Thymosin Beta-4 Treatment Alters Specific Plasma miRNA Following Severe TBI

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DISCLOSURE:

David Poulsen holds equity in and serves as the Chief Science Officer for NeuroTrauma Sciences
INTRODUCTION
Previous studies have demonstrated that thymosin beta 4 (Tβ4) promotes oligodendrogenesis and improves neurological outcomes in preclinical models of traumatic brain injury and stroke\textsuperscript{1-4}. It has also been reported that Tβ4 up regulates the expression of miR-200a \textit{in vitro} in rat brain progenitor cells and \textit{in vivo} in the peri-infarct region of rat brains following middle cerebral artery occlusion\textsuperscript{5}. MicroRNAs (miRNAs) may cross the blood-brain barrier and serve as plasma-based biomarkers. Here, we used ddPCR to investigate changes in the level of miR-200a as well as other members of the miR-200 family in the plasma of rats following severe TBI. In addition, we completed small RNAseq analysis to identify other potential key changes in plasma miRNAs associated with TBI and Tβ4 treatment.

METHODS

We used the rat lateral fluid percussion injury model to generate mild (1.5 atm) and severe (2.8-3.0 atm) TBI. Injury severity was determined based on a neurological severity score functional assessment. Adult, male, Wistar rats (300-350g) were randomly divided into four treatment groups: 1) uninjured, sham operated controls; 2) mild TBI; 3) severe TBI; or 4) severe TBI treated with 30 mg/kg Tβ4 by IP injection at 8 hours after injury. Whole blood was collected at 32 hrs after TBI (24 hrs after treatment) by cardiac puncture with an 18g needle. Plasma was prepared by serial centrifugation (10 min at 1400 rpm, 4°C; 15 min at 12,000 rpm 4°C). Plasma was then filtered through a 0.45µM filter to ensure removal of platelets. Optical density (OD) was determined for all samples at 414nm to determine hemolysis. Only samples with OD 414nm < 0.2 were used. miRNA were purified with the miRNeasy serum/plasma purification kit (Qiagen). cDNA was prepared using stem loop primers for miR-200a-3p; miR-200b-3p and miR-429 and ddPCR was performed with TaqMan primer-probes assays (ThermoFisher). All values were normalized against a housekeeping miRNA (miR-664) that we had previously did not change following TBI or treatment. RNAseq analysis of plasma miRNAs was completed in the UB Center for Bioinformatics and Life Sciences (CBLS).
RESULTS: (Figure 1) The effects of Tβ4 on miR-200 family plasma miRNAs following severe TBI.

ddPCR results demonstrate that Tβ4 treatment significantly increased the plasma levels of miR-200a-3p and miR-200b-3p at 32 hrs after TBI relative to uninjured controls, mild TBI and severe TBI rats. Tβ4 did not increase miR-429 relative to TBI controls. Data is presented as copies of each target miRNA/µl normalized to a house keeping miRNA (miR-664).
(Figure 2) Tβ4 treatment reduces plasma levels of miR-300-3p and increases plasma levels of miR-194-5p following severe TBI.

Small RNAseq analysis of plasma miRNA identified additional significant changes to miRNAs. Figure 2 shows the relative levels of expression detected. The size of the dots represent the fold change (log2) detected in the plasma relative to uninjured controls for specific miRNAs. Blue dots represent decreased expression. Orange dots represent increased expression. P-values are represented by shading of each respective square. Darker squares indicate lower p-values. Tβ4 treatment significantly reduced the level of miR-300-3p and significantly increased the level of miR-194-5p (4.5 fold) relative to severe TBI.
Changes in specific plasma miRNAs induced by Tβ4 treatment alone.

We focused on changes associated with Tβ4 treatment that could serve as potential biomarkers of a physiological response to the drug. Such markers would be valuable as an inclusion/exclusion tool for clinical trials. The RNAseq data set was filtered based on p-values < 0.001 and at least a four fold change in expression relative to uninjured controls. Nine miRNAs with significant changes were identified. Three were significantly increased (miR148a-3p, miR-151-3p, and miR-106b-3p), while six were significantly decreased (miR125a-5p, miR-15b-3p, miR19b-3p, miR-30e-5p, miR-494-3p and let-7f-2-3p). Increases and decreases in the expression of these specific miRNAs with corresponding significance and fold change were also observed in uninjured rats treated with Tβ4 alone.
DISCUSSION

Consistent with previous observations regarding the levels of miR-200a and miR-200b in the brain following stroke, we report that Tb4 treatment caused an increase of both of these miRNAs in the plasma following severe TBI. In addition, we report here that Tb4 treatment caused a significant decrease in miR-300-3p plasma levels. It has recently been reported that an increase in plasma miR-300-3p could serve as a biomarker for transient ischemia attack\textsuperscript{6}. In addition, we observed a significant increase in plasma miR-194-5p plasma levels induced by Tb4 treatment. It has recently been shown that plasma levels of miR-194-5p are decreased in Alzheimers disease patients\textsuperscript{7} and in patients with drug resistant epilepsy\textsuperscript{8}.

Here we have also identified robust changes in nine plasma miRNAs as a consequence of Tβ4 treatment that can be observed in both uninjured and severe TBI rats. These particular miRNA changes could serve as valuable tools to define inclusion/exclusion criteria for data analysis in future clinical trials of Tβ4.


SUMMARY POINTS

• ddPCR analysis indicated that plasma levels of both miR-200a-3p and miR-200b-3p significantly increase following treatment with Tb4 after severe TBI.

• RNAseq analysis suggests that Tb4 treatment significantly increases expression of miR-300-3p and significantly decreases expression of miR-194-5p relative to severe TBI controls.

• RNAseq analysis suggests that Tb4 treatment alone significantly increased expression of miR148a-3p, miR-151-3p, and miR-106b-3p, significantly decreased expression of miR125a-5p, miR-15b-3p, miR19b-3p, miR-30e-5p, miR-494-3p and let-7f-2-3p relative to injured and uninjured controls.