Metabolic changes in the focal brain ischemia in rats treated with human induced pluripotent cell-derived neural precursors

D. Jirak[1,2], K. Turnovcova[3], N. Kozubenko[3], P. Jendelova[3], and M. Hajek[1,2]

[1]Department of Diagnostic and Interventional Radiology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic, [2]Center for Cell Therapy and Tissue Repair, Prague, Czech Republic, [3]Institute of Experimental Medicine, Czech Republic

Introduction

Neural precursors might be a promising tool for the stroke treatment to restore neurological function. We describe the use of human-induced pluripotent cell-derived neural progenitors (iPS-NPs) for transplantation into the rat brain after temporary middle cerebral artery occlusion (tMCAo). Magnetic resonance imaging/spectroscopy (MRI/MRS) is powerful non-invasive tool for monitoring the transplanted cells. Although MRS cannot directly localize the area of transplanted cells, it can give us insight into the metabolic processes in the brain following the cell transplantation. The aim of our study was to determine metabolic changes by proton MR spectroscopy (1H MRS) in the region of striatal tissue of the rat brain after focal brain ischemia during four months.

Subjects and Methods

Neural precursors were derived from iPS through the micro-aggregates stage. Prior to in vivo experiments, in vitro FACS analysis of iPS-NPs was performed. The iPS-NPs were implanted into young Sprague-Dawley rats (n=14, females, 250-280 g) 7 days after tMCAo. Metabolic profiles in the striatal tissue of both hemispheres were assessed by MRS. Ischemic rats together with controls were scanned by a 4.7T Bruker MR spectrometer with a home-made surface coil. 1H spectra were measured using a modified single voxel Point-resolved spectroscopy sequence (PRESS) sequence (with water suppression), with echo time =13 ms and repetition time = 2500 ms. Spectra were acquired from the lesion and from the contralateral hemisphere. The volume of interest was 64 mm^3 for each hemisphere. Proton spectra were evaluated using the LCModel v.6 (1) to obtain absolute metabolite concentrations in laboratory units. Functional recovery was assessed by the apomorphine-induced rotation, tape-removal and rotating pole tests, which were performed up to 16 weeks after cell transplantation. All protocols were approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine and the experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC).

Results

Four months after MCAO, MRS revealed that the concentrations of brain metabolites (Glutamate (Glu) + glutamine (Gln), N-acetyl-aspartate (NAA), (Phospho) creatine (Cr), Taurine (Tau), Choline (Cho) and Inositol (Ins)) in grafted animals with small lesion returned nearly to the values found in unlesioned animals. In animals with large lesions the concentrations of the brain metabolites in the lesioned hemisphere were lower than in healthy animals, however they were higher compared to the non-grafted animals. Concentrations of selected metabolites in animals with large lesion are summarized in Table 1. The grafted animals displayed a decreased number of clockwise rotations in the apomorphine rotation-induced test when compared to control animals. No significant improvement was detected in motor tests, mainly due to spontaneous recovery. iPS-NPs survived and migrated toward the lesion area during four months after transplantation. Some of them differentiated into more mature and tissue-specific neurons (NSE- and DARPP32-positive cells). No tumor formation was observed throughout the whole experiment.

		Ins	Tau	GPC+PCh	NAA+NAAG	Cr+PCR	Glu+Gln
1 months	C_C	4.0±2.3	6.0±1.8	1.4±0.7	7.1±2.1	6.9±2.8	12.9±3.7
	C_L	5.0±3.6	3.9±1.3	1.8±1.7	5.5±5.2	6.5±6.5	10.6±9.9
	iPS_C	3.2±0.3	4.3±0.3	1.0±0.1	5.5±0.1	5.2±0.2	8.1±0.4
	iPS_L	4.7±1.0	3.3±0.0	1.1±0.2	3.7±0.4	4.2±0.3	5.9±0.3
4 months	C_C	5.5±0.8	7.6±0.4	1.8±0.2	11.4±1.0	9.3±0.7	17.5±1.1
	C_L	6.8±0.1	2.9±0.0	1.8±0.1	6.8±0.2	7.3±0.2	9.8±0.2
	iPS_C	7.4±0.3	6.8±1.2	1.9±0.2	11.0±0.7	9.2±0.6	17.2±1.4
	iPS_L	9.2±4.2	5.0±0.4	2.1±0.5	5.2±2.7	6.6±1.8	9.8±2.7
controls		6.4±0.9	8.0±0.7	1.8±0.3	11.4±2.4	9.8±1.4	19.2±4.9

Table 1. Absolute concentrations in mM of selected metabolites in the striatal tissue one and four months after (tMCAo). Data are expressed as mean ± std. C-C: spectra from contra-lateral hemisphere in animals with (tMCAo); C_L: spectra from lesion in animals with (tMCAo); iPS_C: spectra from contra-lateral hemisphere in animals with (tMCAo) and with transplanted cells; iPS_L: spectra from lesion in animals with (tMCAo) and with transplanted cells.

Discussion and Conclusions

These results suggest that iPS-NPs undergo further differentiation after transplantation, integrate into the striatal tissue, partially improve functional outcome and can serve as a safe tool for cell transplantation therapy.

References

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