A DOSE- RESPONSE STUDY TO IDENTIFY THE MINIMAL EFFECTIVE DOSE OF EXOSOMES THAT PROMOTE RECOVERY AFTER STROKE

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Background and Aims

Extracellular vesicles such as exosomes has opened a new field of research.

Exosomes are able to transfer DNAs, mRNAs, microRNAs, proteins, trophic factors and lipids.

Exosomes released by stem cells have shown efficacy in experimental animal models of stroke, promoting brain remodeling and functional recovery.

Aim: To assess the efficacy of 3 doses of exosomes from adipose tissue derived mesenchymal stem cells to identify the minimal effective dose to enhance brain repair and recovery after subcortical stroke in rats

Material/ Methods

Subcortical ischemic stroke was induced by Endothelin-1 into striatum. Intravenous exosomes or saline were administrated at 24h after cerebral infarct. Exosomes were isolated from culture of adipose mesenchymal stem cell and they were characterized by Nanoshight, Electronic microscope, Western blot and Immunofluorescence. We evaluated: motor function (Rotarod, Beam Walking and Roger's test) at 24 hours, 7 and 28 days, lesion volume and tract connectivity were studied by magnetic resonance image at 24 hours and 28 days, cell death by TUNEL and brain repair markers: GFAP (Glial Fibrillary Acidic Protein), Syp (Synaptophysin), BDNF (Brain derived Neurotrophic Factor), MBP (Myelin Basic Protein) and MOG (Myelin Oligodendrocyte Glycoprotein) and analyzed by immunofluorescence at 28 days.

Figure 1: Experimental protocol schematic

Figure 2: Exosomes characterization. A) Histogram representing a distribution graph on a particle-by-particle (events) and size of exosomes using Nanosight, B) Exosomes image by electronic microscope, C) ALIX by Western-Blot and D) ALIX by Immunofluorescence

Results

Biodistribution

Figure 3. Labeled exosomes were found in brain tissue and in peripheral organs such as the lung, liver and kidney at 24 hours after administration

Functional evaluation

Figure 4. A) Animals treated with 50µg, 100µg or 200µg of exosomes showed a significant improvement in the functional evaluation compared to the control. B) Between treatments, high dose of exosomes demonstrates better functional recovery (Rotarod, Beam Walking and Roger's tests) than low and intermediate doses at 24 hours and 7 days, but without significant differences at 28 days

Figure 5. A) No reduction of the lesion size in treated compared to control groups. B) All doses showed the same significant increase in tract connectivity compared to control group at 28 days

Figure 6. Cell death was decreased in all doses compared to control group at 28 days

Figure 7. All doses decreased GFAP and increased Syp, BDNF, MBP and MOG expressions compared to control group

Conclusions

- ❖ 50µg, 100µg and 200µg of exosomes improves the functional recovery, tract connectivity and expression of brain repair markers.
- ❖ Low dose is as effective as intermediate and high doses.
- ❖ 50µg of exosomes is the minimal effective dose to enhance brain repair and recovery in stroke.
- ❖ From a translational point of view, this dose decreases the cost and risk.