[Introduction] The effects of electromagnetic field stimulation on the fracture healing process are widely known, but much remains unknown about its exact mechanisms of action. In our previous study, we applied electromagnetic field stimulation to MC3T3-E1, an osteoblast-like cell line, under optimal conditions and evaluated increases in the cell’s ability to synthesize DNA. To further clarify the mechanism, we evaluated its effects on changes in intracellular calcium concentrations, transforming growth factor-β (TGF-β), type-I collagen, and osteocalcin in the extracellular matrix. In the present study, we evaluated its effects on the key bone growth factor insulin-growth factor-I (IGF-I), and IGF-I receptors.

[Method] The osteoblast-like cell line MC3T3-E1 used in the present study was shown to differentiate in cultures in the same way as in vivo and is capable of inducing calcification. The cells were subcultured in α-MEM containing 10% FBS, penicillin and streptomycin at 37°C and 5% CO2, using 0.05% trypsin and 0.53 mM EDTA. A pulse electromagnetic field stimulator (Riken) was applied under optimal stimulation conditions for DNA synthesis promotion, determined to be 1.0 Gauss, 10 Hz and 25 μsec. Immunologic staining of IGF-I and IGF-I receptors were proved using ABC method. The IGF-I production volume was determined using ELISA and IGF-I receptors were assayed using a flow cytometry system (FACSCalibur). Comparisons were made between with stimulated and non-stimulated groups. Statistical analysis was performed using Student’s t-test. A p-value of less than 0.05 was considered evidence of statistical significance.

[Results] Immunological staining of IGF-I showed that the cell density increased with time showing multiple (double or triple) layers. The cytoplasm was gradually increasingly densely stained due to staining of granular substances. However, no apparent differences of array or morphology were noted in cells of the stimulated and non-stimulated groups. Immunological staining of IGF-I receptors showed that the cell density and stainability increased every week, as in the case of IGF-I. Here again, however, no apparent differences were recognized between the groups.

The amount of IGF-I produced by osteoblasts was determined by ELISA and compared between the stimulated and non-stimulated groups. More IGF-I was produced in the stimulated group than in the non-stimulated group at all time points from 3 days to 4 weeks after stimulation. Differences were most marked in the first week, when the amount produced was 151.2±3.25 ng/10^5 cells in the stimulated group and 82.1±2.04 ng/10^5 cells in the non-stimulated group. Statistical significance was recognized in each week. To examine changes in the production of IGF-I receptors, we determined IGF-I receptors produced by osteoblasts using a flow cytometry system (FACSCalibur) 3 days and 1, 2 and 3 weeks after starting electromagnetic field stimulation. Cells expressing IGF-I receptors increased after starting stimulation, and reached a peak 3 days later. The difference was most marked 1 week after stimulation, with the number in the stimulated group being greater than that in the non-stimulated group. Statistical significance was recognized at 3 days, 1 and 3 weeks.

[Discussion] Chemical agents promoting osteogenesis include parathyroid hormone (PTH), calcitomine, active vitamin D3 and other calcium regulating hormones, cytokines, growth factors, and calcium ions. Physical factors such as pressure, electric stimulation and ultrasonic shocks are also known to promote osteogenesis. Pulse electromagnetic field stimulation has been widely used in the treatment of refractive fractures since Bassett et al reported its beneficial effects for the first time.

In the past, demonstrated that TGF-β and intracellular free calcium ion levels rose and type I collagen increased and osteocalcin decreased on extracellular matrices, and evaluated increases in the cell’s ability to synthesize DNA when the above-mentioned optimal electromagnetic field stimulation was applied. IGF-I showed a great variety of effects such as the promotion of growth, proliferation and differentiation, regulatory function, maintenance of cell functions, and inhibition of apoptosis. IGF-I showed great affinity with IGF-I receptors, and exerted its effects when bound with them. The fact that IGF-I and IGF-I receptors increased in the present study suggests the possibility that cellular DNA synthesis via IGF-I and IGF-I receptors is promoted by electromagnetic stimulation. In the past and also in this study, electromagnetic field stimulation was most effective 7 to 12 days after starting culture, suggesting that the stimulation was effective at an early stage of differentiation.

Regarding its indirect effects, environmental changes caused by electromagnetic field stimulation such as decreases in partial oxygen pressure and increases in pH around negative electrodes favor the formation of callus and calcification. Other indirect factors that contribute to osteogenesis are thought to include increases in local blood flow, functional arrangement of collagen, an important bone element, and chemical changes. Direct effects of electromagnetic field stimulation include increases in the ability of osteoblasts to produce TGF-β and IGF-I, as well as IGF-I receptors. These factors and receptors activate downward signal transmission and increase intracellular calcium, thereby promoting the DNA synthesis DNA and activating their growth and differentiation. TGF-β and IGF-I also promote the synthesis of type-I collagen. Increases of TGF-β, IGF-I and IGF-I receptors thus promote the production of organic matrices, as well as the precipitation and deposit of hydroxyapatite crystals, and accelerate calcification. The direct and indirect effects of electromagnetic field stimulation promoted cellular DNA synthesis, and it was discussed one of the mechanism of promoting osteogenesis.