TECHNICAL PROGRESS REPORTING/FINAL REPORTING TEMPLATE
(Reports must be Cumulative)

SCIRF #: 2015 I-01

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Grant Title: Novel combination therapy for neuroprotection in SCI

6-month Technical Progress Report

Aim 1: Status (Planned, In Progress).

Cumulative detailed description of the progress made towards the aim/sub-aim including the methods utilized:

During the last 6-month period, we made significant progress on Aim 1 of this grant award from the SCIRF. We carried out several experiments in the cell culture model of spinal cord injury (SCI), as we proposed in the Specific Aim 1 of this grant. The only recommended therapy for SCI is methylprednisolone (MP) that shows poor efficacy and its use in SCI is considered highly controversial. So, new therapeutic agents for targeting secondary injury pathways must be developed for neuroprotection in SCI. Recent investigations suggest that retinoids at lower doses are promising neurotherapeutic agents for inhibiting apoptosis (cell death mechanism) and augmenting autophagy (a cell survival mechanism). In our investigation, we examined therapeutic efficacy of our synthetic bororetinoids in cell culture model of SCI. We found that all-trans retinoic acid ATRA, a natural retinoid, at a low dose (1 µM) caused significant cell death while each of our synthetic bororetinoids (BIT-5, BIT-6, and BIT-7) even at 10 µM did not induce significant cell death in ventral spinal cord 4.1 (VSC4.1) motoneurons. In a mono-culture model of SCI, exposure of VSC4.1 motoneurons to 200 nM A23187, a calcium ionophore (CI), for 24 h induced significant neuronal death but 10 µM BIT-5 most significantly prevented the CI induced neuronal death. Our real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) experiments indicated decreases in expression of six neuroprotective microRNAs (miRs) such as miR-34a, miR-138, miR-184, miR-96, miR-98, and miR-133b in VSC4.1 motoneurons after CI insult. Notably, expression of miR-96 was most significantly decreased in the CI insulted motoneurons. So, we hypothesized that overexpression of miR-96 could augment therapeutic efficacy of BIT-5 in the cell culture model of SCI. We are now carrying out the remaining experiments in mono-culture and co-culture models of SCI and also have planned for neuroprotection using our novel combination therapy in acute SCI in rats. Since this award, we published peer-reviewed papers on SCI and also presented results (generated from our studies in cell culture model of SCI) in a scientific meeting.

Describe any data analysis that was performed and include charts/tables when available:

Cytotoxic and neuroprotective effects of natural (ATRA) and synthetic (BIT-5, BIT-6, and BIT-7) retinoids in VSC4.1 motoneurons
Fig. 1. Our MTT assay results showed much lower cytotoxic effect of BITs than ATRA (Fig. 1A). Our study also showed that 10 µM BIT-5 most significantly (p<0.001) provided neuroprotection in VSC4.1 motoneurons exposed to 200 nM CI (Fig. 1B). So, we decided to use 10 μM BIT-5 for neuroprotection in all subsequent experiments.

Changes in expression of neuroprotective microRNAs (miRs) in VSC4.1 motoneurons after CI insult and the most promising miR target prediction
Fig. 2. We observed time-dependent down regulation of six neuroprotective miRs after CI insult, but expression of miR-96 was most significantly (p<0.001) down regulated after day 7 (D-7) (Fig. 2A). So, we decided to use miR-96 overexpression in combination with BIT-5 in all subsequent experiments in this study. Target prediction analysis indicated that mTOR and Cacnb4 mRNA molecules could be two probable targets for their down regulation by miR-96 (Fig. 2B). mTOR and Cacnb4 have significant roles in modulating autophagy and apoptosis pathways, respectively. So, we hypothesized that miR-96 overexpression could enhance the efficacy of BIT-5 through augmentation of autophagy and inhibition of apoptosis.

Aim 2: Status (Planned, In Progress).

Cumulative detailed description of the progress made towards the aim/sub-aim including the methods utilized:

During the last 6-month period, we planned and also initiated work on Aim 2 of this grant award from the SCIRF. We carried out experiments in acute SCI rats using miR-96 and BIT-5 as monotherapy and combination therapy. For in vivo therapeutic studies in acute SCI, adult male Sprague-Dawley rats (weighing 200-250 g, Charles River) were used for induction of moderately severe SCI (40 g.cm force) at T10 by the standardized weight-drop method. Sham (laminectomy without SCI) and SCI plus vehicle (dimethyl sulfoxide or DMSO) animals were used as controls. We used sham (laminectomy without SCI), SCI plus vehicle, and therapeutic agent groups employing miR-96 mimics, BIT-5, and the standard SCI drug MP for 48 h or 2 days. Control SCI animals received <1% DMSO as vehicle. We examined the effects of the treatments in inhibition of apoptosis in acute SCI lesion sections using the in situ ApopTag assay. Spinal cord sections from untreated acute SCI rats showed a lot of dark brown color apoptotic cells. The most significant inhibition of induction of apoptosis was observed following treatment with combination of miR-96 and BIT-5.

Use of the in situ ApopTag assay showed inhibition of apoptosis in acute SCI rats

Fig. 3. In situ ApopTag assay to determine inhibition of apoptosis in acute SCI rats after the treatments. Rats were treated with 50 μg miR-96 mimic via IV injection for the best miR-96 efficacy before induction of SCI. After 12 h, acute SCI (40 g.cm force) was induced following weight-drop method and treated with IP injection of MP (30 mg/kg/day) or BIT-5 (2.5 mg/kg/day) within 15 min of injury. After 48 h, rats were sacrificed, spinal cord (1-cm lesion segment) was collected, and spinal cord lesion sections were investigated via in situ ApopTag assay for determination of amounts of apoptosis.

Combination of miR-96 and BIT-5 most effectively inhibited apoptosis in acute SCI rats
Fig. 4. Combination therapy significantly inhibited apoptosis in acute SCI. Rats in each group (n = 6) were treated following induction of acute SCI, as described in Fig. 3 legend. Apoptotic cells were counted in SCI lesion sections in each treatment group following in situ ApopTag assay. Significant difference between untreated (UNT) group and a treated group was indicated by #p < 0.05 or ##p < 0.01.

List all articles, publications, presentations, grant applications or grant awards related to the SCIRF:

Papers (our recent peer-reviewed publications on SCI):


Abstract (our recent presentation on SCI):