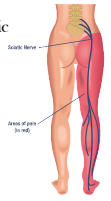
Human Muscle Progenitor Cells Overexpressing Neurotrophic Factors for Improving Intrinsic Neuronal Regeneration in Sciatic Nerve Injury Mice Model

Offen D.[1] , Guy R.[1] , Ben–Zur T.[1], Benninger F.[1], Glat MJ.[1], Panski A.[2], Lamdan R.[2], Danon U.[3], Yaffe D.[4], Grynspan F.[3]

[1] Laboratory of Neuroscience, Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[2] Orthopedics Surgery, Hebrew University Hadassah Medical Centers, Jerusalem, Israel
[3] Stem Cell Medicine Ltd, Jerusalem, Israel
[4] Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

Background

- Peripheral nerve injury can occur in daily life due to mechanical damage resulting from traffic accidents, sports, or surgery. Although the peripheral nervous system has an intrinsic ability to regenerate after injury, the regeneration process is slow, incomplete, and is often accompanied by disturbing motor, autonomic and sensory consequences [1]. The sciatic nerve comprises both motor and sensory fibers; therefore, the **sciatic nerve injury (SNI)** model is one of the most common models for peripheral nerve injury.
- **Neurotrophic factors (NTFs)** are known [2] for their ability to protect peripheral motor neurons and enhance axon regeneration and functional recovery.



Objectives

In this study, we assessed the administration of rats and human muscle progenitor cells (MPC) overexpressing NTF genes, known to protect peripheral motor neurons and enhance axon regeneration and functional recovery, in a SNI model.

Materials and Methods

IN-VITRO

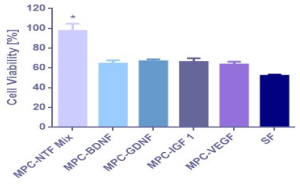
- BDNF, GDNF, VEGF, or IGF-1 gene were amplified and inserted into the destination plasmid, under the CMV promoter and cloned into lentiviral vectors [2]. MPC isolated from L8 rat myogenic cells and human muscles biopsies were transduced with these genes.
- The ability of the MPC conditioned media to protect motor neuron cell-line (NSC-34) from hypoxic stress was assessed. NSC-34 was placed in a hypoxic environment for 48 hours. After 48 hours, Alamar blue 10% was added to the cells for 6 hours. Results were read at wavelengths of 590nm using a fluostar device.

IN-VIVO

- SNI was performed in rats or mice and injected to the lesion site one day later with L8 rat myogenic ectopically expressing the NTF genes or human MPC-NTFs (hMPC-NTFs).
- Motor function was tested in rats using the rotarod test.
- Endplate innervations of the hind limb muscles of the rats were marked by alpha-bungarotoxin and synaptophysin.
- Gait pattern was tested in mice, using the CatWalk XT system, three and seven days post-treatment.
- Sensory nerve response was tested in mice using the hot-plate test 6 days post-treatment. The hot-plate temperature was adjusted to 55 ±0.5 °C, and a cut-off period of 20 seconds was maintained [4].

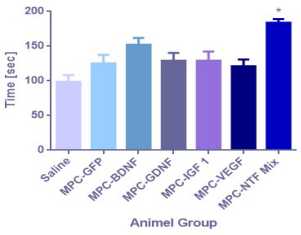
Results

- Exposure of motor neuron cell line NSC-34 to a hypoxic environment, resulted in a 50% reduction in viability.
- Exposure of the cells to conditioned medium collected from cells expressing only a single NTF (BDNF, GDNF, IGF-1 or VEGF) had an non-significant protective effect.
- Cells treated with the MPC-NTFs mix conditioned medium were **almost fully protected**.



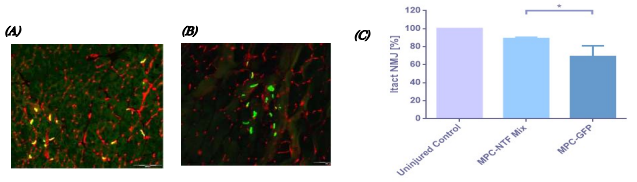
*Conditioned medium of genetically modified L8 cells (MPC-NTFs mix) rescues NSC34 cells in culture. The assay was conducted in triplicate. Data are presented as the mean ± SEM. *P< 0.05, ANOVA test.*

- Three days after SNI, the saline-treated rat group demonstrated poor motor function on a rotarod test.
- Injured rats treated with cells expressing green fluorescent protein (GFP) or cells expressing a single NTF showed an insignificant, moderate improvement.
- In contrast, the rats treated with the MPC-NTF mix demonstrated a significant **improvement in motor performance**. A similar trend was observed six and eight days after the crush.



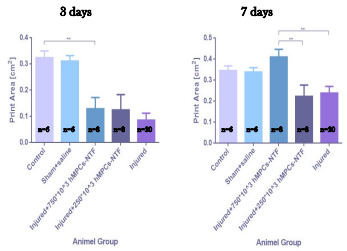
*Transplantation of genetically modified L8 cells expressing NTF improved motor function on a rotarod after sciatic nerve injury. Data are presented as the mean ± SEM, n=9. *P< 0.05, ANOVA test.*

- Injected myogenic cells that secrete NTFs **reduced neuromuscular junctions (NMJs) denervation**, as indicated by double staining of the hind limb muscles using alpha bungarotoxin and antisynaptophysin antibodies.



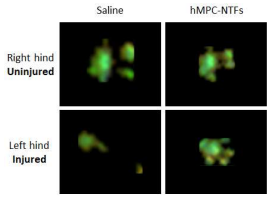
Double staining of the hind limbs muscles using alpha bungarotoxin (green) and antisynaptophysin antibodies (red) antibodies transplanted with (A) MPC-NTF Mix (100µm) or with (B) MPC-GFP.(C) Quantification of intact NMJs. Data are presented as the mean ± SEM, n=5. *P<0.05, one-tailed t-test.

- The paw print area of the injured paws were significantly smaller three days post-treatment than those of the control and sham groups, regardless of the treatment given.
- On the other hand, seven days post-treatment, there was a significant increase in the printed area for the group of injured mice treated with 750x10^3 hMPC-NTFs.



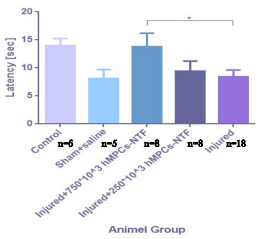
*Left hind paw print areas acquired using CatWalk XT system 3 and 7 days post treatment. Data are presented as the mean ± SEM of n mice per treatment group. *P<0.05, **P<0.01, one-tailed t-test.*

- Improvement in the **gait pattern** of the injured mice treated with 750x10^3 hMPC-NTFs is displayed by two representative images of left hind paw after sciatic nerve crush, assessed seven days post-treatment. The left image in the panel shows an exemplary footprint from the injured mice group treated with 750x10^3 hMPC-NTFs, and the right image is an exemplary footprint from the untreated injured group.



Representative images of paw prints, acquired using CatWalk XT system, 7 days post transplantation of 750x10^3 hMPC-NTFs (left) or without treatment (right).

- The injured mice treated with 750x10^3 hMPC-NTFs showed **less paw sensitivity**, than both untreated injured mice and mice treated with 250x10^3 hMPC-NTFs, and their response resembled that of naïve control mice.



*Nociceptive threshold of the left hind paw was tested by measuring latency of the analgesic response in the hot - plate test. Data are presented as the mean ± SEM of n mice per treatment group. *P<0.05, one-tailed t-test.*

Conclusions

Genetically modified rats and human MPCs were found to synergistically alleviate both motoric and sensory deficits of SNI, suggesting that MPC-NTFs may have therapeutic potential for treating nerve injuries and pain related syndromes.

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