A-6. Effects of Nicotine on the Expression of Cell Cycle Regulatory Proteins of Human Gingival Fibroblasts in virto

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The role of tobacco use in the etiology of periodontal disease is not clear, however most practitioners believe there is strong positive correlation between the use of tobacco and increased incidence and severity of periodontal disease. Recent studies have indeed reported strong associations between smoking and incidence of destructive periodontal disease, alveolar bone loss and response to periodontal therapy. This in vitro study was done to determine the effects of nicotine, a major component of tobacco, on human gingival fibroblast including proliferation, viability, activity, cell cycle, and expression of cell cycle regulatory proteins. A human gingival fibroblast strain derived from a healthy individuals with non-inflamed gingiva was used in this study. Nicotine has been tested for 2 days and 4 days in 5 different concentrations; 0.1$\mu g/ml$; 1$\mu g/ml$; 10$\mu g/ml$; 100$\mu g/ml$; 1000$\mu g/ml$. In control, gingival fibroblasts were supplemented with distilled water. To assess cells proliferation and viability, viable and non-viable cells were counted by hemocytometer; to evaluate cellular activity, MTT assay was employed; to analyze cell cycle distribution, fluorescent propidium iodide-DNA complex were measured; to determine the expression of cell cycle regulatory proteins, western blot analysis was performed. After 2 days and 4 days incubation respectively, at concentrations of 1$\mu g/ml$ - 1000$\mu g/ml$, nicotine significantly inhibited proliferation comparing to non-supplemented controls. The cell viability was significantly decreased after 2 days and 4 days at concentrations of 1$\mu g/ml$ - 1000$\mu g/ml$ and at 10$\mu g/ml$ - 1000 $\mu g/ml$ respectively. After 2 days and 4 days, the cellular activity was significantly decreased at same concentration of 10 $\mu g/ml$ - 1000$\mu g/ml$. The results show that, in vitro, nicotine suppress the proliferation of human gingival fibroblast also it decrease the viability and activity. Accompanying the anticellular effect of nicotine, it induce a significant change in the cell cycle distribution of human gingival fibroblast. Treatment with 100$\mu g/ml$ nicotine for 48 h caused an increase in the proportion of G1-phase cells(from 46.41% to 53.46%) and a decrease in the proportion of S-phase cells(from 17.80% to 14.27%). This means that nicotine causes partial cell arrest in the G1-phase which may in part account for its effects on cell growth. The level of cyclin D and pRB protein in nicotine-treated fibroblast was lower than that of controls. And level of p53, p21 and p16 was higher than that of controls. These results suggests that the decrease of cell proliferation by nicotine may due to the increased expression of p53, p21 and p16 as well as decreased expression of cyclin D and pRB in human gingival fibroblast. Therefore nicotine itself may disturb the maintenance of the periodontal connective tissue as well as wound healing because normal gingival fibroblast function is interfered with nicotine.