Artificial Intelligence Revealed Neuroprotective Drug for Nerve Trauma

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SUMMARY

Single target therapeutic approach have been proved to be insufficient to find out efficient therapies for several neurodegenerative process. Peripheral nerve injuries (PNI) are the major cause of disability worldwide. In order to find out patentable drug combinations to treat PNI we used a network-centric approach. We performed proteomic and System Biology approach couple to the computational tool therapeutic performance mapping system (TPMS) technology. For that purpose, we used two pre-clinical rat models: a Root Avulsion (RA) model that produces retrograde motoneurodegeneration, and a Distal Axotomy (DA) model that promotes MNs survival and regeneration. Comparative proteomic data together with manual curator motivated helped to build up topological and mathematical maps of protein-protein interactions associated to each of these conditions (degenerative or regenerative). By the use of TPMS based on artificial neural intelligence, we screened drug bank libraries to find out the precise stimulus based on drug combinations capable to revert one topological map into the other. Novel in silico drug combinations were found and its therapeutic effect were validated for neuroprotection, anti-inflammation and pro-regeneration in vivo as well as in its synergism in vitro. Furthermore its in silico predicted mechanism of synergetic action was also validated. Thus, we found a promising therapy for neuroprotection to PNI already known to be safe for humans, that cross the brain blood barrier and that activate sirtuin 1.

WORKFLOW

RESULTS

**In silico designed polypharmacology exerted neuroprotection**

**Figure 2.** Left: Nissl stained spinal cord at L4-L5 level ventral horns of control or avulsed side level from untreated or intralesially treated rats. A and B are compounds of C1. Right: Fluorescence MN survival at the ipsilateral side 21 days post-injury.

**The mechanism of Action of C1 involved the cytoskeleton**

**Figure 3.** Left: Microphotographs of fluorescent labelled astrocytes (GFAP), microglia (Iba1) or neurons (GAP-43) at the ipsilateral side in the different conditions. Right: Bar graph of the average of immune-fluorescence quantification of a fixed region of interest in the ipsilateral side normalized to the contralateral.

**C1 reduced gliosis and induced a pro-regenerative neuronal profile**

**Figure 4.** Histogram of the average percentage of cell survival ± SEM after endoplasmic reticulum stress by 1μg/mL of tunicamycin (TN).

**C1 exerts neuroprotection in vitro against endoplasmic reticulum stress and presented synergism**

**Figure 5.** A. Schematic diagram of the workflow followed to elucidate SIRT1 role/s in MN survival after C1 treatment and MN survival after different combinatorial treatments. B. Microphotographs of spinal cord avulsed MNs immunostained for SIRT1 counterstained with Nissl (green) and quantification of the % of MNs with high nuclear immunofluorescence intensity for different markers. C. AAVrh13-SIRT1 overexpression increase MN survival.

**C1 activated sirtuin 1 to exert neuroprotection**

**Figure 6.** A. **Statistical analysis** showing relative expression of Sirtuin 1 (SIRT1) in MNs at different stages of Sirtuin 1 overexpression. B. **Flow chart** showing the process of Sirtuin 1 activation and MN survival. C. **Graph** showing the percentage of cell survival ± SEM after endoplasmic reticulum stress by 1μg/mL of tunicamycin (TN).

**CONCLUSIONS**

- C1 produces neuroprotection in vivo and in vitro.
- C1 reduces inflammation of the nervous system.
- C1 promotes a pro-regenerative profile.
- C1 presents supra-additive effect respect to single components.
- In silico Prediction of C1 MoA is ascertained.

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