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Infusion of human embryonic kidney cell line conditioned medium reverses kainic acid induced hippocampal damage in mice

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Abstract

Background aims. Hippocampal neurodegeneration is one of the hallmarks in neurological and neurodegenerative diseases such as temporal lobe epilepsy and Alzheimer disease. Human embryonic kidney (HEK) cells are a mixed population of cells, including neurons, and their conditioned medium is enriched with erythropoietin (EPO). Because EPO is a known neuroprotectant, we hypothesized that infusion of HEK cells or HEK-conditioned medium (HEK-CM) may provide neuroprotection against kainic acid (KA)-induced hippocampal damage in mice. *Methods.* Adult CF1 mice were treated with KA to induce hippocampal damage. On 3rd and 5th days after KA treatment, HEK cells or HEK-CM was infused intravenously through the tail vein. On the 7th and 8th days after KA treatment, all groups of mice were subjected to cognitive and depression assessment by use of a novel object recognition test and a forced swim test, respectively. Subsequent to this assessment, mice were killed and the brain samples were used to assess the histopathology and messenger RNA expression for EPO and B-cell lymphoma-2 (Bcl-2). *Results.* We found that infusion of HEK cells/HEK-CM improves cognitive function and alleviates symptoms of depression. Histological assessment demonstrates complete neuroprotection against KA-mediated excitotoxicity, and the hippocampal cytoarchitecture of HEK cells/HEK-CM treated mice was comparable to normal control mice. HEK cells/HEK-CM treatment could provide neuroprotection by upregulating the endogenous EPO and Bcl-2 in KA-treated mice. *Conclusions.* Our present data demonstrate for the first time that infusion of HEK cells/HEK-CM can prevent excitotoxic hippocampal damage and alleviate consequent behavioral abnormalities.

Key Words: *conditioned medium, HEK cells, hippocampus, kainic acid, neurodegeneration, neuroprotection*

Introduction

Hippocampal neurodegeneration is one of the hallmarks in neurological and neurodegenerative diseases such as temporal lobe epilepsy and Alzheimer disease [1,2]. Excitotoxicity caused by augmented release of glutamate plays a significant role in neuronal losses in these disease conditions. The majority of the drugs prescribed for neurodegenerative diseases do not have disease-modifying potential. Furthermore, these drugs cause severe side effects, which range from psychological, to cognitive to motor disturbances. Cell therapies are emerging as a potential therapeutic approach for treating various central nervous system diseases. Several studies have shown the beneficial effects of cell therapies in reversing the neurological and behavioral deficits in animal models of central nervous system diseases [3–7]. However, the functional efficacy of grafted cells is a matter of consideration, and the risks of teratoma formation and immune rejections are the major impediments that preclude cell therapies from treating degenerative diseases. Nonetheless, the recent consensus is that the host tissue regeneration may be partly attributed to the paracrine effects of transplanted cells, which in turn activate the host's endogenous reparative mechanisms for cellular and functional recovery [8–10]. In this context, conditioned media (CM) derived from cell cultures are excellent sources of neurotrophic factors and cytokines [11,12]. Though the majority of studies investigated the effects of cell transplantation in animal models of hippocampal neurodegeneration [6,7], there is not much information available on the effects of CM *per se* in cellular and behavioral recovery after hippocampal damage.

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The human embryonic kidney cell line, commonly referred to as HEK-293 cells, was originally derived from human embryonic kidney that was immortalized with sheared adenovirus 5 DNA [13]. Human embryonic kidney cells are globally used as an *in vitro* model to understand normal cellular processes and used mostly for adenovirus transfection studies. Interestingly, a recent study reported by the research group that introduced the HEK cell line to the scientific community demonstrated that this particular cell line also contains a small population of neurons [14]. Furthermore, the CM derived from HEK cells (HEK-CM) could potentiate megakaryopoiesis caused by the presence of erythropoietin (EPO) and other cytokines [11]. Because EPO is a known neuroprotectant [15,16], we hypothesized that infusion of HEK cells or HEK-CM might protect hippocampal neurons against kainic acid (KA)-induced excitotoxicity. Accordingly, in the present study, we compared the cellular and functional recovery after HEK cells or HEK-CM infusion in KA-induced hippocampus-damaged mice. The results of this study demonstrate that HEK-CM *per se* recapitulates the beneficial effects of cell transplantation at molecular-cellular-behavioral levels, thereby supporting the notion that paracrine factors released by the grafted cells contribute significantly to functional recovery by neuroregeneration.

Methods

All animal experiments were carried out in accordance with the guidance provided by the Institutional Animal Care and Use Committee, Manipal University, India. Adult CF1 male mice (2–3 months old) were housed, 4 to 5 in standard polypropylene cages, and paddy husk was provided as bedding material, which was changed every 2 days. Food and water were provided *ad libitum*.

Experimental groups

Adult CF1 mice were grouped into normal control mice (NC; $n = 6$), normal control mice injected with HEK cells (NC+HEK; $n = 6$), normal control mice injected with HEK-CM (NC+HEK-CM; $n = 6$), KA alone—treated mice (KA; $n = 6$), KA-treated mice injected with HEK cells (KA+HEK; $n = 6$) and KA-treated mice injected with HEK-CM (KA+HEK-CM; $n = 5$).

Hippocampal lesion

For induction of hippocampal damage, mice were administered KA (Tocris, Bristol, United Kingdom, 20 mg/kg) intraperitoneally. KA is a glutamate analog that has been used to induce hippocampal neurodegeneration in rodents [17–19]. Control mice (NC, NC+HEK, NC+HEK-CM) received an equal volume of physiological saline (0.9% sodium chloride; Sigma Aldrich, St Louis, MO, USA) solution intraperitoneally.

HEK cell culture and CM derivation

Cryopreserved HEK-293T cells (a generous gift from Dr Anujith Kumar, Manipal University, India) were thawed rapidly at 37°C in a water bath. Dimethyl sulfoxide (Sigma Aldrich) containing HEK cells from the cryovial were transferred into a 15-mL centrifuge tube, and 5 mL of HEK media (Dulbecco's modified Eagle's medium high glucose; Invitrogen, Carlsbad, CA, USA), 10% fetal bovine serum (Hyclone, Thermo Scientific, Waltham, MA, USA), 1% of 200 mmol/L L-glutamate (Invitrogen), 1% non-essential amino acids (Hyclone, Thermo Scientific) and 1% penicillin-streptomycin (Invitrogen) was added to the tube and centrifuged at 1800 rpm (Heraeus Microfuge, Thermo Scientific) for 5 min. The supernatant was discarded, and the pellets were re-suspended in 3 mL of HEK media. The cell suspension was transferred into 25-cm^2 culture flasks (BD Bioscience, San Jose, CA, USA) and incubated at 37°C and 5% CO_2 (Hera Cell 240 CO_2 Incubator, Thermo Scientific) for 24 h. The HEK cells were grown until they attained 70–80% confluency and the CM was collected, aliquoted and stored at −80°C (Hera Freeze, Thermo Scientific) until further use.

Administration of HEK cells and HEK-CM

Three days after KA injection, the KA+HEK group of mice received HEK cells (1×10^6/kg body weight) suspended in 500 μL of phosphate-buffered saline (PBS; pH 7.4, Sigma Aldrich) and the KA+HEK-CM group received 500 μL of HEK-CM through intravenous (tail vein) injections. Each mouse received 2 doses of HEK cells or HEK-CM on the 3rd and 5th days after KA lesion. Likewise, mice from the NC+HEK and NC+HEK-CM groups received an equal volume of HEK cells or HEK-CM on the 3rd and 5th days after saline injection. Mice from the NC and KA groups received an equal volume of PBS through intravenous injection. On the 7th day after KA/saline injection, mice from different treatment groups were subjected to behavioral assessment.

Behavioral assessment

To assess the hippocampal functions after different treatments, the Novel Object Recognition Test (NORT) was performed [20]. NORT is based on the