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Research Report

Enhancing tyrosine hydroxylase expression and survival of fetal ventral mesencephalon neurons with rat or porcine Sertoli cells in vitro

Rania Shamekh[a], *Jennifer Mallery*[b], *Jennifer Newcomb*[a], *Joelle Hushen*[b], *Samuel Saporta*[a,b], *Don F. Cameron*[b], *Cyndy Davis Sanberg*[d], *Paul R. Sanberg*[a,b,c,e], *Alison E. Willing*[a,b,c,*]

[a]*Department of Neurosurgery, Center of Excellence for Aging and Brain Repair, University of South Florida, MDC 78, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA*
[b]*Department of Pathology and Cell Biology, University of South Florida College of Medicine, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA*
[c]*Department of Molecular Pharmacology and Physiology, University of South Florida College of Medicine, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA*
[d]*Saneron CCEL Therapeutics, 3802 Spectrum Blvd. Suite 145 Tampa, FL 33612, USA*
[e]*Department of Psychiatry, University of South Florida College of Medicine, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA*

ARTICLE INFO

Article history:
Accepted 3 April 2006
Available online 15 June 2006

Keywords:
Parkinson's
Development
Cell culture
Dopaminergic neuron

ABSTRACT

Sertoli cells (SCs) are testis-derived cells that secrete trophic factors important for the development of germ cells. Both porcine and rat SCs have been used as graft facilitators — neonatal porcine SCs to support islets in diabetes and 15-day-old rat SCs to enhance dopaminergic neuron transplants in Parkinson's disease models. However, there has never been a study examining the optimal SCs preparation to enhance tyrosine hydroxylase expression in the ventral mesencephalon (VM) neuron. The aim of this study was to compare the ability of both rat and porcine SCs to enhance tyrosine hydroxylase expression (TH) and neuronal survival at the same postnatal developmental ages. The SCs were isolated from 1-, 9-, or 15-day-old rat, or neonate (2–5 days), 2-month, or 4-month-old pig, and co-cultured with VM tissue from 13.5-day-old embryos. Our results showed that VM neurons co-cultured with SCs dispersed over the culture plate and had extensive neuritic outgrowth, while VM neurons cultured alone tended to cluster together forming a mass of cells with limited neurite outgrowth. TH expression was significantly increased when VM neurons were co-cultured with 15-day rat SCs or 2-month pig SCs but not when the cells were co-cultured with other ages of SCs. This suggests that secretion of trophic factors by SCs varies according to the developmental age, and it is critical for the success of graft facilitation that SCs from the appropriate age and species be used.

* *Corresponding author*. Department of Neurosurgery, University of South Florida, Center of Excellence for Aging and Brain Repair, MDC 78, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA.
E-mail address: awilling@health.usf.edu (A.E. Willing).

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doi:10.1016/j.brainres.2006.04.058

1. Introduction

Cell transplantation is a promising approach for restoration of function in neurodegenerative diseases, such as Parkinson's disease. Neural transplantation in PD is based on the idea of transplanting dopamine-producing cells in the striatum. This may substitute for those mesencephalic dopamine neurons that have been lost as a consequence of the disease. The major obstacle to this approach is the extensive cell death during isolation and grafting (Brundin et al., 2000).

The Sertoli cells (SCs) are normal constituents of the testis. By secreting trophic factors, regulatory proteins, and nutritive factors, they support the development of germ cells. These factors include transforming growth factors (TGF-α and β), insulin like growth factor I (IGF-I), basic fibroblast growth factor (bFGF or FGF_2) (Skinner, 1993), platelet derived-growth factor (PDGF) (Loveland et al., 1995), and neurturin (NTN) (Widenfalk et al., 1997). SCs express cytokines such as IL-1α and IL-6 that have also been shown to have trophic effects on dopaminergic neurons (Ling et al., 1998; Von Coelln et al., 1995). Trophic factors such as sulphated glycoproteins 1 (Prosaposin) (Morales et al., 1995) and 2 (clusterin) (Griswold et al., 1988) and desert hedgehog (Dhh) (Bitgood et al., 1996), a member of Sonic hedgehog (Shh) family are expressed by SCs as well, which seem to stimulate the differentiation of dopaminergic neurons (Wang et al., 1995).

These trophic properties of the SCs may enhance survival and differentiation of dopaminergic neurons in transplant treatments for Parkinson's disease (PD). In culture, Cameron and colleagues reported that 16- to 19-day-old SCs enhanced the survival of fetal neurons or human teratocarcinoma cell line hNT neurons upon thaw when they were exposed to conditioned media from the SCs (Cameron et al., 1997). Further, tyrosine hydroxylase (TH+) neurons of both rat and human ventral mesencephalic tissue together with hNT were significantly increased when co-cultured with SCs from 2-month-old pig (Othberg et al., 1998). Furthermore, soma size and neurite outgrowth of neurons were significantly increased in the co-culture group compared to control cultures. When rat SCs were transplanted alone (allograft) (Sanberg et al., 1997) into the 6-hydroxydopamine lesioned striatum, SCs survived and enhanced behavioral recovery. Moreover, SCs promoted graft survival of co-transplanted bovine adrenal chromaffin cells (xenografts) (Sanberg et al., 1996) in the normal rat striatum.

In order to optimize SCs for the process of neuronal transplantation, it is necessary to optimize the SCs population. The ideal SCs to use for human transplants would be human; however, there is little likelihood of obtaining human SCs. They must be obtained postnatally, but to do so would likely render the donor sterile, and a human SC cell-line has proven difficult to develop. This would necessitate the reliance on the organ donor system, which would make this adjunct therapy relatively inaccessible. Thus, alternatives include the use of cells from other species (xenograft). While both rat and pig SCs have been studied for their ability to enhance survival and TH expression of dopaminergic cells, it is unclear which species of cells would be preferable and which developmental age is optimal. Nevertheless, there is no direct comparison of SCs at different ages or from different species to determine if all SCs have the same trophic or immune capabilities across development. In this study, SCs were isolated from three postnatal, prepubertal developmental age groups in both rat and pig. The purpose of this study was to determine the relative potency of rat or pig SCs to enhance tyrosine hydroxylase expression and neuronal survival.

2. Results

2.1. Sertoli cell isolation

We examined the viability of the SCs just after the completed isolation procedure and before co-culture with VM (Table 1). Based on data from all cell isolations performed, we calculated the average number of SCs harvested per animal. There were very few SCs isolated from the neonatal rat pups. The testes were too small, and the yield of cells from 24 pups was negligible. We calculated that it would take approximately 200 pups to provide the number of cells we obtained from 20, 15-day-old pups, which made SCs isolation from neonatal rat pups impractical.

As can be seen in Table 1, the number of cells obtained per animal increased with age at isolation. Further, there were far more cells obtained from the pig than from the rat. The number of SCs isolated from a 4-month pig was a rough estimate, as the tight junction formation made it impossible to obtain a single cell suspension. This decreased viability due to the harsher treatment the cells underwent as we tried to dissociate them.

There were no systematic changes in cell viability between the other preparations.

2.2. Sertoli cell morphology after 1 DIV

As SCs develop, there may be changes in shape, secretory products, presence of tight junctions and sensitivity to endocrine or paracrine factors. When cultured alone, the SCs from 9-day-old rats remained relatively small and rounded (see Figs. 1A and B). At this age, it was more difficult to remove other testicular cell types during the cell isolation,

Table 1 – Sertoli cell yield per animal determined during cell isolation

Species	Yield per animal	Viability
Rat		
Neonate	0	0
9 days	188,000	~76%
15 days	5,517,055	93+%
Porcine		
Neonate	1,880,000	~83%
2 months	33,130,000	~93%
4 months	20,800,000*	~67%