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EXPERIMENTAL STUDY

Effect of Asiasari radix on osteoblastic differentiation of stem cells derived from gingiva

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Abstract

OBJECTIVE: To examine the dose-dependent impact of Asiasari Radix (A. radix) on the cell viability, differentiation and mineralization of stem cells derived from gingiva.

METHODS: Stem cells that were derived from gingiva were grown in the presence of A. radix at final concentrations that ranged from 0.001 to 10 μg/mL. The morphology of the cells was viewed under an inverted microscope and the analysis of cell proliferation was performed by using Cell Counting Kit-8 (CCK-8) on day 1. The alkaline phosphatase activity test was used to assess differentiation and Alizarin red S staining was used to assess mineralization of treated cells.

RESULTS: The control group showed spindle-shaped, fibroblast-like morphology and the shapes of the cells in 0.001, 0.01, 0.1, 1 and 10 μg/mL of A. radix were similar to that of the control group at day 1. The cultures growing in the presence of 0.001 μg/mL of A. radix at day 1 showed an increase in the CCK-8 value ($P < 0.05$). Cultures growing in the presence of 0.001 μg/mL of A. radix presented the highest value for alkaline phosphatase activity ($P > 0.05$). Mineralized extracellular deposits were observed after Alizarin Red S staining and the cultures grown in the presence of 0.001 μg/mL of A. radix showed the highest value for quantitative results for bound dye ($P < 0.05$).

CONCLUSION: Within the limits of this study, A. radix influenced the proliferation of stem cells derived from the gingiva and low concentrations of A. radix might enhance osteogenic differentiation of the stem cells.

Key words: Cell differentiation; Plant roots; Plant extracts; Stem cells; Drugs, Chinese herbal

INTRODUCTION

Asiasari Radix (A. radix) is a traditional herb medicine that is widely used to treat various diseases.[1] It has been used for its anti-inflammatory, anti-allergy, or anti-bacterial effects.[2-5] Moreover, A. radix has been suggested for the treatment of oral diseases, including toothache and aphthous stomatitis.[6-8]

This study was aimed at examining the dose-dependent impact of A. radix on the differentiation and mineralization of stem cells derived from gingiva. In addition, the impact of A. radix on cell viability was also evaluated. The alkaline phosphatase activity test and Alizarin red S staining were used to assess differentiation and mineralization of treated cells.

MATERIALS AND METHODS

Preparation of the materials

The dry roots of Asiasarum heterotropoides (400 g) were boiled in distilled water under reflux for 2 h 30 min. The resulting extract was centrifuged and the supernatant was concentrated to 300 mL under reduced pressure using a rotary evaporator (Eyela NE-1001, Tokya Rikakikai Co., Ltd., Tokyo, Japan). The concentrates were then freeze-dried using lyophilizer (Labconco, Kansas, MO, USA). 65 g of solid residue was obtained, resulting in a yield of 16% (w/w).

Cell culture and stem cells' morphology

Stem cells were obtained from healthy gingival tissue as previously reported.[8] This study was reviewed and approved by the Institutional Review Board of Seoul St. Mary's Hospital, College of Medicine, the Catholic University of Korea, Seoul, Republic of Korea (KC11SISI0348). The gingival tissues from the crown lengthening procedures were de-epithelialized, minced, and digested with collagenase IV (Sigma-Aldrich Co., St. Louis, MO, USA). The cell suspension was filtered and seeded with α-MEM (α-MEM, Gibco, Grand Island, NY, USA), containing 15% fetal bovine serum (Gibco), 200 mM L-Glutamine (Sigma-Aldrich Co., St. Louis, MO, USA), and 10 mM ascorbic acid 2-phosphate (Sigma-Aldrich Co., St. Louis, MO, USA) and 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich Co., St. Louis, MO, USA). The cells were incubated at 37 ℃ in a humidified incubator with 5% CO_2 and 95% O_2.

The stem cells were plated at a density of 2.0×10^3 cells/well in 96-well plates and were incubated in α-MEM (Gibco), containing 15% fetal bovine serum (Gibco), 200 mM L-Glutamine (Sigma-Aldrich Co., St. Louis, MO, USA), and 10 mM ascorbic acid 2-phosphate (Sigma-Aldrich Co., St. Louis, MO, USA) and 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma-Aldrich Co., St. Louis, MO, USA) in the presence of the A. radix at final concentrations that ranged from 0.001 to 10 μg/mL (0.001, 0.01, 0.1, 1, 10 μg/mL), respectively. The morphology of the cells was viewed under an inverted microscope (Leica DM IRM, Leica Microsystems, Wetzlar, Germany) on day 1.

Cell proliferation

The analysis of cell proliferation was performed on day 1. Viable cells were identified by using a Cell Counting Kit-8 (CCK-8, Dojindo, Tokyo, Japan) assay. The spe- ctrophotometric absorbance was measured at 450 nm with a microplate reader (BioTek, Winooski, VT, USA) and the analyses were performed five times.

Alkaline phosphatase activity assays

Stem cells were seeded in a 12-well cell culture plate and incubated overnight and the medium was replaced with osteogenic induction medium supplemented with α-MEM containing 15% fetal bovine serum, 100 μM dexamethasone (Sigma-Aldrich Co., St. Louis, MO, USA), 10 mM ascorbic acid 2-phosphate (Sigma-Aldrich Co., St. Louis, MO, USA), and 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich Co., St. Louis, MO, USA) next day. The medium was replaced with fresh induction medium every 3-4 days. Alkaline phosphatase activity assays were performed at day 7 using an assay kit (SensoLyte® pNPP Alkaline Phosphatase Assay Kit, AnaSpec, Inc., Fremont, CA, USA). In short, lysate was mixed with freshly prepared pnitrophenylphosphate substrate (10 mM) and incubated at 37 ℃ and the optical density of p-nitrophenol at 405 nm was determined spectrophotometrically. The experiments were performed five times.

Mineralization assay

The cells were seeded in a 24-well cell culture plate at a density of 1×10^4 cells/well and incubated overnight. The next day, the medium was replaced with osteogenic induction medium supplemented with α-MEM containing 15% fetal bovine serum, 100 μM dexamethasone (Sigma-Aldrich Co., St. Louis, MO, USA), 10 mM β-glycerophosphate (Sigma-Aldrich Co., St. Louis, MO, USA) and 10 mM ascorbic acid 2-phosphate (Sigma-Aldrich Co., St. Louis, MO, USA), and 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich Co., St. Louis, MO, USA). At day 14 and 21, Alizarin red S staining (Sigma-Aldrich Co., St. Louis, MO, USA) was performed to detect calcium formation. Bound dye was solubilized in 10 mM sodium phosphate containing 10% cetylpyridinium chloride and quantitated spectrophotometrically at 562 nm. The morphological evaluation was performed using an inverted microscope (Leica DM IRM). The experiments were performed in triplicate.

Statistical analysis

The data are represented as the mean ± standard deviation. Tests of normality were performed and Levene's test of homogeneity of variances was performed. One-way analysis of variance with post-hoc test (Tukey test) was used to evaluate the different concentration of A. radix on cellular proliferation and differentiation using commercially available statistical software (SPSS 12.0 for Windows, SPSS Inc., Chicago, IL, USA). Statistical significance level was set at $P < 0.05$.

RESULTS

Evaluation of cell morphology

The control group showed spindle-shaped, fibroblast-like morphology on day 1 (Figure 1). The shapes of the cells in 0.001, 0.01, 0.1, 1, 10 μg/mL of A. radix were similar to that of the control group.