Experimental Neurology 248 (2013) 429–440

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review

Organotypic cultures as tools for optimizing central nervous system cell therapies

Nicolas Daviaud [a,b], Elisa Garbayo [a,b,c], Paul C. Schiller [d,e,f], Miguel Perez-Pinzon [g], Claudia N. Montero-Menei [a,b,*]

[a] INSERM U1066, 'Micro et Nanomédecines biomimétiques—MINT', Angers, France
[b] LUNAM Université, Université Angers, UMR-S1066, Angers, France
[c] Pharmacy and Pharmaceutical Technology Department, University of Navarra, Pamplona, Spain
[d] Geriatric Research, Education and Clinical Center and Research Services, Bruce W. Carter Veterans Affairs Medical Center, Miami, FL, USA
[e] Department of Orthopedics, University of Miami Miller School of Medicine, Miami, FL, USA
[f] Department of Biochemistry & Molecular Biology, University of Miami Miller School of Medicine, Miami, FL, USA
[g] Department of Neurology, University of Miami Miller School of Medicine, Miami, FL, USA

ARTICLE INFO

Article history:
Received 15 February 2013
Revised 15 July 2013
Accepted 18 July 2013
Available online 27 July 2013

Keywords:
Stem cells
Neurodegenerative disorders
Organotypic slices
Cell behaviour
Cell therapy
Tissue engineering

ABSTRACT

Stem cell therapy is a promising treatment for neurological disorders such as cerebral ischemia, Parkinson's disease and Huntington's disease. In recent years, many clinical trials with various cell types have been performed often showing mixed results. Major problems with cell therapies are the limited cell availability and engraftment and the reduced integration of grafted cells into the host tissue. Stem cell-based therapies can provide a limitless source of cells but survival and differentiation remain a drawback. An improved understanding of the behaviour of stem cells and their interaction with the host tissue, upon implantation, is needed to maximize the therapeutic potential of stem cells in neurological disorders. Organotypic cultures made from brain slices from specific brain regions that can be kept in culture for several weeks after injecting molecules or cells represent a remarkable tool to address these issues. This model allows the researcher to monitor/assess the behaviour and responses of both the endogenous as well as the implanted cells and their interaction with the microenvironment leading to cell engraftment. Moreover, organotypic cultures could be useful to partially model the pathological state of a disease in the brain and to study graft–host interactions prior to testing such grafts for pre-clinical applications. Finally, they can be used to test the therapeutic potential of stem cells when combined with scaffolds, or other therapeutic enhancers, among other aspects, needed to develop novel successful therapeutic strategies or improve on existing ones.

Contents

Abbreviations: CI, cerebral ischemia; PD, Parkinson's disease; HD, Huntington's disease; SN, substancia nigra; DA, dopamine; GABA, gamma-aminobutyric acid; MSN, medium spiny neuron; CNS, central nervous system; NSC, neural stem cells; ES, embryonic stem; MSC, mesenchymal stromal cells; iPS cells, induced-pluripotent stem; TH, tyrosine hydroxylase; CA1, Cornu Ammonis layer 1; OGD, oxygen-glucose-deprivation; MPP+, 1-methyl-4-phenylpyridium; 3-NPA, 3-nitropropionic acid; NMDA, N-methyl-D-aspartic acid; NT-3, neurotrophin-3; NGF, nerve growth factor; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; DTG, 1, 3, di-o-tolylguanidine; HUCBC, human umbilical cord blood stem cells; 6-OHDA, 6-hydroxydopamine; QA, quinolinic acid; KA, kainic acid; polyQ, polyglutamine; PAMs, pharmacologically active microcarriers.

* Corresponding author at: INSERM U 1066, IBS-CHU Angers, 4 rue Larrey, 49933 Angers Cx 9, France.
E-mail address: claudia.montero-menei@univ-angers.fr (C.N. Montero-Menei).

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http://dx.doi.org/10.1016/j.expneurol.2013.07.012

Introduction

Neurological disorders such as cerebral ischemia (CI), Parkinson's disease (PD), or Huntington's disease (HD) have in common the loss of neurons in the brain. CI is a condition in which blood flow is curtailed to the brain resulting in neuronal death by oxygen and nutrient deprivation (Barrett and Meschia, 2010). The neurological signs and symptoms of PD are, in large part, a result of selective loss of neurons in the nigro-striatal dopaminergic pathway (for review Lees et al., 2009). In HD gamma-aminobutyric acid (GABA) ergic medium spiny neuron (MSN) death occurs at onset of disease manifestation (for review Walker, 2007). Unfortunately, PD and HD therapies only provide amelioration of symptoms but do not delay or halt neurodegeneration. In CI if the acute phase is not treated on time, the post-injury neuronal death is the cause of many disabilities. As of today, only thrombolytic therapy has shown any efficacy against CI. It is therefore essential to evaluate alternative therapeutic strategies such as cell therapy.

Cell therapy for the central nervous system (CNS) consists in cell injection into a lesioned brain tissue to restore a loss of function (Dunnett and Rosser, 2011). However, apart from poor cell engraftment issues that still need to be addressed cell availability and ethical concerns have limited the development and current clinical application of this therapy (Delcroix et al., 2010b; Liu and Huang, 2007). Stem cells that can differentiate into mature neural/neuronal cells can be used as alternative source of cells, as their self-renewal capacity allows the establishment of a cell bank avoiding availability and ethical difficulties. Neurons and glial cells can be generated from neural stem cells (NSC), embryonic stem (ES) cells, bone marrow-derived multipotent stromal cells also called mesenchymal stromal cells (MSC), and also lately, from induced-pluripotent stem (iPS) cells. Each type of stem cell has advantages and caveats that must be taken into consideration together with the type of application envisaged.

Brain organotypic slices, which can be maintained in culture for several weeks, confer a rapid and simple method to evaluate cellular interactions and mechanisms. Moreover, brain slices can be used to develop *ex vivo* models of neurological disorders and, in this way, they represent a link between *in vitro* studies and animal models. Cells can be grafted in organotypic slices allowing the researcher to understand how implanted cells interact with resident cellular matrix and injured residential cells and to predict how stem cells may behave *in vivo*. Thus, they represent a powerful tool to study cell therapy and neuroprotection.

In this review, after a brief overview of the clinical trials performed using cell therapy to treat cerebral disorders and their current limitations, we discuss how organotypic slices could address some of the key unanswered questions regarding cell therapy. We report the different *ex vivo* models of CI, PD and HD and review the studies carried out using these *ex vivo* models of neurological disorders to evaluate stem cell therapies. Finally, tissue engineering strategies for PD and other neurological disorders tested in organotypic slices are discussed.

Cell therapy clinical trials for neurological disorders

Here we will focus on discussing the clinical evaluation of cell-based therapies in which cells are implanted *via* stereotactic surgery for CI, PD and HD.

The first clinical trials in PD consisted in the striatal stereotactic implantation of adult cells, which may synthesize dopamine (DA) or its precursor, and are thus able to replace the lost DA in the striatum. Over the years, adult cells such as chromaffin cells (for review see Freed et al., 1990), human retinal pigment epithelium cells (for review see Gross et al., 2011; Stover et al., 2005) or carotid body cells (for review see Lopez-Barneo et al., 2009; Minguez-Castellanos et al., 2007) have been evaluated for PD therapy as they lack ethical issues and some of these cell types allow autografts to be performed. Those cell transplantation studies led to a certain improvement of motor functions but cell survival remained too weak limiting their efficacy. Most clinical trials of cell therapy for either PD or for HD consisted of striatal stereotactic implantation of minced foetal tissue or cell suspensions from the ventral mesencephalon or the ganglionic eminence, respectively. They were performed in order to restore lost DA within the striatum for PD patients and lost MSN for HD patients. These trials showed promising results (for review see Barker et al., 2013). However, in PD patients (Mendez et al., 2005), graft induced dyskinesias may occur and Lewy bodies were found in some long survival dopaminergic neurons (for review see Lindvall, 2013; Tomaskovic-Crook and Crook, 2011). But overall, the main limitations concern the poor availability of foetal tissue, the ethical issues associated with their use and the limited survival of the transplanted cells.

Stem cells, which can be isolated from many sources, represent a potential candidate for cell therapy as they self-renew and present a large differentiation potential (for review see Benraiss and Goldman, 2011; Lindvall, 2013). The feasibility of using adult NSCs for PD cell therapy has been demonstrated but their poor availability and an improvement that returns back to baseline after 5 years post-operation suggests that efficacy is limited (Lévesque et al., 2009). Stereotactic implantation of autologous MSCs, which can differentiate into neuronal-like cells and secrete tissue repair and immunoregulatory factors (Tatard et al., 2004), has been evaluated in PD patients (Venkataramana et al., 2010) and in chronic stroke patients for their ability to repair the damaged neuronal tissue (Suarez-Monteagudo et al., 2009). It was concluded that MSCs could safely be grafted into the striatum of patients with good tolerance and no complications. Furthermore, in both cases, a decrease of symptoms associated with the disease was observed.

New clinical trials using adult stem cells are currently on going for the treatment of chronic stroke (NCT01714167 on Clinicaltrials.gov) or for PD (NCT01446614, NCT01453803 on Clinicaltrials.gov). To our knowledge, there are no clinical trials with stem cells for HD.

Cell therapy unanswered questions

As described above, cells from a variety of sources have shown various degrees of efficacy in clinical trials. However, cell therapy also presents some drawbacks that limit its use, such as poor cell survival and *in situ* differentiation, certain undesirable side effects and limited availability of foetal tissue. We now discuss the major issues and some future directions of the research associated with cell therapy for neurological disorders.

First of all, findings in a number of experimental models showed that neuronal precursor cell survival within the host tissue after transplantation was too weak (10–20%) and that cell death occurred within the first 3 weeks (Delcroix et al., 2010b; Liu and Huang, 2007; Olanow et al., 2003). *In situ* differentiation of stem cells was insufficient (at best