Abstract

BACKGROUND:
Periodontal ligament (PDL) cells form mineralized nodules in vitro. Ascorbic acid is known to be required in this process, although its effect on osteoblastic differentiation of PDL cells remains unclear. The purpose of this study was to determine the role of ascorbic acid on the early osteoblastic differentiation of PDL cells, with regard to alkaline phosphatase (ALP) activity, type I collagen production and integrin expression.

METHODS:
Cultured PDL cells were stimulated at confluence with ascorbic acid in the presence or absence of type I collagen inhibitor and blocking antibodies to integrins. After stimulation, the cells and culture supernatants were examined for ALP activity, type I collagen production, and integrin expression. The ALP activity was measured using a colorimetric assay with p-nitrophenyl phosphate and ALP staining. Enzyme-linked immunosorbent assay (ELISA) was used to determine type I collagen production, and ELISA and flow cytometric analysis were employed for assessment of integrin expression.

RESULTS:
Both ALP activity and type I collagen production were upregulated when PDL cells were cultured in the presence of ascorbic acid (200 microM). Inhibitor of the formation of collagen triple helices and blocking antibodies to alpha2beta1 integrin inhibited ALP activity by 50% in ascorbic acid-stimulated PDL cells. Furthermore, ascorbic acid increased the cell surface expression of alpha2beta1 integrin.

CONCLUSIONS:
Our findings indicated that ascorbic acid increases the ALP activity of PDL cells via type I collagen production and also enhances the expression of alpha2beta1 integrin, which is a major receptor of type I collagen. These results suggest that ascorbic acid promotes the osteoblastic differentiation of PDL cells by modulating type I collagen-alpha2beta1 integrin interaction.