ORIGINAL RESEARCH
PERIPHERAL NERVOUS SYSTEM

Gadolinium DTPA Enhancement Characteristics of the Rat Sciatic Nerve after Crush Injury at 4.7T

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ABSTRACT

BACKGROUND AND PURPOSE: Traumatic peripheral nerve injury is common and results in loss of function and/or neuropathic pain. MR neurography is a well-established technique for evaluating peripheral nerve anatomy and pathology. However, the Gd-DTPA enhancement characteristics of acutely injured peripheral nerves have not been fully examined. This study was performed to determine whether acutely crushed rat sciatic nerves demonstrate Gd-DTPA enhancement and, if so, to evaluate whether enhancement is affected by crush severity.

MATERIALS AND METHODS: In 26 rats, the sciatic nerve was crushed with either surgical forceps (6- to 20-N compressive force) or a microvascular/microaneurysm clip (0.1–0.6 N). Animals were longitudinally imaged at 4.7T for up to 30 days after injury. T1WI, T2WI, and T1WI with Gd-DTPA were performed.

RESULTS: Forceps crush injury caused robust enhancement between days 3 and 21, while clip crush injury resulted in minimal-to-no enhancement. Enhancement after forceps injury peaked at 7 days and was seen a few millimeters proximal to, in the region of, and several centimeters distal to the site of crush injury. Enhancement after forceps injury was statistically significant compared with clip injury between days 3 and 7 (P < .04).

CONCLUSIONS: Gd-DTPA enhancement of peripheral nerves may only occur above a certain crush-severity threshold. This phenomenon may explain the intermittent observation of Gd-DTPA enhancement of peripheral nerves after traumatic injury. The observation of enhancement may be useful in judging the severity of injury after nerve trauma.

ABBREVIATIONS: BNB = blood-nerve barrier; DCE = dynamic contrast-enhanced

Approximately 3% of trauma patients sustain an injury to the radial, median, ulnar, sciatic, femoral, tibial, or peroneal nerve. The health care costs associated with traumatic peripheral nerve injury are approximately $150 billion per year in the United States alone. Patients with traumatic nerve injury can have loss of motor function (up to complete paralysis), loss of sensation, and/or neuropathic pain. Direct end-to-end epineurial repair remains the criterion standard treatment for high-grade nerve trauma and is often combined with autologous nerve grafting. However, a recent meta-analysis of median and ulnar nerve repair showed that only 52% of patients achieved satisfactory motor recovery, while 43% achieved satisfactory sensory recovery.

Peripheral nerve injury has been classified by Sunderland on the basis of damage to Schwann cells/myelin, axons, endoneurium, perineurium, and epineurium (grades 1–5, respectively). Nerve conduction studies and electromyography are the standard diagnostic tests for grading traumatic mononeuropathy. However, electrodiagnostics have limited ability to distinguish Sunderland grade 2, 3, and 4 injuries in the acute and subacute settings. Because skeletal muscles begin to atrophy immediately after denervation and are permanently wasted by 18–24 months, and because axons regenerate at only millimeters per day, there is a clinical need to accurately grade nerve injuries in the acute setting so that surgical intervention can be expedited (when appropriate) to prevent permanent loss of function. In addition, there is a clinical and scientific need for noninvasive diagnostic testing to monitor the effects of various novel medical and surgical interventions on nerve repair.

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178
microvascular/microaneurysm clip (of the midfemur by using either surgical forceps (left sciatic nerve was surgically exposed and crushed at the level of 7.2 cm. Animals were imaged between postoperative days 0 and 30; 50 MR imaging sessions were performed (Tables 1 and 2).

Materials and Methods
This study was approved by the University Animal Care and Use Committee (Protocol No. 06–172). Twenty-six male Sprague-Dawley rats were used for the study. In each rat, the left sciatic nerves demonstrate Gd-DTPA enhancement and, if so, to evaluate whether enhancement is affected by crush severity. On the basis of prior literature, we hypothesized that crushed nerves would not show Gd-DTPA enhancement.

Note:—Postop indicates postoperative.

| Forceps: Postop Day No. | No. MRI Sessions (with Limited DCE) |
|-------------------------|--------------------------------------|
| 0                       | 1(0)                                 |
| 1                       | 4(0)                                 |
| 2                       | 2(0)                                 |
| 3                       | 3(2)                                 |
| 4                       | 1(0)                                 |
| 5                       | 1(0)                                 |
| 7                       | 6(5)                                 |
| 12                      | 2(0)                                 |
| 13                      | 2(1)                                 |
| 14                      | 5(4)                                 |
| 19                      | 1(1)                                 |
| 21                      | 1(1)                                 |
| 22                      | 1(0)                                 |
| 30                      | 1(1)                                 |
| Total                   | 31(16)                               |

Table 1: Number of MRI sessions at each time point after forceps (#5 jeweler or toothed Adson) crush injury

| Clip: Postop Day No. | No. MRI Sessions |
|----------------------|------------------|
| 1                    | 3                |
| 2                    | 4                |
| 3                    | 2                |
| 4                    | 3                |
| 5                    | 4                |
| 6                    | 1                |
| 7                    | 2                |
| Total                | 19               |

Table 2: Number of MRI sessions at each time point after clip (10–60 g microvascular/microaneurysm) crush injury

Sequences included axial T1-weighted gradient-echo with fat saturation (TR, 235 ms; TE, 2.6 ms), axial T2-weighted rapid acquisition relaxation excitation with fat saturation (TR, 2500 ms; TE, 23 ms), and axial T1-weighted gradient-echo with fat saturation immediately after 0.1 mmol/kg of intravenous Gd-DTPA (Magnovist; Bayer HealthCare Pharmaceuticals, Wayne, New Jersey). Limited dynamic contrast-enhanced (DCE) T1-weighted gradient-echo imaging with fat saturation was performed on select animals, using the Bruker Small Animal MRI and Spectroscopy sequence (TR, 35 ms; TE, 3.5 ms). Sixteen MR imaging sessions included limited DCE imaging. All acquisitions had a section thickness of 0.8 mm without a gap, an FOV of 5 × 5 cm, and a matrix size of either 168 × 168 or 256 × 256. Gd-DTPA was administered through a surgically placed external jugular central venous catheter. Animals were sedated with isoflurane during imaging and positioned in the prone (normal upright) position, with hips and knees flexed at roughly 90°. Images were acquired in the axial plane, a few millimeters posterior to and roughly parallel to the femurs.

Image postprocessing included manual ROI selection and calculation of the signal-to-noise ratio in each ROI. ROIs included the injured sciatic nerve in the region of the crush injury, sham/nonoperative nerves in the corresponding contralateral location, normal muscle, and background air (Fig 1). The SNR was calculated as the quotient of the mean ROI signal divided by the SD of the noise of background air. Enhancement was calculated as the percentage increase in the SNR after the administration of contrast. Statistical analysis included comparison of enhancement after forceps injury with enhancement of clip injured nerves, sham nerves, nonoperative nerves, and normal muscle using a 2-tailed, 2-sample Student t test. As an internal control, enhancement of sham nerves was compared with enhancement of nonoperative nerves using the same statistical methodology. Significance was set at P < .05.

Results

Forceps Crush Intensity
Measurements of the compressive force delivered by the #5 Jeweler forceps ranged between 650 and 750 g (6.4–7.4 N). Measurements of the compressive force delivered by the Toothed Adson forceps ranged between 750 g and 2.0 kg (7.4–20 N).

T2-Weighted MR Imaging
On T2WI, 14 of 14 (100%) forceps-injured nerves demonstrated increased caliber and signal proximal to, in the region of, and distal to the site of injury (Fig 2). Increased T2 signal extended...
proximally as far as the sciatic notch and distally well into the tibial and peroneal nerve branches. In contrast, only 8 of 12 (67%) clip injured nerves demonstrated qualitative signal hyperintensity on T2WI.

**Gadolinium-DTPA-Enhanced T1-Weighted MR Imaging**

Qualitatively, 14 of 14 (100%) forceps-injured nerves demonstrated avid Gd-DTPA enhancement on T1WI. Enhancement was seen a few millimeters proximal to, in the region of, and several centimeters distal to the site of crush injury (Fig 3). Like T2 signal hyperintensity, Gd-DTPA enhancement extended distally into the tibial and peroneal nerve branches. However, Gd-DTPA enhancement did not extend as far proximally (toward the sciatic notch) as T2 signal hyperintensity. Robust Gd-DTPA enhancement was observed in forceps-injured nerves as early as 3 days after injury. Enhancement peaked at 7 days and subsequently diminished, but it was still evident on day 21 (Fig 4).

Quantitatively, enhancement of forceps-injured nerves was statistically significant \((P < .05)\) compared with normal muscle at days 3, 7, and 14 (Fig 5). Enhancement of forceps-injured nerves was statistically significant compared with sham nerves \((P < .02)\) as well as nonoperative nerves \((P < .01)\) on day 7 only.

In contrast, only 1 of 12 (8%) clip-injured nerves demonstrated Gd-DTPA enhancement on T1WI. This nerve had been crushed with a 60-g microaneurysm clip for 60 seconds and showed Gd-DTPA enhancement only at the crush site on postoperative days 5 and 7. No qualitative enhancement was seen in the sciatic nerve distal to the crush site or the tibial or peroneal nerve branches. Quantitative enhancement of clip-injured nerves was significantly different from that of forceps-injured nerves when comparing all MR imaging sessions between days 3 and 7 \((P < .04)\).

Forceps-injured nerves enhanced both internally and peripherally. Nonoperative nerves did not demonstrate internal Gd-DTPA enhancement. They did, however, demonstrate a thin rim of peripheral enhancement, presumably representing mesoneurium and/or external epineurium, both of which lack a blood-nerve barrier (BNB) (Fig 3C). Some sham nerves demonstrated faint qualitative enhancement only at the surgical site on days 7 \((n = 3)\) and 14 \((n = 1)\), though this was not statistically significant compared with nonoperative nerves at the same time points \((P > .15)\).

Limited DCE MR neurography was performed on a select group of animals to better understand the kinetics of enhancement after contrast injection. On DCE T1WI, forceps-injured nerve SNR reached a plateau approximately 5–10 minutes after the administration of Gd-DTPA (Fig 6) and demonstrated a half-life of approximately 1 hour (Fig 7). Precise mathematic modeling of DCE data was not possible due to the limited temporal resolution and the lack of a reliable arterial input function in the FOV. However, the presence of a delayed, higher peak SNR in forceps-injured nerves compared with nonoperative nerves suggested an enlarged extravascular extracellular space after severe crush injury.\(^\text{13}\)

**DISCUSSION**

Our results demonstrate that Gd-DTPA peripheral nerve enhancement after severe (forceps) crush injury is a robust phenomenon in the rat sciatic nerve at 4.7T. The relative absence of enhancement after mild (clip) crush injury suggests the presence of a crush-severity threshold, below which enhancement is not observed. Therefore, the observation of enhancement after nerve trauma could potentially be useful in judging the severity of injury. In addition, when one performs MR neurog-
raphy for peripheral neuropathy of uncertain origin, the observation of enhancement may not exclude trauma from the differential diagnosis.

In our study, Gd-DTPA enhancement was more specific for higher grade injury compared with T2 hyperintensity. Also, Gd-DTPA enhancement more clearly localized the site of injury because T2 hyperintensity extended proximally to the sciatic notch, while Gd-DTPA enhancement extended only a few millimeters proximal to the site of nerve crush. Gd-DTPA enhancement may provide additional information when added to routine MR neurography protocols in the posttraumatic setting.

Physiologically, peripheral nerve enhancement is a function of increased blood-nerve barrier permeability. The BNB comprises tight junctions between endoneurial endothelial cells and perineurial myofibroblasts. Increased BNB permeability can occur with demyelination (neurapraxia), Wallerian degeneration (axonotmesis), high-grade nerve trauma (neurotmesis), and numerous other causes of nerve injury. In a rat sciatic model, Omura et al observed increased BNB permeability 3 days after crush injury, with maximal BNB permeability after 7 days. Increased BNB permeability was observed 5 mm proximal to the site of injury, at the site of injury, and in the entire nerve distal to the site of injury. In a mouse sciatic model, Seitz et al observed maximal BNB permeability distal to the site of injury 8 days after crush injury. These histologic results closely mirror our present imaging findings.

FIG 3. Axial T1WI with fat saturation, pre- (A) and post- (B–D) Gd-DTPA (same animal as in Fig 2). A, On precontrast images, both injured and nonoperative nerves are isointense to muscle. B, Intense Gd-DTPA enhancement (open arrows) is demonstrated a few millimeters proximal to, in the region of, and distal to the site of crush injury (arrow) (On-line Video 1). C, The nonoperative contralateral nerve (arrows) is well seen in this section and demonstrates a thin rim of peripheral enhancement but no internal enhancement (On-line Video 2). D, Enhancement is seen in a distal branch (arrow) of the injured sciatic nerve (On-line Video 3).
Nerve crush is considered a model of axonotmesis (Sunderland grade 2 injury) with Wallerian degeneration followed by axonal regeneration. Histologic studies have demonstrated a correlation between BNB permeability and axonal degeneration/regeneration. By comparing histologic analysis of tight junction proteins with neurofilaments, Omura et al demonstrated that both restoration of the BNB and axonal regeneration occur from proximal to distal and are closely related in time and space. Bouldin et al have shown that increased BNB permeability persists beyond 14 weeks after complete transection as well as ricin injury; both models have no axonal regeneration. Further studies may be appropriate to determine whether resolution of enhancement after peripheral nerve trauma could be an indicator of axonal regeneration and whether the persistence of enhancement could indicate the absence of axonal regeneration.

Prior studies of Gd-DTPA-enhanced MR neurography after traumatic nerve injury have demonstrated mixed results. Gadolinium-DTPA enhancement has been reported in the facial nerve after crush injury as well as in the median nerve after crush injury in an ex vivo model. However, Aagaard et al performed Gd-DTPA-enhanced MR neurography on 6 rats after sciatic nerve crush, and they were not able to reliably distinguish normal from crushed nerves. Furthermore, Bendszus et al reported no Gd-DTPA enhancement of the rat sciatic nerve after ligation injury. Subsequent studies have also failed to demonstrate reliable Gd-DTPA enhancement in experimental autoimmune neuritis, focal demyelination induced by lysolecithin, and experimental Charcot–Marie–Tooth disease. Our present observations of enhancement very closely parallel prior imaging findings after crush injury, using the nerve-specific contrast agent gadofluorine-M. Possible explanations for prior mixed results with Gd-DTPA include varying injury mechanisms, crush forces, crush durations, magnetic field strengths (4.7T versus 1.5T), and MR imaging protocols. The interplay between crush force and crush duration is an obvious target for future investigation of the crush-severity enhancement threshold.

In recent years, diffusion tensor imaging has emerged as a potential new technique for evaluating injured nerves for evidence of axonal regeneration. The loss and restoration of fractional anisotropy has been correlated with histologic and functional degeneration and recovery. Corresponding observations have been made regarding radial and axial diffusivity.
CONCLUSIONS

In our study, forceps crush injury (6- to 20-N compressive force) to the rat sciatic nerve caused robust Gd-DTPA enhancement between days 3 and 21, while clip crush injury (0.1–0.6 N) resulted in minimal-to-no enhancement. Enhancement after for-
caps injury peaked at 7 days and was seen a few millimeters proximal to, in the region of, and several centimeters distal to the site of nerve crush. Compared with T2 signal hyperintensity, Gd-DTPA enhancement was more specific for higher grade injury and more clearly localized the site of injury. The spatial and temporal characteristics of Gd-DTPA enhancement in our study closely mirror prior histologic studies of blood-nerve barrier permeability after nerve injury. Further studies are needed to determine the scientific and clinical usefulness of this imaging technique.

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