Effects of Placental Extract on Cell Proliferation, Type I Collagen Production, and ALP Secretion in Human Osteosarcoma Cell Line Saos-2

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(Accepted for publication, February 22, 2017)

Abstract: Porcine placenta extract (P-placenta) is widely applied in medicine and cosmetics. However, few studies have examined the effect of the extract on the cellular behavior of the osteoblastic cell line Saos-2. Here, we demonstrated that P-placenta enhances the proliferation, collagen type I production, and alkaline phosphatase (ALP) secretion of Saos-2 in vitro. Proliferation of Saos-2 was assessed by MTT and DNA synthesis assays. Type I collagen production and ALP secretion were evaluated using enzyme-linked immunosorbent assay and ALP assays. The cells were treated with/without 20, 200 and 2000 \( \mu \)g/ml of P-placenta for 24 h. We found that 200 \( \mu \)g/ml P-placenta significantly induced the proliferation of Saos-2 and enhanced type I collagen production and ALP secretion. The results indicate that P-placenta controls the cellular behavior of osteoblasts, resulting in the secretion of early bone-related biomarkers.

Key words: ALP, Collagen, Osteoblast, Placenta, Saos-2

Introduction

The placenta is a temporary organ that supports fetal growth by supplying oxygen and nutrients\textsuperscript{31). The organ stores diverse molecules such as vitamins, bioactive peptides, minerals, and growth factors\textsuperscript{32,33). The placental extract has attracted attention worldwide owing to its potential as drugs for clinical treatments\textsuperscript{34) and as supplements in cosmetics\textsuperscript{35,36). Previous studies showed that placental extract has various therapeutic effects, such as wound-healing\textsuperscript{37), anti-inflammatory\textsuperscript{38), and immune-modulating potential\textsuperscript{39). More recently, we reported that porcine placenta extract (P-placenta) controls type I collagen production, cell proliferation, and anti-inflammatory cytokine production of human gingival fibroblasts (HGFs)\textsuperscript{9). However, its pharmaceutical mechanism in other cells, such as bone-related cells, has not been thoroughly examined.

Bone metabolism is systematically regulated by the complex iteration of bone formation and resorption. Osteoblasts mainly engage in bone formation by secreting extracellular matrix, such as type I collagen and other organic proteins. Alkaline phosphatase (ALP) secreted from osteoblasts degrades inorganic pyrophosphate to phosphate, which is associated with the mineralization of bone\textsuperscript{9). Therefore, type I collagen and ALP are important indicators of bone metabolism\textsuperscript{10,11). However, little information is available regarding the effect of placenta on the cellular metabolism of osteoblasts.

The aim of this study was to evaluate the effect of P-placenta on osteoblastic cells, particularly cell proliferation, type I collagen production, and ALP secretion. To evaluate these issues, we used the osteoblastic cell line Saos-2, which was first established from osteosarcoma in 1987\textsuperscript{12). ALP secretion, type I collagen production, and mineralized matrix production have been evaluated in Saos-2 cells and osteoblasts\textsuperscript{12). These cells present mature osteoblastic characteristics\textsuperscript{13), and thus have been extensively investigated in oncology\textsuperscript{14) and bone biology studies\textsuperscript{13). Additionally, biomaterial function was previously evaluated using Saos-2 cells\textsuperscript{15,16).}

Materials and Methods

Reagents

P-placenta was purchased from Nippon Meat Packers, Inc. (Osaka, Japan). The powder-like extract was composed of natural components from the porcine placenta without any additives. Therefore, we used these reagents for the following experiments.

Cell maintenance

Saos-2 cells (HTB85) were provided by the Riken BRC Cell
Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% fetal bovine serum (FBS) at 5% CO\textsubscript{2} and 37°C.

3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and DNA synthesis assay

For DNA synthesis, Saos-2 seeded at 3.1 × 10\textsuperscript{4}/cm\textsuperscript{2} were cultured in DMEM containing 0.5% FBS with/without P-placenta at doses of 20, 200, and 2000 µg/ml for 24 h. The level of DNA synthesis in cells was determined by measuring BrdU-incorporation using the Frontier BrdU Cell Proliferation assay kit (Millipore, Billerica, MA, USA). For the MTT assay, cells were cultured in DMEM containing 10% FBS with P-placenta at the above concentrations for 24 h. The MTT assay was conducted as reported previously\textsuperscript{17}.

Type I collagen production

Cells were seeded at the 3.1 × 10\textsuperscript{4}/cm\textsuperscript{2} in DMEM with 10% FBS, followed by treatment with DMEM containing 1% FBS with/without P-placenta (200 µg/ml) for 24 h. The levels of collagen type I in the media were measured by an enzyme-linked immunosorbent assay (ELISA), using a biotinylated anti-collagen type I antibody (0.2 mg/ml, Rockland Immunochemicals, Limerick, PA, USA). Total protein levels were quantified in the cell lysate isolated with 0.5% TritonX-100 by using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Collagen production was normalized to the protein content in the cell lysates.

ALP assay

Cells were seeded at a density of 3.1 x 10\textsuperscript{4}/cm\textsuperscript{2} in DMEM containing 10% FBS. Next, the cells were treated with DMEM containing 1% FBS with/without P-placenta (200 µg/ml) for 24 h. The cells were lysed with 0.05% TritonX-100 (200 µl), followed by centrifugation at 14,000 xg for 1 min, and the supernatants were collected. ALP activity in the lysates (20 µl) was measured using a LabAssay ALP kit (Wako Pure Chemicals Industries, Ltd., Osaka, Japan). Protein levels in the cell lysates were also measured using the BCA protein assay kit (Thermo Fisher Scientific). ALP activity was normalized to the total protein level in the cell lysates.

Statistical analysis

Statistical analysis was carried out using StatMate software (ATMS, Tokyo, Japan). Statistical significance was evaluated by one-way analysis of variance with a Dunnett’s test or by student's
Results

Effect of P-placenta on Saos-2 cell proliferation

After 24 h of treatment, 200 µg/ml P-placenta increased the level of DNA synthesis compared to the effect of other concentrations in Saos-2, whereas no obvious change was observed at 2000 µg/ml P-placenta treatment (Fig. 1a). The MTT assay showed that 200 µg/ml P-placenta significantly elevated the cell viability of Saos-2 cells (Fig. 1b). These results suggest that there is optimal concentration of P-placenta that facilitates Saos-2 cell proliferation.

Effect of P-placenta on type I collagen production and ALP secretion from Saos-2 cells

Based on the proliferation assay results, we further evaluated the effect of the P-placenta on type I collagen production and ALP secretion from Saos-2 cells. Addition of P-placenta to the media significantly increased the levels of type I collagen production (Fig. 2) and ALP secretion (Fig. 3) from Saos-2 cells compared to that in media without P-placenta.

Discussion

Although P-placenta may have therapeutic potential not only in medicinal drugs but also in cosmetic supplements, its detailed function in the cellular metabolism of osteoblasts remains unclear. In the present study, we demonstrated P-placenta enhances the cell proliferation and regulates the synthesis and secretion of bone-related proteins in osteoblastic Saos-2 cells.

Under the P-placenta stimulation, 200 µg/ml condition significantly enhanced the proliferation of Saos-2 in vitro. In contrast, 2000 µg/ml P-placenta showed a negligible effect on cell proliferation (Fig. 1). We previously reported that HGFs shows a similar cell proliferation tendency under the same P-placenta concentration. Yoshikawa et al. reported that 0–100 µg/ml P-placenta elevated the proliferation of human skin fibroblasts. These results suggest that concentrations near 100 and 200 µg/ml are sufficient for inducing the proliferation of human somatic cells originating from mesenchymal stem cells by using P-placenta in vitro.

Using 200 µg/ml P-placenta, we found that the extract induced type I collagen production and ALP secretion from Saos-2 cells in vitro. The mechanism by which P-placenta elevated this secretion remains unclear. However, P-placenta is produced from porcine placenta extract without any impurities. P-placenta and human placenta extract are known to contain various minerals, vitamins and growth factors. Including insulin-like growth factor-1, a key regulator of fetal development, are synthesized in placenta. Type I collagen synthesis in Saos-2 cells is reportedly upregulated by insulin-like growth factor-1. Moreover, Saos-2 cells respond to extracellular calcium, hormones, microRNAs, and growth factors. These results support that the multiple molecules in P-placenta synergistically, rather than solely, promote type I collagen production and ALP secretion.

A study conducted in Italy indicated that placenta treatment improved periodontal disease, a major cause of tooth loss. However, few studies have evaluated how placenta extract controls the condition of inflammation and bone loss in periodontal disease. More recently, we reported that P-placenta has an anti-inflammatory effect on HGFs in vitro. The current study indicates that P-placenta can elevate the secretion of bone-related protein (type I collagen and ALP), which are pivotal in bone formation (Figs. 2 and 3). Although there is a wide gap between in vitro and in vivo results, additional studies may reveal the mechanism underlying these effects of placenta on bone formation via protein production of osteoblastic cells, which may be useful for treating periodontal diseases. Peri-implant diseases lead to implant failure due to bone loss and loss of osseointegration. Therefore, preventing or treating these diseases is important in dental implant treatment. Regeneration therapy has been advocated as an approach for curing peri-implant diseases. Given the function of P-placenta in vitro culture of Saos-2 cells in the present study, this extract might be effective for the treatment of not only periodontitis, but also peri-implant diseases, and in bone regenerative therapy. However, because the present study was performed under limited conditions and cell lines, additional studies of primary osteoblasts are imperative. Further detailed in vivo experiments are also required to support these results.

In conclusion, we demonstrated that P-placenta enhanced the cell proliferation, type I collagen production, and ALP secretion of Saos-2 cells in vitro. Although it remains unknown how placenta extract modulates the secretion of other bone-related proteins, our results suggest that the extract modulates the cellular functions of osteoblastic cells.

Acknowledgements

This study was supported by the Grant-in Aid for Scientific Research (15K11438) from JSPS.

Conflict of Interest

The authors have declared that no COI exists.

References


