Selective elimination and analysis of SARM1 inactivation effects in acute optic nerve axonal ischemia

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Purpose

Sterile alpha and (Toll/interleukin receptor (TIR)) motif-containing 1 (SARM1) is an axonal energy level sensor, activated by increased NMN/NAD(+) ratios (Figley, 2020). SARM1 is a key activator of Wallerian degeneration (WLD), in which increased NMN causes rapid axonal collapse in the affected neuron. SARM1 inactivation results in markedly slowed WLD (WLD-*slow; WLDs*) following axonal crush or section. SARM1 knockout inhibits RGC loss in mouse models of pressure-related glaucoma, but does not protect in ON section or crush. No data exists on the effects of SARM1 inactivation following acute axonal ischemia, which occurs in the clinical disease Nonarteritic Anterior Ischemic Optic Neuropathy (NAION). SARM1 is a druggable target. We evaluated SARM1 knockout effects on axonal ischemia using the rodent NAION (rNAION) model.

Methods

Crispr-generated SARM1 knockout rats (SARM1(-)) were unilaterally rNAION induced with 11 seconds of 532nm laser light exposure/532nm wavelength/ 500um spot size/50mW measured at the cornea, following IV administration of 1ml/kg of 2.5mM rose Bengal solution. We induced a similar number of WT animals with identical parameters. Optic nerve head (ONH) edema was evaluated 2d post-induction with SD-OCT, and RGCs were quantified by stereology following retinal flat-mount immunostaining with Brn3a(+) antibody. ON-axonal ultrastructure was evaluated at 5d post-induction with TEM, and ON histology evaluated at 30d post-induction with immunohistochemistry with SMI312 (axons) and MBP (myelin).

Results

ON edema: rNAION-induced SARM1(-) eyes (n=8) had slightly less mean ONH edema than WT (547.6±13.9um sem v. 570.7± 3.9um).

ON Histology: 5d post-rNAION, WT-ONs have severe central loss of SMI312(+) axons and MBP depletion, while SARM1(-) animals show preservation of some, but not all axons (Fig 1).

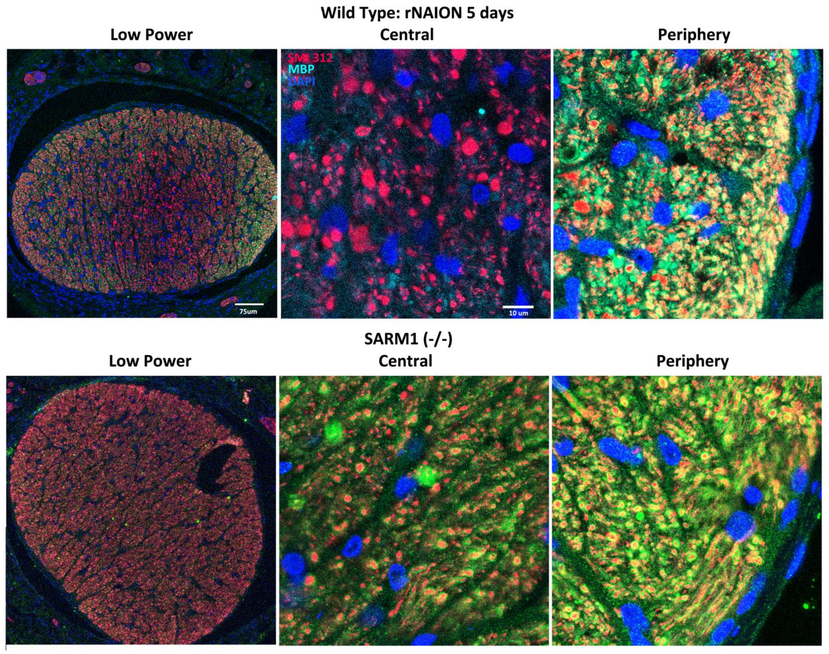

Figure 1. ON Immunohistology 5d post-rNAION in WT (top panels) and SARM1(-) (bottom panels) ONs. There is preservation of SMI312/(red)/MBP (green) axons in the ON periphery of WT animals, as well as in SARM1(-) animals. However, WT axons in the ischemic lesion in the ON central region are degenerated (large red blobs) and there is nearly a complete loss of myelin. In contrast, the central region of SARM1(-) ONs show preservation of many myelinatedf axons.

ON Ultrastructure: A completely different pattern presents in WT vs SARM1 animals 5d post-rNAION induction (Fig 2). WT animals show peripheral axonal preservation (A), with progressively more severe axonal- and myelin- degeneration in the midperipheral to central ON region (B>D). Axons in SARM1(-) animals are also preserved peripherally, and axons also maintain their axoplasm more centrally, with fragmentation, but not loss of myelin. There is preservation of many central axons (arrowheads).

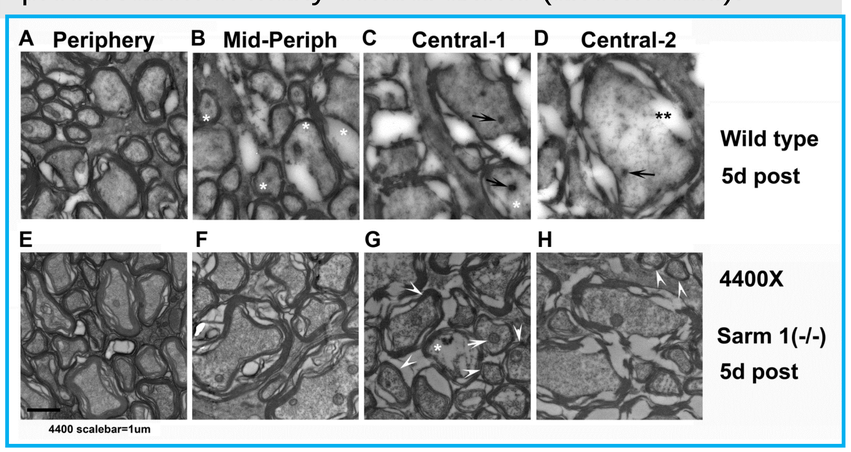

Figure 2: ON-TEM analysis 5d post-rNAION in WT (top) and SARM1(-) (bottom panels) animals. There is peripheral loss in both strains (A,E), but axonal degeneration with swelling and dissolution from mid-periphery to ON center in WT animals (B-D). However, SARM1(-) nerves show axoplasmic preservation, with some myelin fragmentation in the same regions (F-H). There is preservation of occasional central axons (arrowheads).

RGC Stereology: Quantitative analysis of RGC survival in rNAION-induced (n=11) and WT (n=13) eyes 30d post-induction revealed that, in animals with threshold ONH edema (≥500um), WT eyes lost 63±3.9% (sem) vs 49.4±6.1% RGCs. Loss of SARM1 thus provides significant axonal protection difference ($p<0.05$; One tailed Mann-Whitney U test).

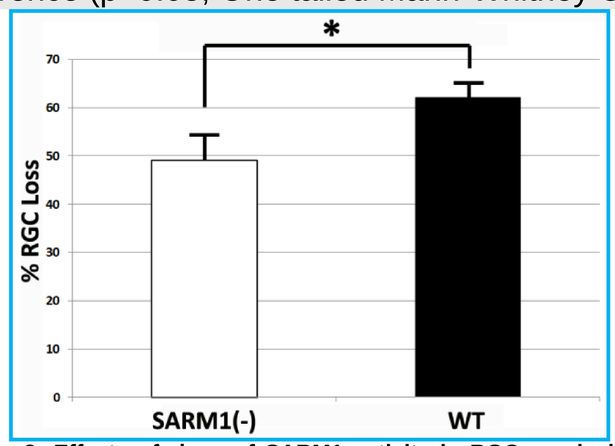

Figure 3: Effects of loss of SARM1 activity in RGC survival following acute axonal ischemia. WT animals expressing SARM1 had a significantly greater loss of RGCs than did CRISPR-generated transgenic rats with a loss of SARM1 activity. Mann-Whitney U test; U value=50; p-value is 0.048 ((p<0.05).

Conclusions

1. Elimination of the SARM1-terminal enzyme activity significantly enhances RGC survival after acute ischemic optic neuropathy. This is different than the lack of effect following ON crush or section.
2. Unlike the original genetic WLDs (NMNAT1 fusion gene), SARM1 is a druggable target, suggesting that pharmacological agents can be used to inhibit SARM1 activity and improve RGC survival following NAION.
3. Combining SARM1 inhibition with other neuroprotective mechanisms with independent action may greatly enhance RGC protection and provide a rational, effective treatment for clinical NAION.

Acknowledgements

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