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Research article

Conditioned medium of dental pulp cells stimulated by Chinese propolis show neuroprotection and neurite extension *in vitro*

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HIGHLIGHTS

- The conditioned medium (CM) of dental pulp cells (DPCs) possessed neuroprotective effects.
- The CM of DPCs also had the activity of neurite outgrowth.
- The benefit of the CM of DPCs was enhanced by the ethanolic extracts of Chinese propolis.
- The CM of DPCs will be an efficient source of new treatments for neurodegenerative diseases.

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ABSTRACT

The purpose of this study was to clarify the effect of Chinese propolis on the expression level of neurotrophic factors in dental pulp cells (DPCs). We also investigated that the effects of the conditioned medium (CM) of DPCs stimulated by the propolis against oxidative and endoplasmic reticulum (ER) stresses in human neuroblastoma SH-SY5Y cells, and on neurite extensions in rat adrenal pheochromocytoma PC12 cells. To investigate the effect of the propolis on the levels of neurotrophic factors in DPCs, we performed a qRT-PCR experiment. As results, *NGF*, but not *BDNF* and *NT-3*, in DPCs was significantly elevated by the propolis in a concentration-dependent manner. H_2O_2-induced cell death was significantly inhibited by the treatment with the CM of DPCs. In addition, the treatment with the propolis-stimulated CM of DPCs had a more protective effect than that with the CM of DPCs. We also examine the effect of the propolis-stimulated CM of DPCs against a tunicamycin-induced ER stress. The treatment with the propolis-stimulated CM as well as the CM of DPCs significantly inhibited tunicamycin-induced cell death. Moreover, the treatment with the propolis-stimulated CM of DPCs significantly induced neurite outgrowth from PC12 cells than that with the CM of DPCs. These results suggest that the CM of DPCs as well as DPCs will be an efficient source of new treatments for neurodegenerative diseases and that the propolis promote the advantage of the CM of DPCs *via* producing neurotrophic factors.

1. Introduction

Propolis is made from a sticky substance that honeybees produce by mixing their own waxes with resinous sap obtained from the bark and leaf-buds of certain trees and other flowering plants. The color of propolis can be green, yellow, brown, or almost black depending on the plants from which the resinous substance is collected. The properties and constituents of propolis also differ with its geographical origin. The putative therapeutic properties of propolis could be related to its anti-bacterial [1], anti-inflammatory [2], anti-oxidative [3,4] and/or tumoricidal [5,6] activities.

Dental pulp tissue is thought to be derived from migrating neural crest cells during development [7,8], and has been shown to harbor various populations of multi-potential stem/progenitor cells [9–11]. *In vitro* neural differentiation studies of rat and human adult dental pulp cells (DPCs) demonstrated that these

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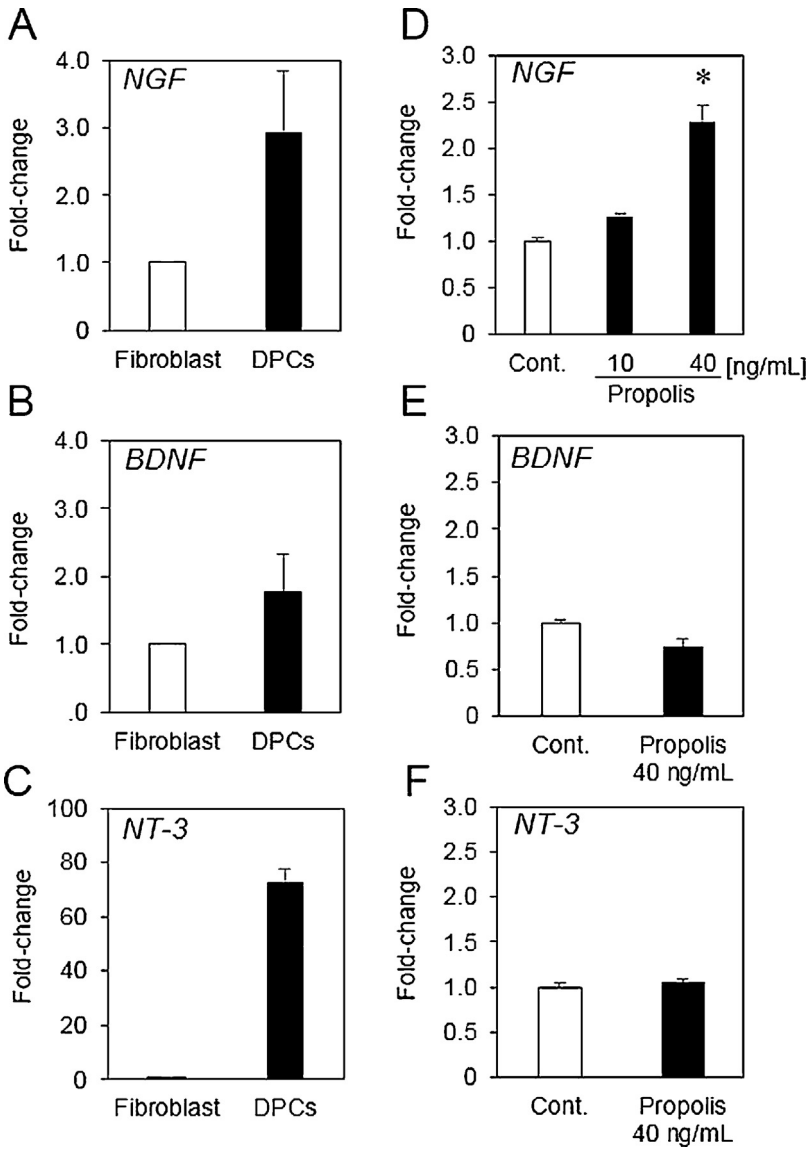

Fig. 1. Effect of Chinese propolis on the neurotrophic factors of DPCs. Real-time qRT-PCR analysis of the expression of neurotrophic factors for *NGF* (A), *BDNF* (B) and *NT-3* (C). Results were expressed as fold increased compared with the level expressed in skin fibroblasts. Real-time qRT-PCR analysis of the expression of neurotrophic factors of DPCs for *NGF* (D), *BDNF* (E) and *NT-3* (F) at 4 h after the propolis treatment. Data are normalized to the amount of 18s *rRNA*. Results were expressed as fold increased compared with that in control.

stem/precursor cell populations were able to differentiate into neurons based on cellular morphology and the expression of early neuronal markers [10,11]. In addition, cultured DPCs have the ability to produce neurotrophic factors, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) [12]. Previous studies suggest that neurotrophic factors and/or unknown factors, rather than neurodifferentiation, expressed by DPCs may play major roles in the neuroprotection [12,13]. Therefore, these previous reports support the exploitation of neuroprotective factors secreted by DPCs as a unique cellular resource for neuroregeneration therapies. However, to our knowledge, no examination of the effects of propolis has been carried out using DPCs.

Here, the purpose of the present study was to clarify the effects of propolis on the neurotrophic factors of DPCs. In addition, we also investigated that the effects of the conditioned medium (CM) of DPCs stimulated by propolis against oxidative and endoplasmic reticulum (ER) stresses in human neuroblastoma SH-SY5Y cells, and on neurite extension in rat adrenal pheochromocytoma PC12 cells.

2. Materials and methods

2.1. Isolation and establishment of dental pulp cell lines

Canine DPCs was used in this study since it was easy to isolate dental pulp from canine cuspid. The pulp tissues of adult canine cuspid (1 year old, Japan SLC Inc., Hamamatsu, Japan) were minced into small clumps and digested with 1 mg/mL collagenase type I (Sigma, St. Louis, MO, USA) for 1 h at 37 °C. Several colonies were obtained after these small clumps were transferred to culture dishes containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), then incubated at 37 °C in humidified 5% CO_2/95%, and then fibroblastic cells that grew out from these colonies were expanded in the medium.

2.2. Conditioned medium and propolis treatment

For conditioned medium, at 70–80% confluence, the cell culture medium was changed to serum-free DMEM, and then DPCs was