MRI evaluation of periperal nerve regeneration in rodents

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Authors: A. Fischmann, M. Tremp, M. Meyer zu Schwabedissen, D. F. Kalbermatten, C. Stippich; Basel/CH
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Purpose

Nerve injuries affect several hundred thousand people each year in Europe with serious economic implications for both the individual patient and society. Despite limited improvements in surgical technique, traumatic nerve injuries represent a major clinical challenge.

Following injury, the distal part of the nerve undergoes Wallerian degeneration (within 10 - 20 days), thereby forming columns of Schwann cells (bands of Bunger) that serve as guiding structure for the regenerating axons. Most commonly used treatments include end-to-end suturing and autologous nerve grafting [1].

To date, nerve reconstruction can only be assessed after nerve regeneration. As this might take several months without evidence for surgical success, earlier evaluation would be valuable. We investigated whether MRI is able to evaluate nerve growth in a rat model.
Methods and Materials

All experiments were performed according to the guidelines for experiments in animals by the University of Basel. All animals were sacrificed in a series of trials on the influence of stem cells on nerve growth.

Eight female Sprague Dawley rats were anaesthetized and the left sciatic nerve exposed. The nerve was transected and a fibrin conduit of 14-mm length and 2-mm lumen with culture medium alone, rat or human adipose-derived stem cells (1 x 10^6 cells) was connected to the proximal and distal nerve stumps (Figure 1). Animals were sacrificed at two weeks post surgery by CO2-chamber euthanasia.

MRI was performed on a clinical 3 Tesla whole body scanner (Siemens Magnetom Verio, Erlangen, Germany) using a human approved 8 channel wrist coil. Sagittal T2 weighted fat saturated sequences (TR = 4250ms, TE = 71ms, matrix 128 x 256 leading to a voxel size of 0.25 x 0.25 x 1mm) parallel and orthogonal to the thigh were acquired, as well as T1 weighted (TR = 500ms, TE = 28ms) and T2 weighted (TR = 4000ms, TE 25ms) fat saturated images (both with a matrix 320 x 320 leading to a voxel size of 0.25 x 0.25 x 3mm) orthogonal to the calf.

Nerves were harvested less than 12 hours after MRI for morphological (immunohistochemistry) analysis. Implants were retrieved and the harvested conduits were fixed over night in 4% paraformaldehyde/1% glutaraldehyde solution. The conduits were subsequently transferred into phosphate-buffered saline and put into sucrose 30% for 24h. The specimens were then embedded in O.C.T. compound (Sakura Finetek, Tokyo, Japan) for cryosectioning. Longitudinal sections of 12#m thickness were obtained by cryosectioning the explant (Cryostat, Leica CM 1850, Meyer Instruments, Houston, TX, USA) on cryoslides. Serial sections were collected and processed for single staining of either PGP9.5, a marker for regenerating axons, or S100, a marker for migrating Schwann cells. First, tissue sections were washed thrice with PBS and incubated with 5% blocking serum albumin for 1 h at room temperature. The specimens were then incubated with primary antibody (anti-PGP9.5 and anti-S100 IgG from rabbit; 1:500) at 4°C overnight. The sections were washed again thrice with PBS and incubated at room temperature for 40 min with Alexa Fluor 488-labelled goat anti-rabbit IgG (1:1000). Dapi (4’-6-diamidino-2-phenylindole, blue) was used to stain the nucleus. Washes were repeated as before, and slides mounted with Vectashield. Results were entered to spreadsheets. Means and median as well as Pearsons correlation coefficient were calculated using statistical software (JMP 9, SAS Institute, Cary (NC), USA).
Fig. 1: Intraoperative image of the dissected nerve with the nerve conduit (arrows).

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Results

All animals showed calf atrophy and T2 hyperintensity in the treated leg (arrowheads in figure 2a), while the distal nerve stumps were clearly visible as strongly T2 hyperintense (arrows in figure 2a). We were able to visualize the conduit in all animals with MRI (figure 3). However due to extensive edema and inflammation around the graft (figure 2b) measurement of the growth cone was not possible with MRI in one and histology in two rats.

In the remaining 6 animals MRI showed a small T2 hyperintensity inside the conduit, which was assumed to represent the end of the growth cone. Average length from the proximal conduit to this hyperintensity was 6.2mm (5 mm to 7.5mm). The growth cone could clearly be visualized in immunofluorescence microscopy (figure 4) with an average length of 3.9mm (2.5 to 5.5 mm). Histology and MRI showed a good correlation of growth cone length ($r^2=0.49$, figure 5).
Fig. 2: a) T2 weighted image of the calves in one rat, the distal ischiadic and tibial nerves (arrows) are clearly visible as hyperintense structures representing Wallerian degeneration. Atrophy and Hyperintensity of the treated leg (arrowheads) is also visible in the calves. b) T2 weighted sagittal image in another animal with extensive fluid around the conduit (arrows) preventing measurements of the growth cone.

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Fig. 3: T2 weighted sagittal images of the thigh. The conduit is clearly visible as linear signal voids (arrowheads). The distal and proximal nerve stumps are visible as a T2
hyperintense structure (small arrows), while a small T2 hyperintensity can be detected centrally in the conduit representing the growth cone (arrow outline).

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Fig. 4: Immunoflourence image of the growth cone. Nerve cells are sprouting from left to right into the conduit

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Fig. 5: Comparison of growth cone length in histology and MRI with a good correlation between both imaging methods.

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Conclusion

MRI with human approved equipment was able to visualize the fibrin conduit and growth cone, which might be a valuable clinical tool.

Future clinical and animal trials are necessary to validate our findings.
References

Personal Information

Arne Fischmann MD MHBA, Christoph Stippich MD

University of Basel Hospital, Department of Radiology and Nuclear Medicine, Division of Diagnostic and Interventional Neuroradiology, Petersgraben 4, CH-4031 Basel, Switzerland
mailto: arne.fischmann (at) unibas.ch

Mathias Tremp MD, Moritz Meyer zu Schwabedissen MD, Daniel F. Kalbermatten MD PhD

University of Basel Hospital, Department of Plastic, Reconstructive and Aesthetic Surgery, Hand Surgery, Petersgraben 4, CH-4031 Basel, Switzerland

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