Fundació La Marató TV3

23rd SOCIAL RETURN OF THE RESEARCH
Strokes and traumatic spinal cord and brain injury

CLINICAL GRADE COMBINED CELL, TRANSPLANT THERAPY WITH SMALL NEUROPROTECTIVE CHEMICAL PRODUCTS IPSC-DERIVED INA RAT MODEL FOR CENTRAL SCI REGENERATION OF SPINAL CORD PATHWAYS

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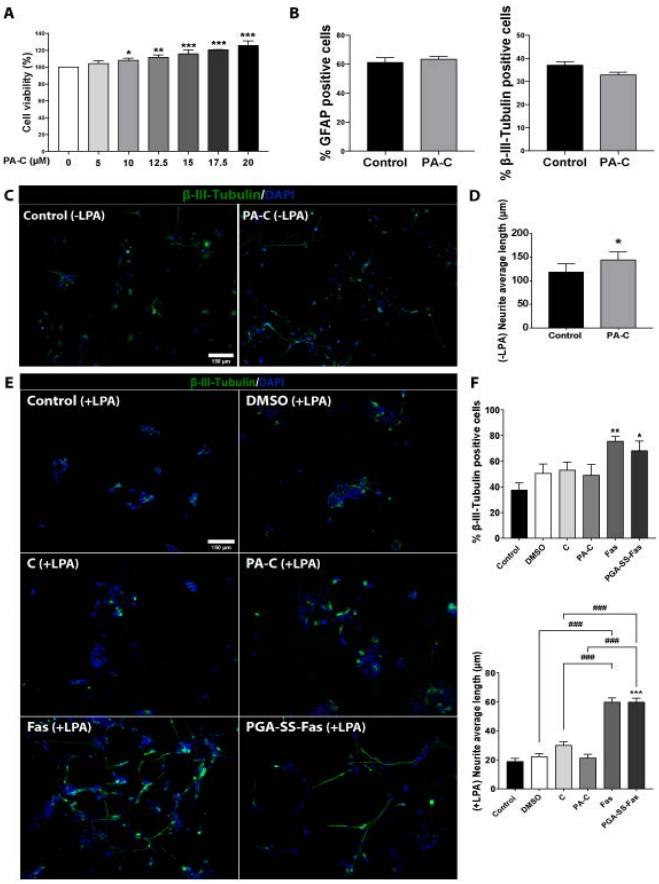
1. Summary

We currently lack effective treatments for the devastating loss of neural function associated with spinal cord injury (SCI). In this study we evaluated a combination therapy comprising human neural stem cells derived from induced pluripotent stem-cells (iPSC-NSC), human mesenchymal stem cells (MSC), and a pH responsive polyacetal-curcumin nanoconjugate (PA-C) that allows the sustained release of curcumin. In vitro analysis demonstrated that PA-C treatment protected iPSC-NSC from oxidative damage in vitro, while MSC co-culture prevented lipopolysaccharide-induced activation of nuclear factor-κB (NF-κB)in iPSC-NSC. We then evaluated the combination of PA-C delivery into the intrathecal space in a rat model of contusive SCI with stem cell transplantation. While we failed to observe significant improvements in locomotor function (BBB scale) in treated animals, histological analysis revealed that PA-C- or PA-C and iPSC-NSC+MSC-treated animals displayed significantly smaller scars, while PA-C and iPSC-NSC+MSC treatment induced the preservation of β-III tubulin-positive axons. iPSC-NSC+MSC transplantation fostered the preservation of motoneurons and myelinated tracts, while PA-C treatment polarized microglia into an anti-inflammatory phenotype. Overall, the combination of stem cell transplantation and PA-C treatment confers higher neuroprotective effects compared to individual treatments.

2. Results

PA-C treatment increases iPSC-NSC viability, enhances neurite elongation, but fails to induce neural and glial differentiation: We previously described that polyacetal-curcumin conjugate (PA-C, curcumin loading 3.8% w/v), displayed significantly lower cytotoxicity than free curcumin (C) in primary cultures of adult rat spinal cord NSC (Requejo et al, 2017). To elucidate whether PA-C influences the viability of iPSC-NSC, we performed an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell viability assay at 24 h after treatment with increasing concentrations (up to 20 μM) of PA-C at curcumin equivalent concentrations. We discovered that the evaluated concentrations of PA-C failed to induce cytotoxicity in iPSC-NSC; moreover, PA-C treatment significantly increased iPSC-NSC metabolic activity in a dose-dependent manner at concentrations higher than 10 μM (Figure 1A).Next, we studied whether a 24 h treatment with 10 μM



PA-C influenced iPSC-NSC fate determination. Staining for cells positive for GFAP or beta-III-tubulin indicated that PA-C treatment failed to alter the proportion of astrocytes and neurons, respectively, compared to control vehicle-treated iPSC-NSC (Figure 1B). We also analyzed gene expression profiles using an RT2 Profiler PCR Array that includes a set of genes related to neural differentiation such as brain-derived neurotrophic factor (BDNF), oligodendrocyte transcription factor (Olig2), SOX2, achaete-scute family BHLH transcription factor 1 (ASCL1), dopamine receptor D2 (DRD2) and apoptosis related genes such as adenosine A1 receptor (ADORA1) and BCL2. PA-C treatment failed to influence the expression of genes involved in apoptosis, which agrees with the results of the cytotoxicity assays (Figure 1A). Furthermore, from this select group of genes, only DRD2 expression became significantly downregulated after treatment with PA-C ((*p<0.05 compared to control vehicle-treated iPSC-NSC), indicating that PA-C treatment did not significantly induce neural differentiation of iPSC-NSC. Previous studies established that PA-C treatment induced neurite outgrowth. While the incubation of iPSC-NSC with 10 μM PA-C for 24 h failed to induce significant neural differentiation, we found that PA-C treatment significantly induced neurite elongation in beta-III tubulin-positive cells (Figure 1C-D). We also evaluated the functional relevance of induced neurite elongation by PA-C treatment in an inhibitory environment by treating iPSC-NSC with lysophosphatidic acid (LPA), which activates the rho/ROCK pathway and induces growth cone retraction and neurite collapse. We employed free fasudil (Fas), a well-known rho kinase inhibitor, and a nanoconjugated form of fasudil (PGA-SS-Fas) as positive controls for neurite elongation. In a similar manner to curcumin, we previously reported that polymer conjugation enhanced the stability of fasudil and supported sustained release, which improved the neuroprotective and

regenerative activity of fasudil in SCI models. While treatment with PGA-SS-Fas or fas (Figure 1E-F)permitted a significant increase in axonal elongation in the presence of LPA, we found no differences following the treatment of iPSC-NSC with PA-C (Figure 1D) or C (Figure 1E-F). Representative images of beta-III-Tubulin immunostaining in the absence of LPA treatment show neurite elongation following PA-C treatment (Figure 2C), abundant and long neural extensions following PGA-SS-Fas treatment (Figure 1E-F), and the absence of morphological changes in PA-C-treated iPSC-NSC in the presence of LPA in comparison with vehicle-treated control iPSC-NSC (Figure 1E-F).

PA-C and PA-C combined with iPSC-NSC+MSC transplantation preserve beta-III-tubulin positive fibers, limit inhibitory scar size, and increase functional synapse number after SCI: We analysed longitudinal spinal cord sections, including the epicenter of the injury and rostral and caudal segments to the injury, by double immunostaining with beta-III-tubulin (representative images shown in Figure 2A; left panels, green) and GFAP (right panels, red) to quantify the nerve fiber preservation. The quantification of beta-III-tubulin staining (Figure 2B; top panel) revealed a significantly larger positive area present in animals receiving iPSC-NSC+MSC+PA-C treatment than control animals or those receiving iPSC-NSC and MSC but not in comparison with PA-C treatment. PA-C-treatment prompted a significantly larger beta-III tubulin-positive area than iPSC-NSC or MSC treatments. Of note, we observed a significant reduction in the number of neuronal fibers following iPSC-NSC or MSC treatment compared to the control or iPSC-NSC+MSC+PA-C treatment.

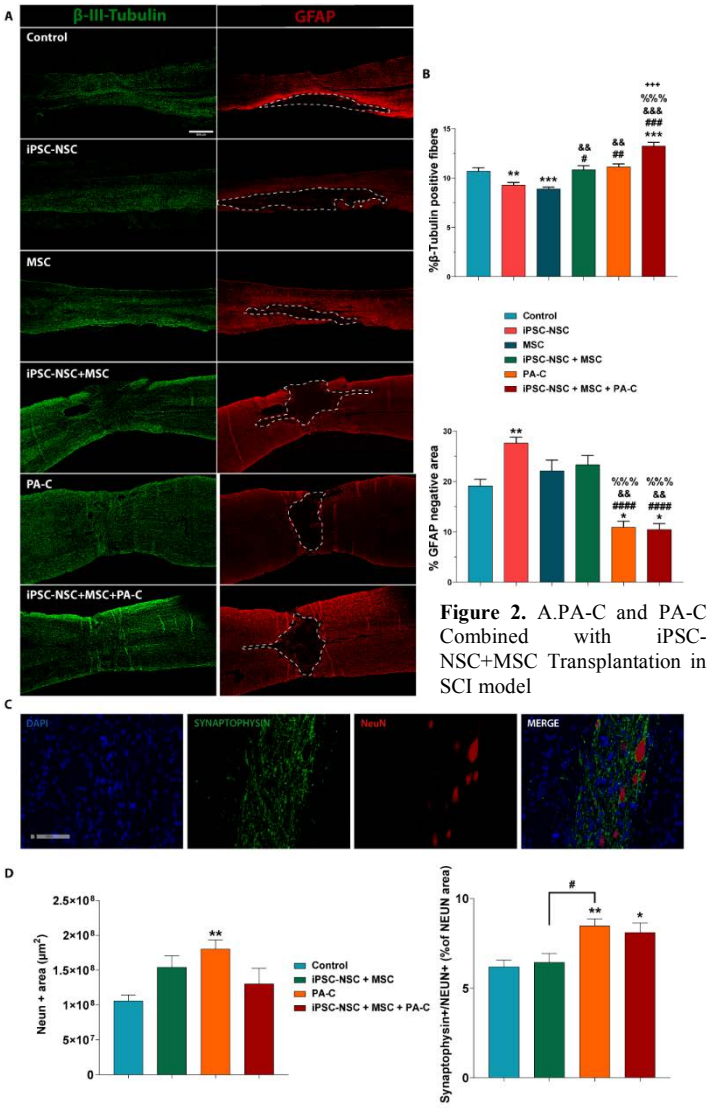


Figure 2. A.PA-C and PA-C Combined with iPSC-NSC+MSC Transplantation in SCI model

We also evaluated the size of the inhibitory scar by quantifying the lack of GFAP staining, expressing this value as a percentage of the total quantified tissue area (Figure 2A - right panel, dashed line; Figure 2B - bottom panel). PA-C and iPSC-NSC+MSC+PA-C treatments significantly reduced scar area compared to other treatments. iPSC-NSC treatment resulted in an increased scar area compared to control; however, iPSC-NSC+MSC or MSC treatments failed to induce any significant alterations.

To evaluate the preservation of functional synapses we quantified the synaptic bottoms at neuronal somas by co-localizing synaptophysin and NeuN for those treatments providing better outcomes in the previous neuronal fiber preservation analysis (Figure 2C shows representative images of each of the tested stainings from the control group). Analysis of preserved NeuN-positive cells, expressed as the percentage of NeuN-positive area, demonstrated a significant increase following PA-C treatment compared to control (Figure 2D; left panel). Furthermore, the quantification of co-localization (synaptophysin/NeuN+) normalized to the total NeuN-positive area demonstrated a significant increase following PA-C or iPSC-NSC+MSC+PA-C treatment when compared to control (Figure 2D; right panel).

PA-C combined with iPSC-NSC+MSC transplantation induces white matter sparing after SCI:

We determined white matter sparing by Luxol fast blue (LFB) staining - LFB binds to the myelin sheath lipoproteins and allows quantification of the remaining myelinated areas. LFB analysis (Figure 3A-B) demonstrated that iPSC-NSC and MSC treatments increased spinal cord white matter sparing compared to control and PA-C treatment. Furthermore, iPSC-NSC+MSC+PA-C treatment also increased white matter preservation compared to control and PA-C treatment.

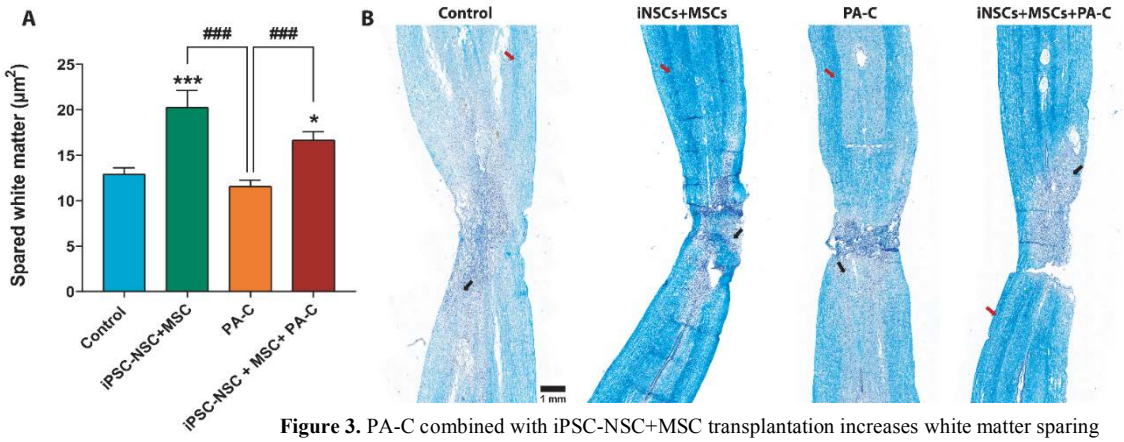


Figure 3. PA-C combined with iPSC-NSC+MSC transplantation increases white matter sparing

PA-C and PA-C combined with iPSC-NSC+MSC transplantation promotes microglia polarization towards an anti-inflammatory profile: We next evaluated the potential in vivo anti-inflammatory activity of PA-C or iPSC-NSC+MSC+PA-C by studying the presence of activated microglia with an anti-inflammatory profile by the co-localization of IBA1 expression (microglia marker) *and arginase-1 (Arg1) (*Figure 4A-B). After quantifying the percentage of cells expressing IBA1, Arg1, or IBA1 and Arg1 (Figure 4C), we found that both treatments (PA-C or iPSC-NSC+MSC+PA-C) increased the presence of anti-inflammatory microglia (IBA1+/Arg1+) in the spinal cord nine weeks post-SCI.

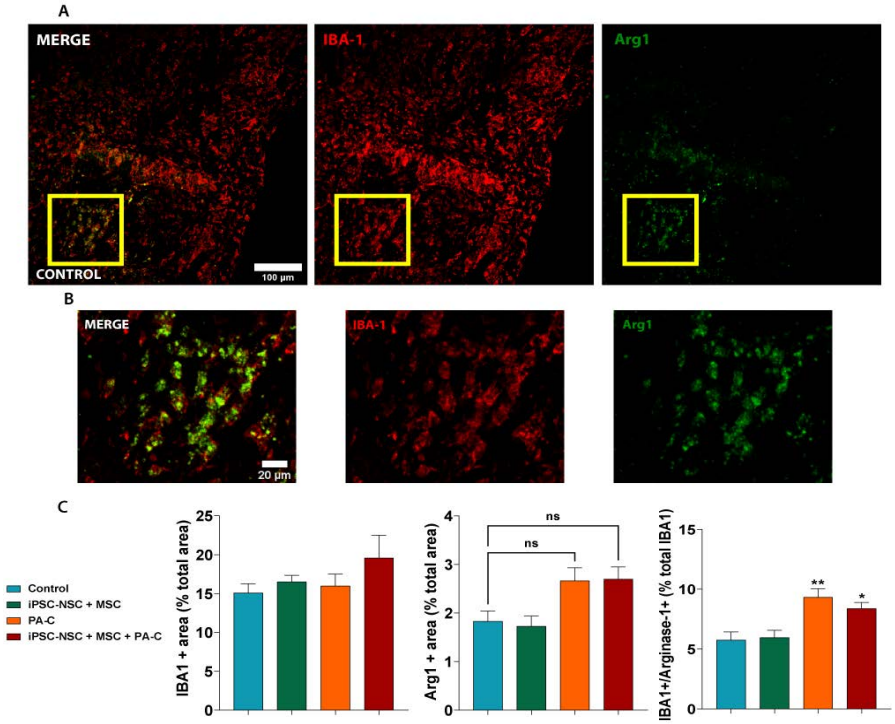


Figure 4. PA-C and PA-C combined with iPSC-NSC+MSC transplantation prompt microglial polarization towards an anti-inflammatory profile

3. Relevance and possible clinical applicability of the final results

The complex pathological nature of the traumatic spinal cord injuries requires the implementation of a multifaceted and versatile therapeutic perspective regarding the development of treatments. The single application of unique strategies based on cell transplantation of either mesenchymal cells (MSC) or neural precursor cells (NPC) has already shown beneficial effects on clinical applications. We have accomplished a combination of already tested treatments in individual applications in order to enhance their functional outcomes. We have demonstrated within this project that the combined treatment comprising PA-C (a water soluble and stable conjugation of curcumin), iPSC-NSC, and MSC provides immunomodulatory and neuroprotective effects to prevent axonal degeneration, neuronal death, and loss of neuronal connectivity. Furthermore, this combinatorial strategy reduced the injury area and prevented the expansion of the glial scar in chronic stages, thereby providing a versatile and clinically relevant approach to near future been implemented in the treatment of acute SCI.

4. Publications, communications and training of personnel derived from this research

A synthetic mRNA cell reprogramming method using CYCLIN D1 promotes DNA repair generating improved genetically stable human induced pluripotent stem cells.
Alvarez-Palomo AB, Requena-Osete J, Delgado-Morales R, Moreno-Manzano V, Grau-Bove C, Tejera AM, Otero MJ, Barrot C, Santos-Barriopedro I, Vaquero A, Mezquita-Pla J, Moran S, Naya CH, Garcia-Martínez I, Pérez FV, Blasco MA, Esteller M, Edel MJ. Stem Cells. 2021 Feb 23. doi: 10.1002/stem.3358.

Human-Induced Neural and Mesenchymal Stem Cell Therapy Combined with a Curcumin Nanoconjugate as a Spinal Cord Injury Treatment.
Bonilla P, Hernandez J, Giraldo E, González-Pérez MA, Alastrue-Agudo A, Elkhenany H, Vicent MJ, Navarro X, Edel M, Moreno-Manzano V. Int J Mol Sci. 2021 May 31;22(11):5966. doi: 10.3390/ijms22115966

Doctoral thesis, title: Human-Induced Neural and Mesenchymal Stem Cell Therapy Combined with a Curcumin Nanoconjugate as a Spinal Cord Injury Treatment.

Doctoral student: Pablo Bonilla (under preparation, expected to be defended in December 2021)