NOVEL NEUROTROPIC CONTRAST AGENTS FOR VISUALIZATION OF PERIPHERAL NERVES BY MAGNETIC RESONANCE IMAGING (MRI)

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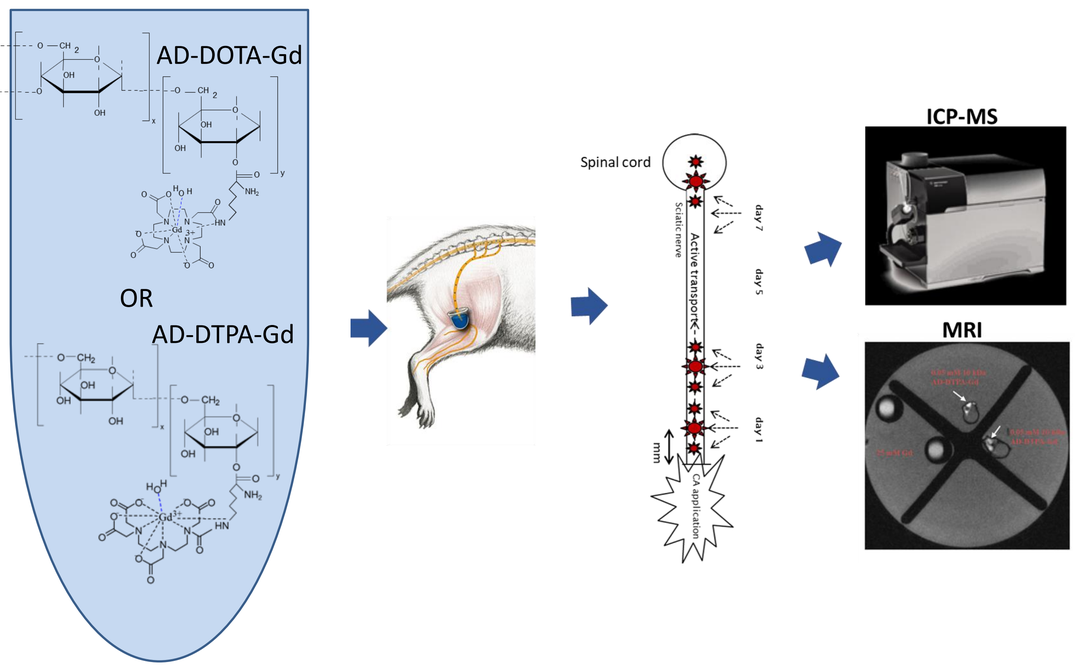
Objective

Amino-dextrans (AD) conjugated with gadolinium (Gd(III)) were developed as neuro-specific contrast agents (CA) for the visualization of the sciatic nerve in rats by magnetic resonance imaging (MRI). AD with 3, 10, and 70 kDa molecular weights were assessed as carrier molecules known to be transported with various speed by axonal microtubules.

Material and Methods

Detailed spectroscopic characterizations, analyses by Fast Protein Liquid Chromatography (FPLC), Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), and inductively coupled plasma-mass spectrometry (ICP-MS), were carried out. For MRI, the paramagnetic Gd(III) ion was coupled as a T1 signal enhancer. The well-established linear chelator, diethylenetriaminepentaacetic acid (DTPA), was used and subsequently replaced by the more stable cyclic chelator 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). In addition, a fluorescently labeled AD-DTPA-Gd was prepared to demonstrate an active transport to the spinal cord by histochemistry. After successful synthesis and characterization, molecular migration of the AD-DTPA-Gd in the sciatic nerve of healthy Sprague Dawley rats was monitored by MRI for up to seven days.
Enhancement of nerve structures was evaluated by MRI and correlated with ICP-MS analyses. To investigate the distribution of CA along the neuraxis, all animals were sacrificed after the final MRI monitoring. Nerves, spinal ganglions, and corresponding spinal cord sections were harvested, to determine the localization and concentration of the paramagnetic element.



Results

Our study demonstrates successful synthesis and characterization of three AD, coupled to either DTPA-bisnahydride or to DOTA-NHS ester and finally complexated with paramagnetic Gd(III). The *ex vivo* and *in vitro* measurements of the three compounds tested verified contrast uptake and highlighted the rat's sciatic nerve on MRI. This is the first assessment of active uptake and transport of AD-based, nerve-specific contrast agents visualized with MRI and quantified by ICP-MS.

Conclusion

Coupling of different Gd chelates to AD was successful and their potential as nerve-specific imaging probes in a completely novel setting for intra-axonal transport was demonstrated. This is the first report that demonstrates the active uptake and transport of AD-Gd conjugates within the sciatic nerve. This new concept may serve as a potential diagnostic tool for the direct visualization and monitoring of the continuity of injured nerves.

References

[1]Wessig C, Bendszus M, Stoll G. Exp Neurol. 2007 Mar;204(1):14-9; [2]Liu Y, Liu J, Zhang J , Li X, Lin F , Zhou N, Yang B , Lu L . Biomater Sci. 2019 Mar 26;7(4):1574-1583.

The first syntheses of AD-DTPA-Gd, AD-DOTA-Gd, FR-DTPA-Gd and FR-DOTA-Gd conjugates for the evaluation of uptake and retrograde transport into the N. ischiadicus were successfully performed. The newly produced compounds were purified by FPLC, lyophilized, and Gd content was determined by ICP-MS. SDS-PAGE gel electrophoresis was carried out to check the molecular weight and the presence of aggregates. The compounds were well-soluble in water and PBS with no indication of aggregates.

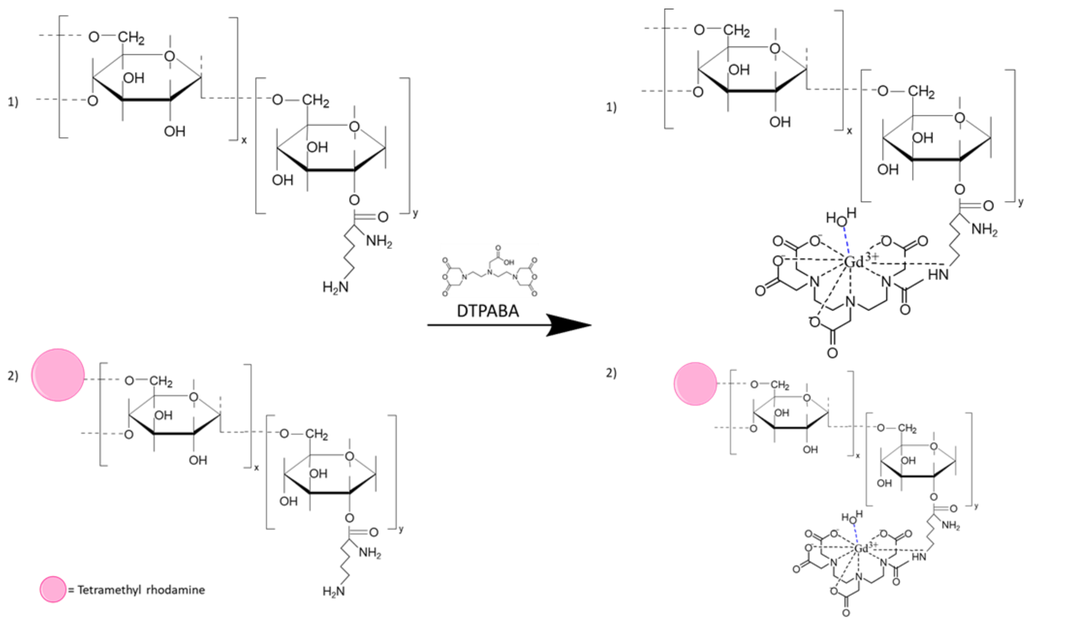


Figure 1. Synthetic scheme of the preparation of AD-DTPA by bis-anhydride mediated conjugation of the Gd(III) chelator DTPABA at the ε-amines of lysine residues (1) and tetramethyl rhodamine (Fluoro ruby) FR-AD-DTPA-Gd (2).

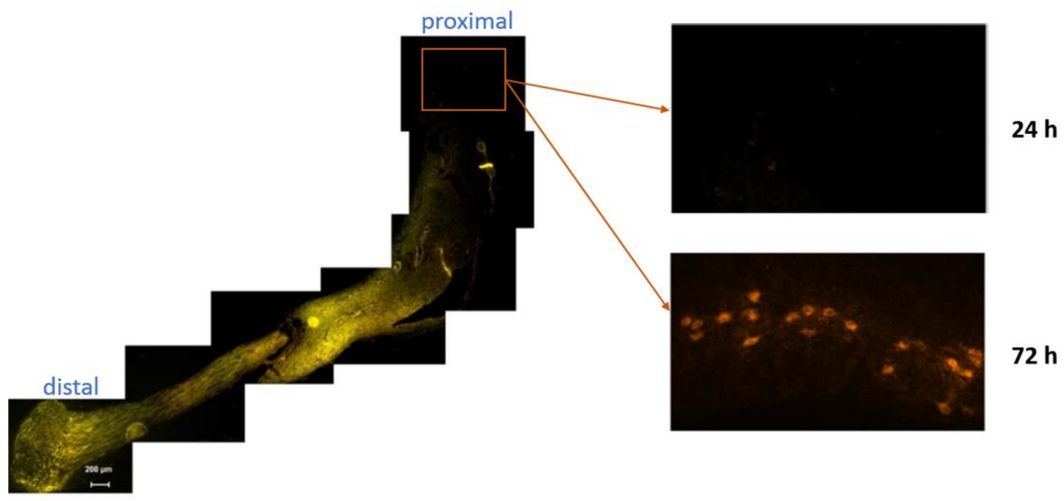


Figure 2. Fluorescence microscopy images of a magnified Nervus ischiadicus (longitudinal cross-section) 24 and 72 h after staining with 10% 10 kDa FR-AD-DTPA-Gd and corresponding spinal cord section. After 24 h, no labeling of the proximal nerve end (directly at the spinal cord entrance) could be detected, whereas, at 72 h, the corresponding spinal cord (SC) part glowed orange due to active retrograde transport of the fluorescently labeled 10 kDa AD-Gd compound, indicating a non-hindered neural transport.

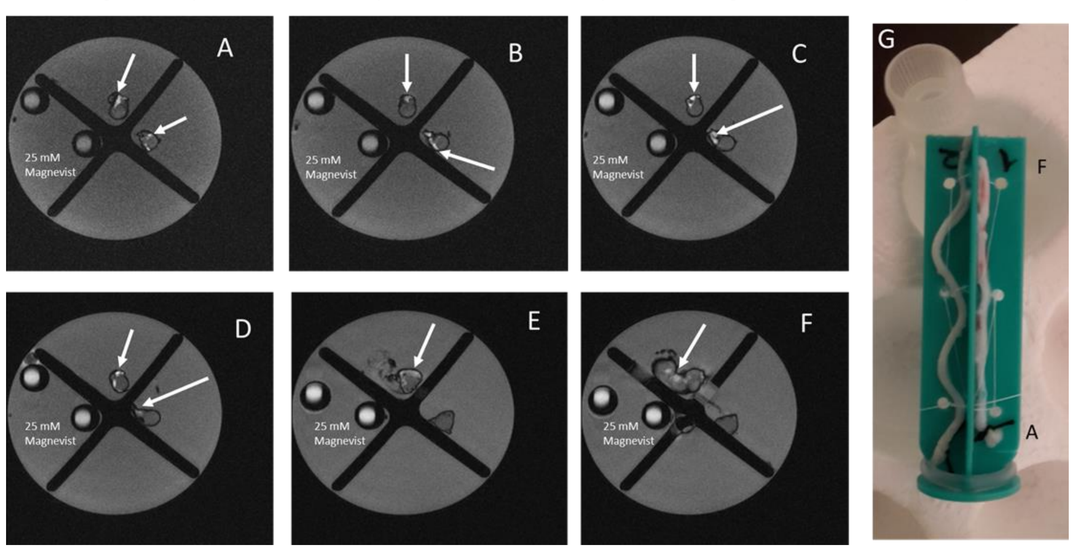


Figure 3. T1 weighted ex vivo MR images using the microimaging insert (7Tesla) of an N. ischiadicus sample (2D IR-Seq: TE= 6.6 ms, TR1= 3500 ms TI= 1000 ms in-plane resolution of 78µm SL 1mm). Cross-sections of two nerves incubated with 10 kDa AD-DTPA-Gd (0.05 mM) versus control tubes filled with gadopentetate dimeglumine (Magnevist, 25 mM) as a positive control. Nerve segments and control tubes were placed in a syringe (G) and measured by MRI. Images shown start from the distal part (A) to the proximal (F) end of the nerve. Six out of a total of 30 slices are shown. The contrast-to-noise ratio (CNR) between the epineurium (the nerve rim) (white arrows) and the non-enhanced inner region of the nerve is about 41. Note the strong signal enhancement coming from the epineurium, which demonstrates the active transport reaching up to the spinal cord entrance (proximal nerve site F) after the application of a 500 times lower Gd concentration compared to the positive control (25 mM Magnevist).