Effect of Richlocaine on Proliferative Activity of Osteoblasts and Intracellular Calcium Content in Rats

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We studied the effect of local anesthetic richlocaine on proliferation and intracellular calcium content in cultured osteoblasts from rat parietal bone. In a concentration of 1 mg/ml this drug produced a cytotoxic effect on osteoblasts. In concentrations of 0.01 and 0.001 mg/ml richlocaine in the absence and presence of subtoxic dose of sodium cyanide (0.2 mM) increased the number of osteoblasts by 15.4 and 36.6 or 13.8 and 38.6%, respectively. In a concentration of 1 mg/ml, richlocaine increased the content of cytosolic calcium in osteoblasts by 105%. These effects of richlocaine in low concentrations (0.01 and 0.001 mg/ml) can be related to stimulation of metabolic processes in osteoblasts.

Key Words: *richlocaine; rat osteoblasts; cytotoxicity; free intracellular calcium; fluorescence*

We previously showed that richlocaine improves the efficiency of complex therapy in patients with chronic generalized parodontitis: it accelerates remission, decreases the number of relapses, and stabilizes pathological processes in theparodontium, specifically, in alveolar processes [2]. It was interesting to examine the effect of richlocaine on functional state of osteoblasts.

Our aim was to study the effect of richlocaine on osteoblasts, specifically, on their viability and intracellular free calcium content.

MATERIALS AND METHODS

Experiments were carried out on parietal bone osteoblasts from newborn albino rats. Osteoblasts were isolated and cultured as describes elsewhere [1]. The shapes of the cultured osteoblasts corresponded to published data [5].

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Stock solutions of richlocaine were prepared on RPMI-1640 medium. The medium (3 ml) was placed into am ampoule and diluted to 1:10, 1:50, 1:100, and 1:500.

For evaluation of the effect of richlocaine on the number of cells in culture and their metabolism, the monolayer was treated with trypsin, the cell suspension (10^4/ml, 180 μl) was transferred to wells of a 96-multiwell flat-bottom plastic plate (Costar). Richlocaine (20 μl) in the above concentrations was placed into the corresponding wells. For evaluation of the effect of the drug under hypoxic condition, sodium cyanide (0.2 mM) was added to wells.

Cell viability assessed by trypan blue staining was 85-90%. Cytotoxicity of richlocaine was evaluated using MMT-test [6]. The effect of richlocaine on the level of free cytosolic calcium was studied with fluorescent probe FURA-2/AM (Calbiochem) as described elsewhere [4].

For evaluation of the effect of richlocaine on intracellular calcium content, the drug was added to a cuvette with osteoblast suspension to final concentrations of 1-0.0001 mg/ml. Four measurements were made over 10 min after addition of the drug.

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The data were analyzed statistically using nonparametrical Wilcoxon—Mann—Whitney *U* test.

RESULTS

Richlocaine (1-0.001 mg/ml) added to the incubation medium dose-dependently changed proliferation and metabolism of osteoblasts. In a concentration of 1 mg/ml richlocaine produced a cytotoxic effect and caused cell death, which was confirmed by microscopy. By contrast, in concentrations of 0.01 and 0.001 mg/ml richlocaine increased the number of cells by 15.4 and 36.6%, respectively. It can be hypothesized that in low doses richlocaine stimulated proliferation and metabolism of the parietal bone osteoblasts (Table 1).

Simulation of hypoxia by adding sodium cyanide in a subtoxic dose (0.2 mM) showed that richlocaine (0.001 mg/ml) abolished the effect of hypoxant on osteoblasts and stimulated their proliferative activity (Table 1). Histological study showed that these osteoblasts were viable and virtually did not differ from the control.

The study of the effect of richlocaine on intracellular calcium content showed that only in a concentration of 1 mg/ml the drug significantly increased intracellular calcium content to 205.0±12.9 nM (by 105%). After addition of 0.1, 0.01, and 0.001 mg/ml richlocaine intracellular calcium contents were 98.7± 8.5, 90.0±7.5 and 84.30±4.35 nM, respectively. The control level of cytosolic calcium was 89.2±6.5 nM.

Within the concentration range of 10^{-1} to 10^{-4} mg/ml richlocaine was nontoxic either under normal conditions or in the presence of subtoxic doses of sodium cyanide. In high concentration (1 mg/ml) it produced a cytotoxic effect, which manifested in markedly increased intracellular calcium concentration probably caused by abnormal work of ionic channels and disturbances in membrane integrity.

Thus, in a concentration of 1 mg/ml richlocaine produces a cytotoxic action on osteoblasts from rat parietal bone. In concentrations of 0.01 and 0.001 mg/ml it increased the number of osteoblasts both in the absence and presence of sodium cyanide. In a concentration of 1 mg/ml the drug increased intracellular calcium content in osteoblasts. Probably, the effects of richlocaine in low concentrations (0.01 and 0.001 mg/ml) reflect stimulation of metabolic processes.

TABLE 1. Effect of Richlocaine on the Number and Metabolism of Osteoblasts Assessed by Optical Density of Formazan Formed in MTT-Test and in the Presence of Sodium Cyanide over 24 h (*M*±*m*)

Concentration of richlocaine, mg/ml	Optical density, %	
	formazan test	sodium cyanide test
Control	100	100
0.1	84.6±7.5	85.8±5.7
0.01	115.4±8.1	113.8±10.5
0.001	136.6±11.4*	138.6±14.2*
0.0001	102.2±9.2	89.3±15.1

Note. *p=0.05 compared to the control.

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