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Hypertonic saline attenuates cord swelling and edema in experimental spinal cord injury: A study utilizing magnetic resonance imaging

Yvette S. Nout, DVM PhD DACVIM DACVECC, Georgeta Mihai, PhD, C. Amy Tovar, BA, Petra Schmalbrock, PhD, Jacqueline C. Bresnahan, PhD, and Michael S. Beattie, PhD

Brain and Spinal Injury Center, Department of Neurological Surgery, University of California, San Francisco, San Francisco, California (YN, JB, MB); Heart & Lung Research Institute (GM), Department of Medical Virology, Immunology and Medical Genetics (AT), and Department of Radiology (PS), The Ohio State University, Columbus, Ohio

Abstract

Objective—To use magnetic resonance imaging (MRI) to characterize secondary injury immediately after spinal cord injury (SCI), and to show the effect of hypertonic saline (HS) on MRI indices of swelling, edema, and hemorrhage within the cord.

Design—A prospective, randomized, placebo-controlled study.

Setting—Research laboratory.

Subjects—Twelve adult Long-Evans female rats.

Interventions—Rats underwent a unilateral 12.5mm SCI at vertebral level C5. Animals were administered 0.9% NaCl (n=6) or 5% NaCl (n=6) at 1.4ml/kg intravenously (IV) every hour starting 30min after SCI. Immediately after SCI, rats were placed in a 4.7T Bruker MRI system and images were obtained continuously for 8hrs using a home-built transmitter/ receiver 3cm Helmholtz coil. Rats were sacrificed 8hrs after SCI.

Measurements and Main Results—Quantification of cord swelling and volumes of hypointense and hyperintense signal within the lesion were determined from MRI. At 36 minutes after SCI, significant swelling of the spinal cord at the lesion center and extending rostrally and caudally was demonstrated by MRI. Also, at this time point, a hypointense core was identified on T1, PD, and T2 weighted images. Over time this hypointense core reduced in size and in some animals was no longer visible by 8hrs after SCI, although histopathology demonstrated presence of red blood cells. A prominent ring of T2 weighted image hyperintensity, characteristic of edema, surrounded the hypointense core. At the lesion center, this rim of edema occupied the entire unilateral injured cord and in all animals extended to the contralateral side. Administration of HS resulted in increased serum [Na], attenuation of cord swelling, and decreased volume of hypointense core and edema at the last time points.

Conclusions—We were able to use MRI to detect rapid and acute changes in the evolution of tissue pathophysiology, and show potentially beneficial effects of HS in acute cervical SCI.

Contact Author: Michael S. Beattie, UCSF - Neurosurgery, 1001 Potrero Ave Rm, 101 San Francisco California 94110, United States, T: 415-206-9753, F: 415-206-3948, michael.beattie@ucsf.edu.

Work performed at: Brain and Spinal Injury Center, Department of Neurological Surgery, University of California, San Francisco, San Francisco, California and Department of Neuroscience and Department of Radiology, The Ohio State University, Columbus, Ohio.

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Keywords

nervous system trauma; diagnostic imaging; hypertonic solutions; critical care; sodium

Introduction

Spinal cord injury (SCI) is a debilitating and costly condition. In human SCI, injuries to the cervical region of the spinal cord are the most common (52.4%), and estimated lifetime costs reach USD 1.7-3.1 million (1). We recently studied a rodent model of cervical SCI using magnetic resonance imaging (MRI) to monitor and quantify lesion development over a 3 week time course (2,3). From this work we hypothesized that MRI would also be a valuable tool for assessment of early secondary injury events in the acute phase of SCI and that MRI would provide information that could be used for therapeutic intervention assessment as has been suggested in human SCI (4). The current investigation demonstrates the effects of hypertonic saline (HS) on lesion development depicted by MRI during the acute phase of unilateral cervical SCI.

HS has been investigated for resuscitation in traumatic shock and for reducing intracranial pressure (ICP) to treat cerebral edema after traumatic brain injury (TBI) (5-12). HS mobilizes free water from the intracellular into the extracellular space by osmotic force and reduction of peripheral vascular resistance. In shock, the result is a rapid improvement of arterial pressure and cardiac output. In cerebral edema, HS lowers ICP by establishing an osmotic gradient between the intracellular and intravascular space. In addition, improved cerebral blood flow and increased delivery of oxygen (DO_2) cause a compensatory vasoconstriction and a reduction in cerebral blood volume which further lowers ICP (6). There have been few investigations into the use of HS in SCI, and although most studies report positive effects of HS on behavioral and histopathological outcomes, this has not led to widespread use of HS in human or experimental SCI (13-16). HS appears a promising addition to a combinatorial treatment strategy in SCI, and further research is required to examine its potential beneficial role in SCI.

We recently showed that MRI is a valuable imaging modality to assess temporal evolution of SCI and to distinguish different severities of cervical SCI in rats (2). In that study, quantification of cord swelling, hypointense and hyperintense signal, and lesion length from MR images appeared to be the most valuable parameters to determine since these parameters were highly correlated to locomotor function outcomes and histopathological characteristics of the lesion. Indeed, studies using MRI in human SCI also demonstrate that hemorrhage and cord swelling are significantly correlated with functional outcome (4,17). The aims of the present study were to use MRI to describe the events that occur within the first 8 hours after unilateral cervical SCI and to determine whether administration of HS would reduce spinal cord swelling and/or hemorrhage and edema using our previously developed quantification techniques.

Materials and Methods

Surgical Procedures

Twelve adult, female Long-Evans hooded rats (Simonsen Laboratories, Gilroy, CA, USA) age 84 days (range 83-86) and weighing 229 ± 4 g were used in this study. Rats were housed individually in plastic cages, maintained on a 12 hour light/dark cycle, and had access to food and water ad libitum. All animal experiments were conducted after approval by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University and were performed in compliance with NIH guidelines and recommendations.

Surgical procedures were carried out aseptically under deep anesthesia induced and maintained by inhalation of isoflurane (IsoFlow, Abbott Laboratories, North Chicago, IL, USA; 2-3%). Anesthetic plane was determined by foot pinch. Lacrilube ophthalmic ointment (Allergan Pharmaceuticals, Irvine, CA, USA) was applied to the eyes prior to surgery and body temperature was monitored using a rectal thermal probe and maintained at $37.5 \pm 0.5^{\circ}\text{C}$ using a heating pad.

A long catheter (PE-50-polyethylene tubing, Clay Adams, Division of Becton-Dickinson Co., Parsippany, NJ) was placed in the right jugular vein and kept in place with 2 sutures. A dorsal midline incision was made and a dorsal laminectomy at C5 was performed to expose the entire right side and most of the left side of the spinal cord. A right-sided contusion injury was produced using a MASCIS/NYU injury device with a modified 2.0mm diameter impounder rod as previously described (3,18). The rod was centered over the right side of the spinal cord so that the medial curve was aligned with the midline tangent. The spinal cord, with the dura mater intact, was impacted with the 10g rod from a height of 12.5 mm. After injury, the muscle layers were closed with 2 sutures and animals were immediately transferred and positioned in the magnet for imaging under continuous inhalant anesthesia with Isoflurane (maintenance 1 – 2%).

MRI Procedure and Treatment

A Bruker 4.7 Tesla/40cm horizontal bore MRI System, with a 400mT/m, 120mm inner diameter gradient insert was used with a laboratory-built 3cm diameter Helmholtz coil for transmitting and receiving the MRI signal. Rats were placed in prone position (ventral recumbency) on a laboratory-built Plexiglas holder that also incorporated the matching and tuning electronic circuitry of the radiofrequency (RF) coil. The cervical region of the animal was placed between and parallel to the two loops of the RF coil, and fixed in place with masking tape. Exterior landmarks (occipital bone and shoulder blades) were used to ensure C5 was placed at the center of the coil. Animals received serial boluses of either NaCl 0.9% (normal saline, control; n=6) or NaCl 5% (hypertonic saline, HS; n=6) at 1.4 ml/kg intravenously (IV) every hour starting 30 min following SCI. The IV catheter was positioned so that administration was possible from outside the MRI system without the need to move the animal. Anesthesia was maintained in all animals with inhaled Isoflurane at 1–2%. Body temperature was maintained at $33 - 37^{\circ}\text{C}$ using a warm water blanket (TP12; Parkland Scientific, Coral Springs, FL) connected to a circulator. Respiratory rate was monitored continuously using the monitoring & gating system for small animals (Model 1024; S.A. Instruments Inc., Stony Brook, NY).

Injury location (C5) was carefully positioned at the isocenter of both the RF antenna and within the magnet. Tuning and matching of the Helmholtz coil was performed for each animal, prior to and after insertion into the magnet. Acquisition of a three plane localizer for identifying the vertebral column was followed by a higher resolution 2.2 minute sagittal gradient echo, T1 weighted localizer (TR/TE=500/4.5ms; flip angle=90) that allowed depiction of vertebral bodies which were used as anatomical landmarks. Axial MRI studies covered a 16.4mm region of the spinal cord, starting just rostral to the second thoracic spinous process, and extending to the third cervical vertebra.

Consecutive series of axial T1, T2 and PD weighted images were acquired each hour for 8 hours. T1 weighted (Gradient Echo: TR/TE/flip angle=500/4.9ms/90°, 5 averages) and proton density weighted (PD; Spin Echo: TR/TE=2000/15ms, 2 averages) images were acquired with $175 \times 175 \mu\text{m}$ in-plane resolution and 1mm slice thickness with a 0.1mm gap between slices. The 3D T2 images (Spin Echo, TR/TE/RARE factor= 1629.2/59.7ms/16, 2 averages) were acquired with $179 \times 175 \times 625 \mu\text{m}$ resolution.

Sacrifice

At 8 hours after SCI, animals were anesthetized with xylazine (TranquiVed™, Vedco Inc., St. Joseph, MO, USA; 10 mg/kg IP) and ketamine (ketamine HCl, Abbott Laboratories, N.Chicago, IL, USA; 80 mg/kg IP) and transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde in phosphate buffered saline. Prior to perfusion, blood was collected from the heart for determination of serum [Na].

Data Analysis

Axial MR images were used to quantify the evolution of the pathology in the two SCI groups. Axial images were reconstructed from the raw data, magnified 4 times, and cropped to retain just the vertebral column using IDL (Research System Inc., Boulder, CO, USA). Data were analyzed blind to treatment condition. Seven consecutive T1 and PD weighted images around the lesion center were overlaid and regions of interest were manually traced using MetaMorph software (vs.6.3; Molecular Devices Corporation, Downingtown, PA, USA). This combination of images provided the most detail to clearly determine areas of the whole cord and areas of hypointense (commonly thought to reflect hemorrhage; (19) and hyperintense (edema) signal within the lesioned cord. Pixel counts were converted into area units (mm^2) by scaling with the in-plane pixel size. Volume measurements (mm^3) were obtained by adding the individual slice areas and multiplying by 1.1mm slice plus gap thickness.

Images from axial 3D T2 weighted MR images were used to assess and compare the evolution of hyperintense signal using MetaMorph. The area of the lesioned spinal cord containing hyperintense signal was first manually traced by a blinded observer. Subsequently the area of hyperintense pixels was determined by implementing a threshold for the pixel intensities based on visual spread of hyperintensity within this traced region. Areas were multiplied by the slice thickness, 0.625mm, to obtain the cord volume containing hyperintense pixels.

Histopathology

Immediately after sacrifice (8 hours after SCI), the injured region of the cervical spinal cord was dissected, post-fixed in 4% paraformaldehyde for <48 hours, cryoprotected in 30% sucrose in PBS for 48-72 hours, and then frozen at -80°C until sectioning. The lesioned region (14mm) was sectioned transversely at 20 μm on a cryostat and sections were stained with cresyl echt violet for Nissl substance. Lesion area, and area of total, left, and right hemicord were determined at the lesion epicenter using MetaMorph. The areas filled with red blood cells were traced in every 6th section throughout the lesion and volume of hemorrhage within the lesion was calculated. In addition, large motor neurons (diameter: 25 – 70 μm) with a discernable nucleus that were present in the ventral gray matter, were counted throughout the lesion using MetaMorph.

Statistics

Quantitative MRI data and histopathology measurements, are presented as means $\pm$ standard error of the mean (SE) for all rats in each group. A 2-way repeated measures analysis of variance (ANOVA) was used to analyze all MRI data. A 2-way ANOVA was used to analyze the histopathological data. The null hypothesis was rejected at $\alpha = 0.05$. A t-test was used to compare endpoint serum Na concentrations between the 2 groups of animals. Significant differences identified by the ANOVA were isolated using the Holm-Sidak procedure for pairwise multiple comparison post-hoc test. Statistical computations were performed with software packages (Sigmastat 3.0, SPSS, Chicago, IL).

Results

All animals survived the surgical procedures and 8-hour continuous MRI protocol. The time between injury and generation of the first image by MRI was not different between groups and was 36 ± 4 min for both groups (range: 21-54 min). Body temperature during surgery and MRI was not different between groups. Administration of HS according to our protocol resulted in a serum [Na] of 152 ± 1 mMol/L which was significantly higher than the serum [Na] in control animals (138 ± 4 mMol/L) at the end point of our study ($p=0.003$).

Immediately after injury, asymmetry of the spinal cord due to ipsilateral swelling, extending rostrocaudally well beyond the level of SCI, and an ipsilateral core of hypointense signal were detectable by MRI in all animals of both groups (Figure 1). The rostrocaudal extent of the hypointense core was visible over a length of 3 slices (3.3 mm). T2 weighted MR images showed a rim of hyperintense signal surrounding the hypointense core (Figure 2). Unlike the hypointense core that was limited to the ipsilateral side, the hyperintense signal extended to the contralateral side of the cord. The spread of hyperintense signal into the contralateral cord was particularly visible in the central area of the cord, around the central canal. The rostrocaudal extent of the hyperintense signal was visible over a length of 7 slices (7.7 mm). Over the 8 hour course of our study, an interesting finding, directly visible from MRI, was that in 4 animals the hypointense core reduced in severity (2 animals in each group) and in 3 of these animals (1 control, 2 HS) this hypointense core had completely disappeared towards the endpoint of our study.

Figure 3 shows examples of the tracing method used for quantification of cord swelling and volumes of hypo- and hyperintense signal. Quantification of cord volumes over the 8 hour study period (Figure 4) demonstrated that in both groups, cord volume significantly increased over time; cord volume at hour 1 was significantly lower than at hours 3 – 8 ($p<0.002$). Furthermore, in animals that received HS, cord volume was significantly smaller than in control animals at all time points ($p=0.017$). Quantification of the volume of hypointense signal throughout the cord over time (Figure 5) showed that in animals that received HS this volume was significantly smaller at 8 hours than during the first 6 hours (Figure 5A; $p=0.002$). When examining the slices taken at the lesion epicenter, in both groups the volume of hypointense signal was smaller at hours 7 and 8 compared to hours 1, 3 – 5 and 1 – 6, respectively (Figure 5B; $p<0.001$).

Quantification of hyperintense signal showed an increase in volume over time in the control group. In these animals the volume of hyperintense signal was significantly higher at 8 hours than during the first 2 hours (Figure 6; $p=0.001$). In animals that received HS, this volume was significantly smaller when compared to the control animals at hours 7 and 8 ($p=0.008$ and $p=0.003$, respectively).

Histopathological analysis showed severe disruption of the normal spinal cord cytoarchitecture and vasculature at 8 hours after acute unilateral SCI (Figure 7). Hemorrhage was apparent in a radial pattern, with red blood cells accumulated along the tracts of penetrating arteries/veins (Figure 7A). Closer towards the lesion epicenter, large accumulations of red blood cells were present throughout the ipsilateral cord, with most present in the core of the lesion (Figure 7B). Volume of hemorrhage determined from histopathology was not different between the 2 study groups (control: 4.1 ± 0.4 mm³; HS: 4.3 ± 0.3 mm³) and length over which hemorrhage was present was not different between the 2 groups (control: 4.4 ± 0.2 mm; HS: 4.3 ± 0.2 mm). Infiltration of the cord with red blood cells was seen throughout the ipsilateral cord and in 9 of 12 animals red blood cells were also present on the contralateral side of the central canal (Figure 7C). In these animals red blood cells were present dorsal ($n=6$) and/or ventral ($n=3$) to the central canal in the gray matter or in the white matter along the ventral sulcus ($n=1$). In 2

animals, red blood cells were present within the central canal. Motor neuron counts in the ipsilateral cord were significantly lower than in the contralateral cord (Figure 7D; $p < 0.001$). In both groups, there were significantly fewer motor neurons ipsilaterally over a length of 2.8 mm around the lesion epicenter ($p < 0.001$). There was no significant contralateral loss of motor neurons in any group and no effect of treatment was seen in the number of ipsilateral or contralateral motor neurons.

Discussion

In this study we show that 8 serial bolus treatments of 5% HS delivered every hour for 8 hours starting 30 min after SCI in rats reduced spinal cord swelling and edema. Few other studies have examined HS in experimental SCI, and they used single bolus treatments of 7.5% HS delivered 1-15 min after SCI (13-16). We chose to examine a treatment strategy that would be clinically applicable and similar to ones used in treatment of patients with cerebral edema. HS solutions are commercially available in a variety of concentrations (3%, 5%, 7.5%, 23.4%) and most have been used in the treatment of patients with cerebral edema and/or elevated ICP (5-7,20,21). We chose to use a 5% HS solution that provided a Na load that we considered safe to deliver and high enough to maximize the chance of showing positive effects of hyperosmolar therapy.

Previous studies have shown positive effects of a single bolus administration of 7.5% HS (5 ml/kg) on spinal cord blood flow, spinal cord conduction, and somatosensory evoked potentials after SCI in rats (13,15,22). Follow up experiments showed faster recovery of bladder and hind limb locomotor function, higher locomotor outcome scores, and attenuation of histopathological outcomes in animals treated with 7.5% HS (5ml/kg bolus) 1 minute after injury (14,22). In 2001 Legos *et al* also showed that co-administration of 7.5% HS (5 ml/kg bolus) with methylprednisolone (MP) may enhance delivery of MP. Additionally, this combinatorial treatment had positive effects on survival and locomotor outcome (16). All these reports suggest HS would improve spinal cord blood flow much like it improves cerebral perfusion in cerebral edema. Moreover, Spera *et al* (1998) showed that leukocyte adhesion after SCI was attenuated by HS and suggested that HS may reduce leukocyte swelling similar to the effect it has on endothelial cells (23,24). There is mounting evidence that, in addition to the osmotic and perivascular aquaporin modulating effects of HS, its other actions such as its antiinflammatory and immunomodulatory properties contribute to its neuroprotective effects (6,25).

Similar to what has been shown to occur in the brain after injury, HS appeared to reduce the severity of spinal cord swelling and edema during the 8 hour period after SCI. We did not determine spinal cord water content or specific gravity post mortem since our aim was to determine the effect of HS on parameters that were measurable *in vivo*, however future studies using HS should include additional measures of edema. We suggest that the beneficial effects of HS identified in the present study, i.e. reduction of cord swelling and enhanced resolution of edema, should improve spinal cord perfusion and increase DO_2 with the ultimate goal of improving recovery. Future studies should include long-term functional outcome data.

The first MR images that we obtained in this study showed loss of normal cord cytoarchitecture, characteristic evidence of hemorrhage, and accumulation of edema. Hemorrhage and edema were recognized by hypointense signal in T1 weighted images and hyperintense signal in T2 weighted images, respectively. Over the time course of this study we showed that in untreated SCI, the injured cord continued to increase in volume with a simultaneous increase in edema. This is consistent with the relationship between edema and cord swelling we demonstrated in our previous study. In that study we showed increase in volume and increase of edema in the acute stage (24 hours and 7 days) after cervical SCI, followed by a decrease in both parameters

over the 3 week study period (2). In the current study we did not show a difference between the two groups in volume of hyperintense signal during the first few hours after SCI. However, the cord volume in animals treated with HS was significantly smaller than in the untreated animals. Our first administration of HS was at 30 minutes after SCI, just before obtaining the first images. Our results suggest that perhaps free water is initially drawn from normal appearing tissue and in the later stages also from edematous areas. This may explain the difference in volume of hyperintense signal at the later time points.

The hypointense MR signal visible in the lesion center in the ipsilateral cord has typically been considered characteristic of hemorrhage (19,26). Similar to what has been shown by other groups, in our study the hypointense core was surrounded by extravascular fluid accumulation (26-28). Interestingly, this hypointense core was present in all animals at the first time point but appeared to resolve in 4 animals and was no longer visible in 3 of those animals at the end point of the study. This is consistent with the fact that at 24 hours after injury, we did not see a hypointense core in all animals in our previous study (2). In fact, only in a subset of more severely injured animals did we see evidence of hypointensity at 24 hours after injury. However, in that study, a hypointense core was present in all animals from 7 to 21 days after injury. These findings, together with the discrepancy we found in the present study between the endpoint MR images (i.e. a significantly smaller volume of hypointense signal in HS treated animals) and histopathology (i.e. no difference in volume of red blood cells in the lesion) suggests that the MR hypointense signal is produced by more than just red blood cells. Moreover, histopathology at 8 hours demonstrated the presence of red blood cells in the spinal cord lesion of all animals, including those in which there was no hypointense core visible using MRI. We propose that the changes in the hypointense core may be due to, not only red blood cell accumulation, but also properties of the surrounding fluids and changes in local perfusion. Attenuation and resolution of the hypointense core identified by MRI may indicate enhanced perfusion or enhanced recovery of microvascular properties after treatment with HS. It is also possible this change reflects different properties of the red blood cells left in the lesion area such as iron binding properties and subsequent relaxation times in response to the MR signal.

Bilgen *et al* (2000) examined the spatial and temporal evolution of hemorrhage for a period of 6 hours after a mid-thoracic compression injury. They found that the volume of hemorrhage, characterized by hypointensity, increased over time and encompassed 12.5% of the cord volume at the lesion center at the start (~32 min after SCI) and 25% at the end of their study (6 hours after SCI). This is different from our study in which we did not find an increase in volume of the hypointense core over the first 6 hours. It is possible that the difference in evolution of hypointense core is related to the different cytoarchitecture and vascular pattern of the thoracic cord and cervical hemicord. As far as the authors are aware, these are the only two *in vivo* studies that have looked at hemorrhage after SCI in rats at these early time points. Further investigations are required to determine what exactly the hypointense core consists of, and what the reason is for the disappearance/attenuation of the hypointense core between 8 hours and 7 days after SCI, as suggested by this and our prior study (2).

Although we found differences between the two study groups in volumes of hypointense and hyperintense signal using MRI, the number of motor neurons counted throughout the cord was not different. Future studies need to determine whether there would be long term effects of HS on histopathological outcomes such as motor neuron survival. If an early decrease in hemorrhage and edema would be indicative of attenuation of secondary insult, this may lead to fewer cells undergoing delayed cell death. The concern that HS may be detrimental because of development of a reversed osmotic gradient in the face of a damaged blood brain barrier, and subsequently increasing water content of injured tissue should also be addressed in further studies into the use of HS in SCI.

Conclusion

Here we demonstrate beneficial effects of HS in acute SCI that were quantifiable using MRI. In this study serial bolus administration of 5% HS resulted in increased serum [Na] and attenuation of cord swelling and edema. This is a finding that may have significant clinical implications considering the fact that HS is already widely used in intensive care and neurocritical care patients and can be relatively easily implemented in acute SCI patients.

As far as the authors are aware, this is the first report to document developing cord pathology after cervical SCI throughout the first 8 hours of injury using continuous MRI, and to demonstrate how HS affects this lesion. The outcomes of this work suggest that *in vivo* longitudinal MRI can be used to not only assess evolution of injury site, but also to monitor experimental treatment strategies based on knowledge of MRI appearance of pathologic events. Moreover, MRI could be applied as a screening tool to either administer goal-directed therapies, or enable even group distribution. This should prove valuable in developing strategies for assessing the evolution and repair of SCI in the clinical setting.

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Figure 1.

Representative T1 weighted MR images at the level of the lesion epicenter. Consecutive images taken every 60 min show lesion development over time from 36 min after injury (left) to 8 hours after injury (right) in a control animal (top row) and an animal that received HS (bottom row).

Figure 2.

Representative T2 weighted MR images at the level of the lesion epicenter. Consecutive images taken every 60 min show lesion development over time from 36 min after injury (left) to 8 hours after injury (right) in a control animal (top row) and an animal that received HS (bottom row).

Figure 3.

Examples of tracings of the regions of interest for determination of volume measurements. The left is an overlay of a T1 and PD weighted image in which the whole cord and hypointense core are traced. The right is a T2 weighted image which demonstrates thresholding and tracing of the hyperintense area.

Figure 4.

Cord volume determined for 7 consecutive slices over time. There was a significant difference between treatment groups over time (★★ $p=0.017$) and for both groups the volume at the time of the first image was significantly lower than at hours 3 – 8 (★ $p<0.002$). Data are presented as mean + standard error of the mean.

Figure 5.

Volume of hypointense signal determined for 7 consecutive slices (**A**) and for 1 slice at the level of lesion epicenter (**B**) over time. In **A**, volume of hypointense signal throughout the cord was significantly smaller at 8 hours than at 1 – 6 hours in animals that received HS (★ $p=0.002$). In **B**, volume of hypointense signal at the level of lesion epicenter was smaller in both groups at 7 and 8 hours compared to hours 1, 3 – 5, and 1 – 6, respectively (★ $p<0.001$). Data are presented as mean + standard error of the mean.

Figure 6.

Volume of hyperintense signal throughout the lesion (7 slices). In the control group, the amount of hyperintensity at 8 hours is significantly increased when compared to 1 and 2 hours (★ $p=0.001$). Furthermore, the volume of hyperintensity was significantly smaller in the animals that received HS compared to control animals at 7 (★ $p=0.008$) and 8 (★ $p=0.003$) hours. Data are presented as mean + standard error of the mean.

Figure 7.

A: Representative section from the lesion showing the radial pattern in which red blood cells infiltrate the spinal cord. **B:** Representative section at the level of lesion epicenter. Severe disruption of normal anatomy and accumulation of red blood cells is clearly visible. **C:** Representative section showing red blood cell accumulation in the ipsilateral right hemicord and contralateral accumulation of red blood cells in the central gray area dorsal and ventrolateral to the central canal (arrows). **D:** Motor neuron counts in the right hemicord are significantly lower than in the left hemicord ($p < 0.001$). Furthermore, within the right hemicord, motor neuron counts were significantly lower in the 2.76mm length of cord around lesion epicenter than in the rostral and distal ends of the right hemicord ($\star p < 0.001$). Within the left hemicord there was no significant difference in motor neuron count throughout the lesion. Data are presented as mean + standard error of the mean.