



In vitro effects of date palm (*Phoenix dactylifera* L.) pollen on colonization of neonate mouse spermatogonial stem cells

Maryam Mahaldashtian^a, Majid Naghdi^b, Mohamad Taghi Ghorbanian^a,
Zohreh Makoolati^{b,*}, Mansoureh Movahedin^c, Seyedeh Momeneh Mohamadi^c

^a Department of Molecular & Cellular Biology, Faculty of Biology, Damghan University, Semnan, Iran

^b Department of Anatomical Sciences, Faculty of Medicine, Fasa University of Medical Sciences, Fasa, Iran

^c Department of Anatomical Sciences, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Article history:

Received 25 October 2015

Received in revised form

11 April 2016

Accepted 11 April 2016

Available online 13 April 2016

Keywords:

Spermatogonial stem cell

Date palm pollen

Co-culture

Sertoli cell

Colonization

Chemical compounds studied in this article:

Collagenase (PubChem CID: 18680304)

Hyaluronidase (PubChem CID: 91820602)

Triton X-100 (PubChem CID: 5590)

ABSTRACT

Ethnopharmacological relevance: Date palm (*Phoenix dactylifera* L.) pollen (DPP) is widely used as a folk remedy for male infertility treatment, and has well known medicinal effects.

Aim of the study: This study aimed to determine the in vitro effects of DPP on the efficiency of neonate mouse spermatogonial stem cells (SSCs) proliferation.

Material and Methods: Sertoli and SSCs were isolated from 6 to 10-days-old mouse testes, and their identity was confirmed using immunocytochemistry against cytokeratin for sertoli cells and PLZF, Oct-4 and CDH-1 for SSCs. Isolated testicular cells were cultured in the absence or presence of 0.06, 0.25 and 0.62 mg/ml concentrations of DPP aqueous extract for 2 weeks. The number and diameter of SSC colonies were assessed during third, 7th, 9th and 14th day of culture, and the expression of the Mvh, GFR α -1 and Oct-4 was evaluated using quantitative PCR at the end of the culture period. The significance of the data was analyzed using ANOVA and paired samples *t*-test and Tukey and Bonferroni test as post hoc tests at the level of $p \leq 0.05$.

Results: Pattern assay of colony formation showed that SSCs numbers increased in the presence of 0.62 mg/ml concentration of DPP extract with higher slope relative to other groups ($P < 0.05$). Colony diameters had no significant difference between groups in 3th, 7th, 9th and 14th days after culture. The Mvh and Oct-4 genes expression had no significant difference between groups, while GFR α 1 expression was increased significantly in cells treated with 0.06 mg/ml concentration relative to other groups ($P < 0.05$).

Conclusion: It seems that co-culture of SSCs with sertoli cells in the presence of low doses of DPP can increase SSCs proliferation and keep their stemness state, while higher concentrations can differentiate the treated cells.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Spermatogonial stem cells (SSCs), also known as male germline stem cells, are a self-renewing and differentiating population of adult stem cells. These cells have the potential of producing progeny cells for sperm production throughout the life in male (Kanatsu-Shinohara et al., 2005).

The isolation of SSCs is difficult. One of the limitations of SSCs includes the low number of these stem cells in the mature testis

* Corresponding author at: Department of Anatomical Sciences, Faculty of Medicine, Fasa University of Medical Sciences, Fasa, Iran.

E-mail addresses: mahaldashtian.m@gmail.com (M. Mahaldashtian),

majidnaghdi@yahoo.com (M. Naghdi),

mt.ghorbanian000@gmail.com (M.T. Ghorbanian),

zohreh1438@yahoo.com (Z. Makoolati).

(Morena et al., 1996). Therefore, many methods for in vitro enrichment of SSCs developed, and success in germline stem cells proliferation is an attractive treatment for male infertility, particularly the one caused by the long-term adverse effects of cancer treatments as chemo- or radiotherapy (Sadri-Ardekani et al., 2009).

Previous culture experiments demonstrate that SSCs proliferation was significantly improved by the addition of cytokines (Golden et al., 1999; Trupp et al., 1995; Viglietto et al., 2000). Similarly, increased proliferation of SSCs was observed using the feeder layer such as sertoli cells (Anjamrooz et al., 2006; Aponte et al., 2006; Mohamadi et al., 2012). Recent studies have shown that some soluble growth factors, especially GDNF, that is secreted by sertoli cells stimulates SSCs proliferation in animals (Ebata et al., 2011; Kanatsu-Shinohara et al., 2005; Koruji et al., 2007, 2009; Kubota et al., 2004; Meng et al., 2000; Nagano et al., 2003).

Infertility is one of the major health problems in married couples (Radford et al., 1999) and recent clinical and epidemiological evidence suggests that male reproductive problems are growing (Oyeyipo et al., 2014).

During the last years, increasing attention has been paid to the use of herbal medicine for male infertility treatment. One of the herbs that is widely used as a folk remedy for this purpose is Date palm (*Phoenix dactylifera* L.) pollen (DPP) (Shafieesarvestani, 2000).

A large and growing body of literature has reported a history of use of DPP as male tonic to improve fertility (Bahmanpour et al., 2006; Dostal et al., 1996; Mehraban et al., 2014; Shafieesarvestani, 2000). DPP contains retina, cholesterol and carotenoids which cause gonadotropin activity in rats (Dostal et al., 1996) as well as estrogenic compounds as gonad-stimulating composites that can improve male infertility (Soliman and Soliman, 1958). It has been demonstrated that DPP can increase the plasma levels of oestradiol and testosterone (Abedi et al., 2012; Mehraban et al., 2014; Kazemini et al., 2014; El-Neweshy et al., 2013). DPP also contains facilitator factors: alkaloids, saponins and flavonoids that increase sexual excitement and libido (Abedi et al., 2012).

The aforementioned in vivo studies permitted evaluation of the effect of DPP administration on male reproductive system. So far, however, no research has been found that surveyed the in vitro effects of DPP on SSCs enrichment. Thus, this study was designed to evaluate the in vitro effects of aqueous extract of DPP on the colonization of immature mouse SSCs in the presence of sertoli cells.

2. Materials and methods

2.1. Reagents

Collagenase, trypsin, hyaluronidase, normal goat serum, cyto-keratin and fetal bovine serum (FBS) were supplied by Sigma-Aldrich (St Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM) was offered by Gibco-BRL, (Grand Island, NY, USA). Secondary FITC goat anti-rabbit and goat anti-mouse antibodies were the product of Raybiotech (Tehran, Iran). RevertAid™ First strand cDNA synthesis kit, DNase and PCR master mix were purchased from Fermentas (St Leon-Rot, Germany). RNXPlus™ was the product of Cinnagen Co. (Tehran, Iran). Mouse CDH1 antibody was supplied by Calbiochem (San Diego, CA, USA). Mouse Oct-4 antibody was obtained by Chemicon (UK). Triton X-100 was prepared from ICN Biomedicals and PLZF antibody was purchased from R&D Systems.

2.2. Preparation of aqueous extract of date palm pollen

Date palm pollen grain, a small oval shape gametocyte with a fine bark, was collected from Jahrom Province Botanical garden, South of Iran, authenticated and deposited in the Herbarium of the Fasa University with the voucher specimen number of 100–1. Then, the grains were separated from the bark, washed with distilled water, dried and blended. The powders (8.55 g) was dissolved in distilled water (80 ml, 30 °C), shake for 5 h with magnetic shaker, filtered and lyophilized in oven (55–60 °C) for vaporization of water. Dry extract was resolved in normal saline to give the required concentrations (0.06, 0.25 and 0.62 mg/ml) (Mahaldashtian et al., 2014).

2.3. Animals

Eighty male mice with 6–10-days-old were used in the experiment. The animals were kept at the animal house of Fasa

University of Medical Sciences (Fasa, Iran) at a 22–25 °C temperature with a 12-h light/dark cycle. Free access to standard laboratory drinking water and foods was provided. Care of animals was in accordance with institutional guidelines and the protocol was approved by the committee on the ethics of animal.

2.4. Isolation and cultivation of testicular cells

Six-day-old mice testes were digested in the two steps (Scarpino et al., 1998). In the first step of enzymatic digestion, interstitial fibroblasts and endothelial cells were removed (Fig. 1a). For this purpose, tunica albuginea was separated; testes were minced and suspended in DMEM containing 0.5 mg ml⁻¹ collagenase, 0.5 mg ml⁻¹ trypsin and 0.5 mg ml⁻¹ for 20 min at 37 °C. A little pipetting and shaking was done every 10 min for better digestion. Then, the testes were centrifuged at 1200 rpm for 2 min. In the second step, fresh mixture of mentioned enzymes and DMEM was added into the seminiferous cord component for 60 min at 37 °C. A mixture of germ cells and sertoli cells prepared by repeated pipetting were centrifuged at 400 rpm for 2 min in order to separation from the residual fragments of seminiferous tubule (Fig. 1b). Immediately after cell isolation, testicular cell suspension was assessed for cell viability and cultured in DMEM containing 4% FBS in the absence and presence (0.06, 0.25 and 0.62 mg/ml) of DPP extract for 14 days. The primary cell number was equal in all groups (5.55×10^5) and culture medium was renewed every 2 days. The prepared cell suspension contained two different cell types. The first type, sertoli cells, formed a monolayer of cells and the second, SSCs, created prominent colonies after proliferation. Sertoli cells were used as feeder layer for the culture of SSCs colonies.

2.5. Immunocytochemistry for identity confirmation of the sertoli and spermatogonial stem cells

After 2 weeks of culture, the cells were fixed with 4% paraformaldehyde for 20 min at the room temperature, washed with PBS, incubated for 30 min in HCl (2 N) at the room temperature and washed with borate buffer. Nonspecific antibody reaction was blocked with 10% normal goat serum and permeabilisation was done using 0.3% triton X-100 in PBS.

The specimens were incubated in 1/100 diluted cytokeratin antibody for 1 h at 37 °C, as a marker of sertoli cells, or mouse Oct-4; and PLZF antibodies diluted 1/100 in PBS as markers for undifferentiated cells and mouse CDH1 monoclonal antibody diluted 1/100 in PBS for type A spermatogonial cells (de Rooij, 1998; Guan et al., 2009; Mohamadi et al., 2012; Nagano et al., 1998). After being extensively washed with PBS, The slides were incubated in 1/10 diluted secondary FITC goat anti-rabbit antibody against Oct-4 and PLZF and goat anti-mouse antibody against cytokeratin and CDH1 for 2 h at room temperature. Nuclei were counterstained with ethidium bromide (5 µg/ml). Washing and mounting with 90% glycerol in PBS were done finally. The control groups were processed under similar conditions except for the removal of the first antibody.

2.6. Colony assay

The diameter and the number of spermatogonial colonies were assessed on the third, 7th, 10th and 14th days of the culture. The number of clusters and their diameters were measured by inverted microscope (Carl Zeiss; Oberkochen, Germany) using Image J software (version 1.240; National Institutes of Health, Bethesda, MD, USA).

Download English Version:

<https://daneshyari.com/en/article/2544728>

Download Persian Version:

<https://daneshyari.com/article/2544728>

[Daneshyari.com](https://daneshyari.com)