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Embryonic Stem Cells and Retina: Neurosphere Method

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PURPOSE
The purpose of this study is to evaluate the potential for survival, migration, differentiation and neural protection of murine neural progenitor cells (mNPC) in a pharmacological degeneration of the retinal pigmented epithelium and photoreceptor model in rats.
MATERIALS AND METHODS
Harvesting and culturing GFP-mouse NPC were obtained from E14 (embryonic day 14) C57BL/6-GFP mouse embryos. The fetuses were placed in a Petri dish containing PBS/2% glucose, and the dissection was made under magnifying lens. The brains were sectioned and the tissue was incubated with Trypsin-EDTA solution (Gibco, 15400-054) for 15min at 37ºC. Trypsin was inactivated with fetal bovine serum, and, after cell sedimentation, the supernatant was removed and the cells were dissociated in 70% DMEM (Gibco 11965-118), 30% F12 (Gibco 11765-062), 1% PSA (Gibco 15240-062), 2% B27 (Gibco 17504-044), 20ng/mL EGF (Sigma E9644), 20ng/mL FGF-2 (R& D 233-FB), and 5 mg/mL heparin (Sigma H3149 100KU). The cell suspension was counted in a hemocytometer and the cells were seeded in a T25 flask at a density equivalent to 100,000 cells/mL. The spheres were transferred to conical tubes and washed carefully 3 times with 8 mL pre warmed DMEM. The spheres were put in growth factors free medium (DMEM/F12/B27) and kept in those conditions in suspension for 10 days. Eight transgenic C57BL/6-GFP mouse (green fluorescent protein) with 8 weeks y-o was submitted to a pharmacological degeneration of the retinal pigmented epithelium and photoreceptor with systemic application of NaIO3, after 72 hours was applied intra vitreus mNPC (100.000 células/µL). In 7 days, their eyes were dissected and cryoprotected in 30% sucrose in PB for at least 24 hours at 4°C. After they were embedded in OCT compound, retinas were sectioned perpendicularly to the vitreal surface on a cryostat (12-µm sections). The material was analyzed with immunohistochemistry, primary antibodies anti-GFP, anti b – tubulina III and anti-GFAP
RESULTS
Survival and migration of the murine neural progenitor cells (mNPC) was

observed after 7 days following a single application with neurosphere method.

CONCLUSION

Current results point to a possible role for mNPC in the treatment of some forms of human retinal degenerative diseases and highlight the versatility and efficacy of these cells as therapeutic tools in a broad range of neurodegenerative disorders.