBLEMS WITH ASSESSMENT OF RECOVERY IN THE SPINAL CORD**

A major problem, both in the medical diagnosis of spinal cord injuries and in studies aimed at inducing some recovery in the injured spinal cord, is the difficulty of evaluating the true state of the injured cord, its morphology and function, and the kinetics of its possible recovery (14). Various noninvasive spinal cord monitoring techniques are being used to uncover different aspects of this puzzle.

Most of the techniques for monitoring function, such as evaluation of locomotion (15,16) and electrophysiology (17,18), depend on an integrated activity beyond the integrity of the spinal cord. These techniques are masked by phenomena such as the animal’s general physiological state. In addition, they would be insensitive to any regeneration that might be induced, until synapses are formed by the regrowing axons, and function is regained. For evaluation of morphological changes in the spinal cord, $T_2$-weighted spin-echo MRI is probably the most sensitive tool currently available (19–22). $T_2$-weighted spin-echo scanning can differentiate between white and gray matter. However, after spinal cord injuries, it is used mainly to determine the integrity of the cord and the existence and location of bone fractures, edema, hemorrhage, and other injury-related structures on a 0.1-mm scale. The achievable spatial resolution and the enhanced signal intensities associated with spinal cord injuries make it difficult to differentiate white matter from other tissues under these conditions. Thus, the imaging of white matter-related processes such as recovery or degeneration is difficult (19,21,22).

A general problem in quantifying spinal cord function and morphology is the limited resolution for assessment of the contribution of cell- or fiber-associated structures. Thus, despite the availability of multiple monitoring tools, there are still wide gaps in our ability to track the state of the tissue. Diffusion-weighted MRI may provide a partial solution to this problem. The uniqueness of the diffusion-weighted MRI technique lies in its sensitivity to motion (of water molecules) on the scale of microns. Because of this feature, the acquired signal can reflect structures at the cellular level and is therefore relatively well equipped to assess cellular morphology in the spinal cord.

**DIFFUSION-WEIGHTED MRI FOR MONITORING OF SPINAL CORD INJURIES**

The use of diffusion anisotropy MRI for the assessment of neuronal damage is based on the finding that the amount of anisotropy is diminished following a lesion or other damage to the nerve fibers. Using this technique, it was possible to detect neuronal damage that could not be detected by $T_2$-weighted MRI after events such as brain injury, ischemia, and demyelination (23–25). Several studies of the injured spinal cord (26–30) have already characterized the changes in apparent diffusion coefficients (ADCs) as a function of the degree of injury. A reduction in spinal cord AI reflects both a decrease in the ADC values in the longitudinal direction (ADC$_L$) and an increase in the ADC values in the transverse direction (ADC$_T$). Multi-exponential analysis was applied (31) to study the continuous distribution of diffusion coefficients in a multi-coefficient model. The diffusion coefficients were found to be separated into two major components, related to intracellular (slow) and extracellular (fast) regimes, in which the slow regime was found to be anisotropic. AI in both regimes was affected by injury.

Diffusion tensor imaging of the rat spinal cord has been previously described. Inglis et al. (32) characterized the anisotropy by trace images for a pseudo-3D view of the rat spinal cord. Clark (33) showed that for a cylindrically symmetric object oriented in the MRI gradient axes (such as a spinal cord straightened ex vivo), it is possible to estimate the principal diffusivities by using a pair of diffusion-weighted scans (instead of the $N > 6$ scans in the general case).

Here we show that measurements and analysis of diffusion anisotropy can yield simple and scalable parameters that document the degenerative processes and the induced recovery. The experiments in this study were conducted in the injured spinal cords of rats, with and without neuroprotective treatment. The MRI scans were performed ex vivo.

**FIG. 1. Schematic presentation of the progression of secondary degeneration after spinal cord injury. (i) Degeneration of neuronal elements associated with the initial damage (a). (ii-iii) Pre-synaptic and post-synaptic longitudinal degeneration of elements (b-c). (iv) Lateral degeneration of neurons that escaped the primary lesion (d).**
Within an hour of the contusion, the rats were randomly housed in a light- and temperature-controlled room and matched for age.

Injury and Treatment

Rats were anesthetized and their spinal cords were exposed by laminectomy at the level of T7–T8. One hour after induction of anesthesia, a 10-g rod was dropped onto the laminectomized cord from a height of 50 mm. The rod’s diameter was 2 mm. The NYU impactor was used to ensure a well-controlled injury. The degree of impact was monitored through measurements of the rod trajectory parameters (height, time, and velocity). As differences in the force of impact within this experiment varied in most cases by much less than 5%, the initial compression of the force of impact varied in most parameters (height, time, and velocity). As differences in the force of impact within this experiment varied in most cases by much less than 5%, the initial compression of the cord was virtually identical in all animals. (The reproducibility of the contusion is manifested by the low variations in the functional outcome among injured-untreated rats).

Animals

Inbred female adult Lewis rats (24 rats, 10–12 weeks old, 180–220 g) were supplied by the Animal Breeding Center of the Weizmann Institute of Science. The rats were housed in a light- and temperature-controlled room and matched for age.

Assessment of Recovery

Starting on the first day after the injury and thereafter approximately twice a week, the locomotor activities of the trunk, tail, and hind limbs were evaluated in an open field by observers blinded to the rats’ identity. Each rat was placed for 4 min in the middle of a circular enclosure made of molded plastic with a smooth, non-slip floor (90 cm diameter, 7 cm wall height). Behavioral recovery was scored on a scale of 0 (complete paralysis) to 21 (complete mobility) (15,16).

Diffusion Anisotropy MRI

After a follow-up period of more than 3 months, when the locomotor activity scores had reached a plateau, the structural integrity of the sites of injury of nine rats was analyzed by diffusion-anisotropy MRI. The rats were killed and their excised spinal cords were cut ~2 cm below the assumed site of lesion, immediately fixed in formalin, and inserted into an NMR tube (inner diameter 4.2 mm). Special care was taken to perform the excision in a reproducible way. Morphological examinations of other fixed spinal cords verified that the overall structure of the cord was maintained after excision, and that any changes observed in the spinal cord were therefore a result of the contusion.

Diffusion anisotropy was measured in a Bruker DMX 400 widebore spectrometer, using a microscopy probe with a 5-mm Helmholtz coil and actively shielded magnetic field gradients. A multislice pulsed gradient spin echo experiment was performed with nine axial slices, with the central (fifth) slice positioned at the assumed center of the site of injury. Images were acquired with a TE of 31 msec, TR 2000 msec, diffusion time (Δ) 15 msec, diffusion gradient duration (b) 3 msec, FOV 0.6 cm, matrix size 128 × 128, slice thickness 0.5 mm, and slice separation 1.18 mm. Four diffusion gradient (Gd) values (0, 28, 49, and 74 G/cm) were applied along the “read” direction (transverse diffusion) and along the “slice” direction (longitudinal diffusion). The frequency encoding gradient (Gf) was 9.78 G/cm and was operated for a period of 2.85 msec (β). The slice selection gradient (Gs) was 16 G/cm and was operated for 4.54 msec (2α). The diffusion sensitivity values (b-values) (34) were calculated, including the cross terms of the diffusion and imaging gradients (35):

\[
b = \gamma^2 \cdot (b_{\text{diffusion}} + b_{\text{cross term}})
\]

\[
b_{\text{diffusion}} = G_d^2 \cdot \delta^2 \cdot (\Delta - \delta/3)
\]

\[
b_{\text{cross term,read}} = 2 \cdot G_d \cdot G_f \cdot \beta \cdot \delta \cdot \Delta
\]

\[
b_{\text{cross term,slice}} = 2 \cdot G_d \cdot G_s \cdot \alpha^2 \cdot \delta
\]

where γ refers to the gyromagnetic ratio. To extract and analyze the AI, a Matlab-based routine was developed. The only non-automated task was the definition of an area of free water (formalin solution) in each tube, to allow precise calibration of the ADC, and ADC maps for calculation of diffusion anisotropy.

Diffusion Anisotropy Analysis

For analysis of pulsed gradient diffusion-weighted data, the use of high b-value, low signal-to-noise data should be optimized, while at the same time the error in the resulting ADCs should be minimized (36). In the interests of quantitation, a basic guideline of our pixel-by-pixel analysis was to minimize any possible false contribution of ADC and AI values, with minimal loss of useful information.

For the analysis (see Fig. 2), multislice data were acquired using four b-values and two orthogonal diffusion gradient directions (Fig. 2a). Noise masks were derived for each slice and each b-value (Fig. 2b). The corresponding ADC maps (ADC, and ADC, ) were derived (Fig. 2c). Using the ADC histograms for water and tissue (Fig. 2d), the calculated ADC values were normalized (Fig. 2e) and free-water ADCs were eliminated. Finally, the maps were combined to yield an AI map (Fig. 2f). All corrections were performed automatically to minimize the effects of noise and other variable experimental conditions on the final results. Details of the analysis are described below.

Noise Rejection

A region of interest (ROI) outside the tube was manually defined and its noise histogram was characterized as an
average $I_{\text{noise}} = \sigma$. This noise histogram was used for the following operations:

1) A tube border’s mask (Fig. 2b, left) was defined by the least diffusion-weighted image ($b_1 = 0$) of the middle (fifth) slice. We defined this mask by a threshold value of $I_{\text{noise}} + 3\sigma$ on the pixels in this slice. The mask was then applied to all slices.

2) Pixels within the tube’s area with intensities lower than a fixed threshold of $2\sigma$ were eliminated (Fig. 2b, right). The higher the diffusion weighting ($b$-value) in the DWI, the greater the elimination of pixel values. The application of this mask removed up to 68% of the pixels for the DWIs with the highest $b$-value (Fig. 2b, left). Since at high $b$-values the amplitudes of the free-water pixels were smallest (due to its relatively high ADC), the pixels removed by the mask were mainly in the area occupied by free water.

### Extraction of ADCs

The four diffusion gradients applied in each direction were used to derive the ADC for each pixel by a two-parameter non-linear fit (Nelder-Mead optimization) to a mono-exponential function,

$$S(b) = S_0 \cdot e^{-b \cdot \text{ADC}}. \quad [2]$$

where $S(b)$ refers to a pixel’s diffusion-weighted intensity for a given $b$-value, and $S_0$ is the pixel’s intensity with no diffusion gradient applied. This operation yielded two ADC maps (Fig. 2c): a diffusion map parallel to the cord’s longitudinal axis ($\text{ADC}_i$) and a transverse diffusion map ($\text{ADC}_t$).

In cases where the use of the mask had nullified data in more than two $S(b)$ values of a pixel, the ADC of this specific pixel was not calculated. If two $S(b)$ values were nullified, only three values (where the last one is 0) were used to calculate the exponential fit. Goodness of fit was indicated by the ADC values obtained for the tube’s free water. The difference between the $\text{ADC}_i$ and $\text{ADC}_t$ for water was $\sim 4\%$ on average, and never exceeded $7\%$. Note that the removed pixels correspond to both water and damaged tissue.

### Normalization and Removal of Free-Water ADC

#### Normalization

The histogram of ADC values in the images was bimodal, corresponding to the high diffusion rate of free water and the low diffusion rate of tissue. By defining an ROI in the area of the tube’s free diffusing solution (done manually by comparing intensities), the histogram of ADCs was calculated (Fig. 2d), smoothed, and the common value was
defined as the solution ADC (ADCsolution). A similar calculation over all pixels yielded an ADC for the tissue (Fig. 1d). The ADCsolution was used to normalize all ADC values by:

$$ADC(i, j)_{\text{normalized}} = ADC(i, j) \cdot \frac{D_{\text{solution}}(13^\circ\text{C})}{ADC_{\text{solution}}}$$

where $D_{\text{solution}}$, the diffusion coefficient at $13^\circ\text{C}$, is the normalization factor. This normalization compensated for nonphysiological ADC differences between the scans, which could result from differences in temperature as well as from possible errors in calculation of b-values and ADCs.

**Removal of Free-Water ADCs**

The minimum value of the ADC histogram between tissue ADCs and solution ADCs was defined as a threshold. ADC values higher than this threshold, which correspond to free water, were ignored.

The normalization protocol and the removal of free-water ADCs prevented the contribution of any residual false difference in the water ADC to the calculation of total anisotropy. Such a contribution, although small for each pixel, could accumulate to produce a significant error in the calculation of SAI. Differences obtained in the values of ADCsolution in subsequent scans confirmed that these processes neutralize the effect of residual differences.

**Estimation of the Degree of Anisotropy**

Using the two ADC maps corresponding to the longitudinal (“slice”) and transverse (“read”) diffusion scans, an AI map was calculated according to:

$$AI = \frac{ADC_i - ADC_c}{ADC_i + ADC_c}$$

In this AI map, the walls of the tube were found to create a false AI contour superimposed on the cord AI. Since this could affect the calculation of SAI, the pixels representing the tube walls were removed using a fitted ring mask. Positive AI values correspond to an increased ADC along the primary cord axis while negative values correspond to preferential axial diffusion. The total AI in a slice was defined by three parameters:

- The average of the AI values in the slice (Mean-AI ± SE).
- The area occupied by pixels showing anisotropy (Area).
- The sum of AI values over all pixels in a slice (SAI).

**Statistical Analysis**

In each rat, the slice with the lowest SAI was defined as the site of lesion. This slice may contain the true site of the initial impact, or it may reflect a secondary effect (e.g., cystic degeneration) that reduces the SAI in this area to minimum. Averages of the three derived parameter values, at the assumed site of the lesion, were compared by a two-tailed Student’s t-test for evaluation of the differences among groups. Normal distribution of the compared parameters was assumed since they are all defined using a sum of multiple pixels.

In the derivation of average values of all the parameters, and in the depicted graphs, slices were positioned by the lowest SAI. The derived AI matrices were displayed as $128 \times 128$ AI maps, using a fixed color code scaled over the practical range of AI values $[-0.4, 1]$.

**RESULTS**

Three AI maps that best demonstrate the differences between the spinal cords of an injured-treated, injured-untreated, and a naive rat are shown in Fig. 3. Bright colors mark the pixels showing higher values of AI. The two nine-slice maps of the injured spinal cords show that closer the slice to the site of injury, the smaller the area occupied by anisotropic tissue, and apparently the lower the AI values. A comparison of these two maps suggests that the area occupied by anisotropic tissue at the site of injury and around it, is smaller in the spinal cords of untreated rats. In addition, the anisotropic tissue at that site is less organized, less symmetrical, and its borders are less defined than in the spinal cords of treated rats. In all the slices of injured spinal cord, from both treated and untreated rats, the AI values are much lower than those of the naive rat.

Figure 4 depicts the AI values of an averaged histogram of the three central slices, normalized on the basis of the total number of values in each slice. The averaged AI histogram of the treated group (solid line) and that of the untreated group (dashed line) are shown. In all slices, the histograms are Gaussian and are narrower (and thus reach higher counts) for the untreated group than for the treated group (and, obviously, than for the naive rat). In addition, in the untreated group, there is a clear increase in the number of negative AI values, reflecting a growing number of pixels with a preference for diffusion in the transverse direction.

The narrowing effect and the decrease in AI values is clearly shown in Fig. 5a, where the averaged mean-AI values are depicted as a function of the slice number. Toward the site of the lesion the average gradually declines, with an increasing difference in its value between the two injured groups. As expected, the difference in the averages at the central slices is extremely high, reflecting the effect of the growing number of negative AI values ($P = 0.047$, two-tailed $t$-test) mentioned above. The average AI-area values as a function of the slice number (Fig. 5b) is also U-shaped, reflecting the gradual decrease in anisotropy near the site of the lesion. However, this parameter did not differ between the two injured groups.

As to the SAI, analysis showed that the two groups differed significantly at the site of the lesion with respect to the SAI values (Fig. 6a, $P = 0.028$, two-tailed Student’s $t$-test). Like the other parameters, the SAI value declines approaching the site of lesion (Fig. 5c), and differs between the two groups, being higher in the treated group.
The values of the mean-AI, AI-area & SAI, expressed as percentages of the corresponding values in the naive rat, of the two furthest slices (top and bottom) were lower in the spinal cords of untreated (66%, 66%, and 45%) than in treated rats (80%, 66%, and 52%). These ratios differed significantly for the mean-AI ($P < 0.04$, one-tailed $t$-test) between the two injured groups. Differences between the naive cord and both injured groups were statistically significant for all the parameters ($P < 0.001$). These results suggest that the injury affects even areas which are far from the lesion site, and that the treatment was apparently beneficial throughout the spinal cord. In addition to the differences in values of the AI parameters, the SAI values are shown to be consistent with the locomotor function scores (Fig. 6b) (a relation that is not necessarily treatment-dependent). There is no reason to assume a linear correlation (or other parametric relationship) between the SAI values and the locomotor scores. The locomotion test

![Treated AI maps](image1)

![Control AI maps](image2)

![Naive AI maps](image3)

**FIG. 3.** Representative AI maps of injured rat spinal cords treated with anti-MBP T cells (top), PBS (middle), and a naive rat’s spinal cord (bottom). The assumed site of lesion is located at the fifth slice. Left-to-right corresponds to top-to-bottom spinal cord slices. Slice thickness = 0.5 mm; distance between slices = 1.18 mm. For convenience, color maps were scaled over the range [-0.4,1] and nullified pixels were set to minimum value.

**FIG. 4.** AI histogram along the cord axis, averaged for each group. Histograms were calculated using a 50 bins count, smoothed using an averaging window (5 values), and normalized by the total AI pixel count (i.e., AI area). The three depicted slices correspond to the site of lesion (central slice) and the slice on either side of it. The solid line corresponds to the treated group; dashed line = injured-untreated; and dotted line = naive.
grades an ensemble of behavioral parameters that are step-wise and are not equally spaced with reference to the recovery process. However, the relationship between the SAI values and the locomotor scores is important to validate that the reported differences in AI values are related to the amount of preserved neural tissue as reflected by the MRI measurements, rather than an artifact of the excision or processing.

The injury experiment included 24 rats, of which nine were used for MRI. The high statistical significance of the results of the MRI analysis allowed us to use other rats for various other tests and measurements, such as staining of fibers, astrocytes, and red nuclei.

DISCUSSION

The results of this study demonstrate that it is possible to develop an objective analysis of diffusion anisotropy MRI as a tool for quantitative assessment of the final outcome of spinal cord degeneration and possible neuroprotective therapy.

The contusion of the spinal cord in this study led to an apparent transient complete paralysis, which was followed by a spontaneous steady improvement in locomotor ability of all rats. However, differences were observed in the locomotor abilities of the treated and untreated groups, starting in the third week after the injury. These differences are likely to be the result of differences in the extent of degeneration, i.e., neuroprotection achieved by the treatment with the autoimmune T cells. In other experiments, using a rat model of complete transection of the spinal cord, we are currently examining the promotion of regeneration. Differences in locomotor function in those experiments are observed not earlier than in the eighth week after injury. Thus, differences in locomotion observed in the present study could have arisen only from differences in the degree of neuroprotection.

Changes in the AI histograms were observed after injury (Fig. 4). A number of reasons have been suggested for such changes (26,27), including the primary mechanical disruption and tearing of fibers and myelin sheaths that interfere with diffusion along the pre-injury anisotropic structure. There are also injury-related processes such as axonal swelling, demyelination, loss of spatial organization of the white matter in reference to the longitudinal (z) axis, Wallerian degeneration, and processes related to the tissue matrix, including edema, hemorrhage, liquefication of tis-

FIG. 5. The three AI parameters values along the cord, around the site of injury. The x-axis corresponds to the slice number. Spinal cord slices are marked 1–9 from top to bottom, where the fifth slice refers to the assumed site of the lesion. The y-axis corresponds to the mean ± SEM of each parameter. a: Averaged mean-AI value; b: averaged AI area; c: averaged SAI.

FIG. 6. Comparison of the SAI values at the site of lesion of the two injury groups. a: Bar plot depicting the significance of the difference between the two groups ($P = 0.028$). b: The relation between the open field locomotor scores (with values ranging from 0 to 21) and the SAI values at the site of lesion.
sue, and cystic degeneration. These processes result in a secondary decrease in the AI parameters values following the injury.

We observed significant differences between treated and untreated rats in the averaged SAI values and in the mean-AI histograms at the site of injury (Figs. 4 and 6). These differences point to an apparent reduction of lateral degeneration in the autoimmune T cell-treated group relative to the untreated group. In other words, it seems that neurons in the treated rats (but not in the untreated group) were essentially protected from secondary degeneration. The overall change in the mean-AI and SAI values reflects two morphological differences related to the results of T cell treatment: enhanced survival of anisotropic myelinated fibers, and—possibly as a by-product of the decreased degeneration—decreased formation of isotropic cysts.

Confirmation of the validity of this quantification procedure and its parameters (SAI and mean-AI values) comes from the gradual decrease in the averaged SAI and mean-AI values observed near the site of the lesion. These gradual longitudinal changes correspond to both retrograde and anterograde degeneration, which occur in both ascending and descending sensory and motor fibers (Fig. 5). The longitudinal changes differ between the treated and untreated groups, the difference being greatest at the central area.

Thus, our method of analysis allows us to demonstrate that the neuroprotective effect is expressed not only in the sparing of uninjured axons (i.e., lateral degeneration, Fig. 1iv) but also in slowing down of the degeneration of injured axons (i.e., longitudinal degeneration Fig. 1–iii). In addition, the total extent of the degeneration in both injured groups (treated and untreated) can be evaluated by the comparison to the naïve cord.

From our data, it appears that the AI-area is not a valid parameter for quantification of the degree of degeneration or rescue of fibers. Primarily, the AI-area is a binary accumulation of anisotropic pixels, and thus it cannot reflect partial volume effects that result in differences in the pixels’ AI values. Secondly, possible physiological effects such as shrinkage and condensation of the SC bias the AI-area value, with no correlation to the survival of anisotropic tissue.

We have recently adapted this procedure, as mentioned above, for analysis of the completely transected spinal cord treated by stimulated homologous macrophages to induce regeneration (10). The derived SAI, mean-AI, and AI maps confirmed the validity of the method. Moreover, it seems that this method allows us to distinguish between an organized spared tissue and a regenerating tissue. These results will be published separately (Lazarov-Spiegler et al., unpublished results).

We have not yet fully exploited the data contained in the images acquired in this study. Additional image processing techniques can be applied to measure degrees of symmetry, characteristics of edges, and similarity of the contracted AI map of the injured rat to that of healthy white matter. Such an analysis could add parameters that would quantify additional aspects of the degeneration or of possible neuroprotection. We also hope to extend this work by calculating the AI and SAI values in small ROIs in particular spinal cord tracts and evaluating their specific degeneration.

In view of the variety of pharmacological and operative techniques being developed for remediation of spinal cord injuries, it becomes even more urgent to solve the quantification problem so that these treatments can be optimized. Diffusion MRI experiments have already been performed on the injured spinal cord in vivo (30, 37–40), and they highlight the need to address some additional technical considerations: Care should be taken to choose an optimal pulse sequence and b-values to allow an optimal signal-to-noise ratio (SNR) within a reasonably short imaging session. The problem of motion artifacts should be addressed, using anesthetics, restraining movements, ECG, and respiration gating, and by applying a navigated pulsed-gradient technique. In addition, we have used the tube to straighten the spinal cord, thereby aligning it along a single axis: the slice-selection imaging axis (i.e., we aligned the diffusion–ellipsoid principal frame with the laboratory frame). In so doing, we circumvented the need to calculate the tensor of anisotropy. In vivo experiments have been reported in which either the operated field gradients were aligned with specific spinal cord segments, or multiple scans were performed to compute the diffusion tensor. Diffusion-weighted experiments in spinal cords making use of these techniques have already been reported. These experiments, as well as the present study, demonstrate the potential usefulness of automated analysis of diffusion anisotropy MRI in quantifying spinal cord injury.

CONCLUSIONS

This work describes the use of diffusion anisotropy MRI for the quantification of processes that occur after spinal cord injury. The post-traumatic longitudinal and lateral degeneration of white matter were both documented through the use of simple parameters related to the anisotropy of the tissue. The technique enabled us to differentiate between treated and untreated rats in terms of the outcome of injury. The results suggest that MRI can serve as a tool for evaluation of the extent of axonal loss within the white matter after spinal cord trauma.

The implications of the therapeutic approach used in this study are beyond the scope of this work. Briefly, T cells specific to MBP were found to have a positive effect on the final morphological and functional outcome of spinal cord injury in rats. The effect has even wider implications for the general role of autoimmunity, as it suggests that autoimmunity is a purposeful physiological response, which becomes harmful and causes autoimmune disease only when it gets out of control (12).

We plan to perform an in vivo experiment with slices taken much further from the site of the lesion, and at different times after the injury. We believe that the technique described in this study can yield unique parameters allowing quantification of recovery for both experimental and clinical use. As a by-product, this should allow us to gain a deeper insight into the course of degenerative processes in the injured spinal cord.

REFERENCES

Diffusion Anisotropy MRI of CNS Injury