Edaravone Reduces TDP-43 Levels but not Stress Granule Formation in Sodium Arsenite-Treated TDP-43 Overexpressing Cells

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BACKGROUND

The RNA-binding protein TDP-43 is linked to neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Studies have shown that cytoplasmic TDP-43 aggregates co-localize with stress granule (SG) markers. SGs are cytoplasmic inclusions that repress translation of a subset of RNAs during cellular stress. Since it was shown that SG formation contributes to accumulation of TDP-43, inhibition of SG formation and/or recruitment of TDP-43 to SGs are pathways that are currently in the focus of ALS research. To be able to support this research, we have developed a respective *in vitro* model in TDP-43-overexpressing neuroblastoma cells.

MATERIAL & METHODS

Human TDP-43 overexpressing neuroblastoma cells (SH-SY5Y-hTDP-43) were stimulated with the well-described SG inducer sodium arsenite (SA, 200 µM) and treated with 1 µM edaravone (EDA) or 10 µM cycloheximide (CHX) as possible rescuing agents. TDP-43 aggregation in soluble and insoluble protein fractions was analyzed by ProteinSimple WES™ technology. SG formation and TDP-43 recruitment were investigated via immunocytochemistry.

RESULTS

WES analysis showed a strong shift from soluble to insoluble TDP-43 species upon SA stimulation.

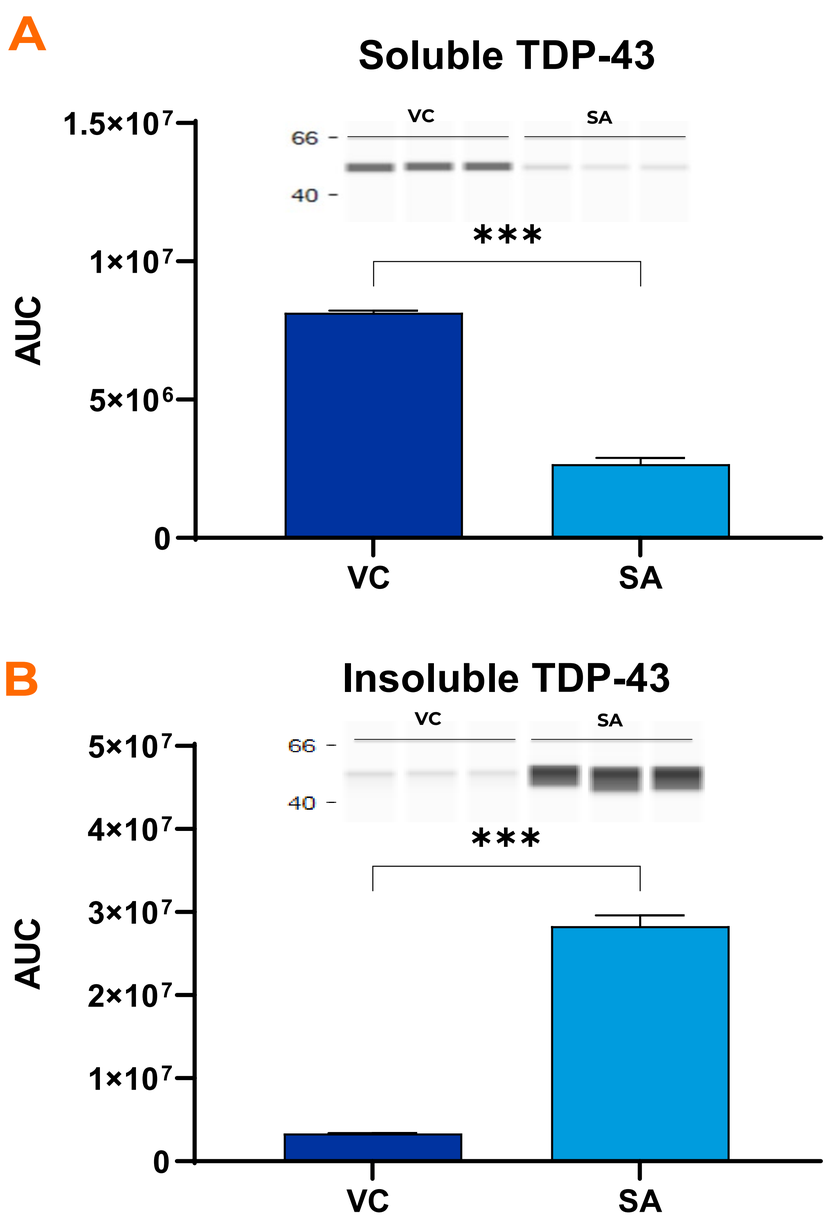

Figure 1: Effect of sodium arsenite (SA) treatment on soluble and insoluble TDP-43 levels. SH-SY5Y-hTDP-43 cells were harvested after treatment with 200 µM SA or vehicle (VC) and soluble and insoluble protein fraction were prepared. Both fractions were analyzed for TDP-43 on ProteinSimple WES™. WES lane view of TDP-43 signal in soluble and insoluble fraction. Data are shown as area under the curve (AUC) and presented as bar graphs with mean + SEM (n=3 per group). Two-tailed unpaired *t-test;* ***p<0.001.

Immunocytochemical staining for the SG marker G3BP revealed substantial SG formation in SA-treated cells compared to the respective vehicle control. Cycloheximide counteracts SG formation, whereas edaravone had no impact on stress granule formation, but on TDP-43 immunoreactive area.

Cycloheximide (CHX)

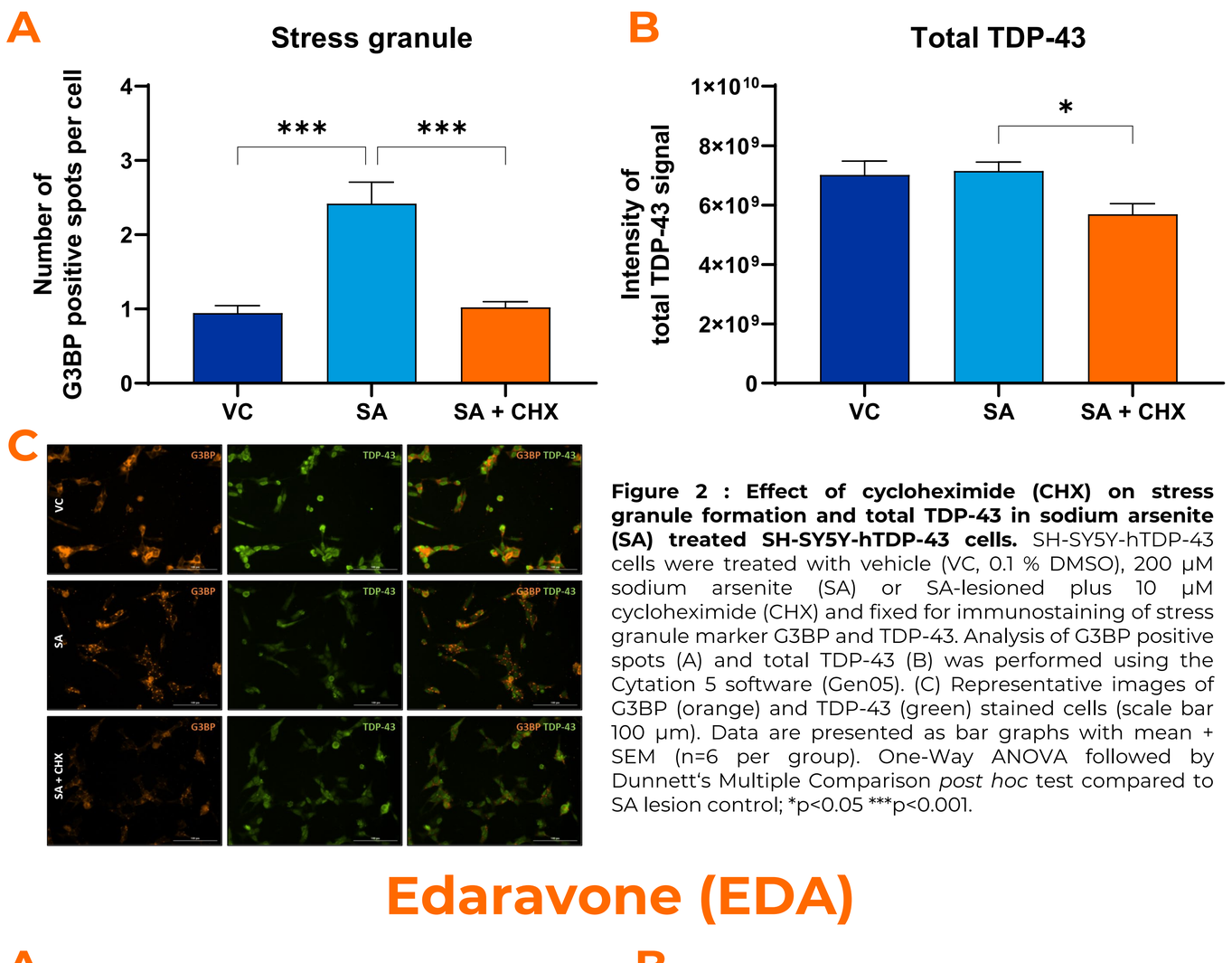

Figure 2 : Effect of cycloheximide (CHX) on stress granule formation and total TDP-43 in sodium arsenite (SA) treated SH-SY5Y-hTDP-43 cells. SH-SY5Y-hTDP-43 cells were treated with vehicle (VC, 0.1 % DMSO), 200 µM sodium arsenite (SA) or SA-lesioned plus 10 µM cycloheximide (CHX) and fixed for immunostaining of stress granule marker G3BP and TDP-43. Analysis of G3BP positive spots (A) and total TDP-43 (B) was performed using the Cytation 5 software (Gen05). (C) Representative images of G3BP (orange) and TDP-43 (green) stained cells (scale bar 100 µm). Data are presented as bar graphs with mean + SEM (n=6 per group). One-Way ANOVA followed by Dunnett's Multiple Comparison *post hoc* test compared to SA lesion control; *p<0.05 ***p<0.001.

Edaravone (EDA)

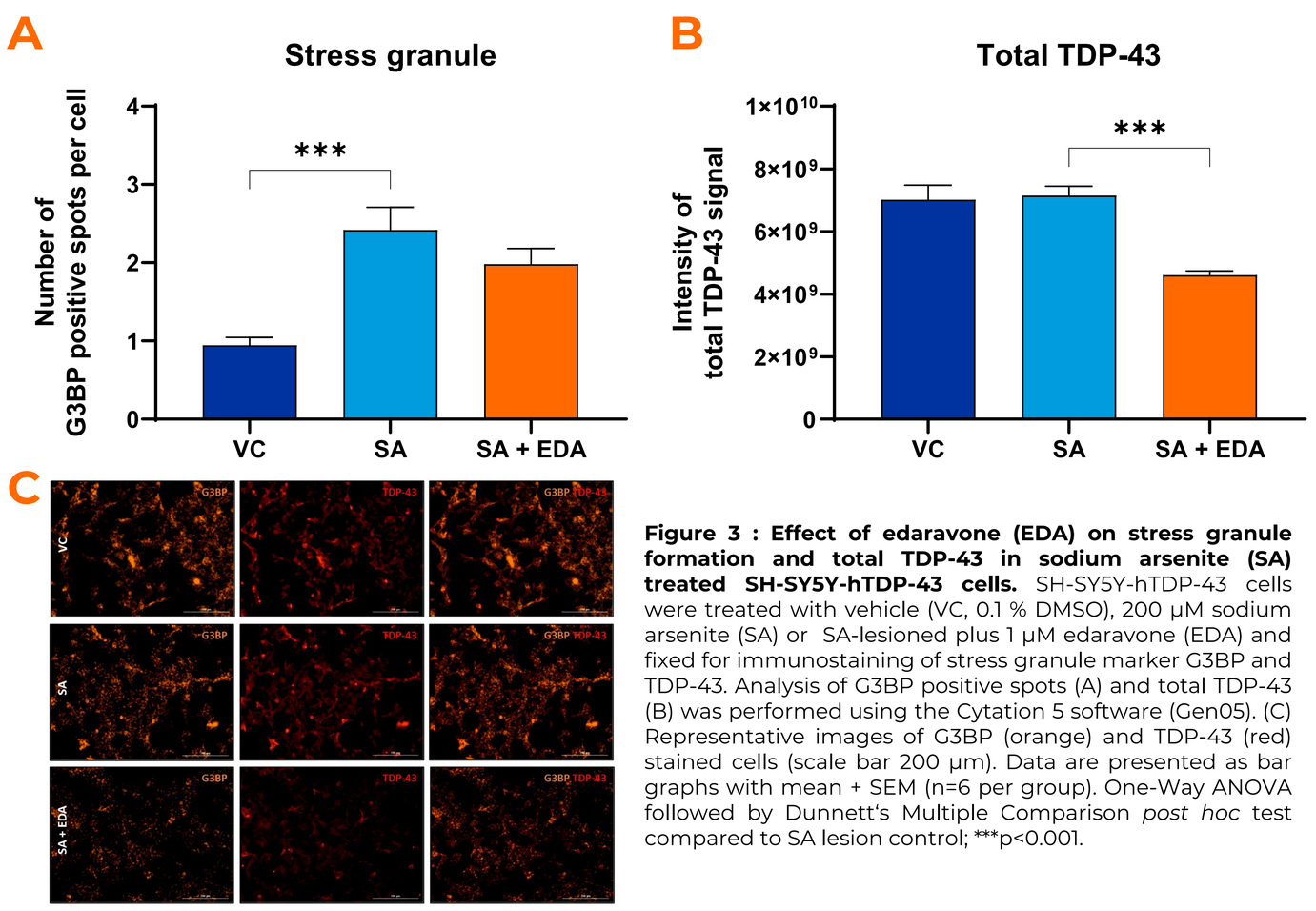

Figure 3 : Effect of edaravone (EDA) on stress granule formation and total TDP-43 in sodium arsenite (SA) treated SH-SY5Y-hTDP-43 cells. SH-SY5Y-hTDP-43 cells were treated with vehicle (VC, 0.1 % DMSO), 200 µM sodium arsenite (SA) or SA-lesioned plus 1 µM edaravone (EDA) and fixed for immunostaining of stress granule marker G3BP and TDP-43. Analysis of G3BP positive spots (A) and total TDP-43 (B) was performed using the Cytation 5 software (Gen05). (C) Representative images of G3BP (orange) and TDP-43 (red) stained cells (scale bar 200 µm). Data are presented as bar graphs with mean + SEM (n=6 per group). One-Way ANOVA followed by Dunnett's Multiple Comparison *post hoc* test compared to SA lesion control; ***p<0.001.

CONCLUSION

The presented *in vitro* model using SA as stressor to induce SGs is a suitable system to study SG formation and TDP-43 aggregation, which are differently affected by the tested agents edaravone and cycloheximide.

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