電磁場刺激對造骨細胞影響之機制探討

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電磁場刺激對造骨細胞影響之機制探討

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計畫主持人： 孫 瑞 昇
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Abstract

The use of electricity in the medicine was accelerated in 1953 when Yasuda induced osteogenesis by electrical stimulation. Increased osteogenesis has been reported using all major forms of electrical stimulation; direct current, capacitively coupled and inductively coupled electromagnetic fields. The manner in which electric fields influence the behavior of cells remains poorly understood. The purposes of this research were to assess the stimulatory effect of pulsed electromagnetic field (PEMF) on bone cells and to validate that bone tissue-like formation was associated with the increase in the number of cells and/or with the enhancement of cellular differentiation. The osteoblast-like cells were isolated and pulsed electromagnetic field (PEMF) stimulation was stimulated from the 3rd to 14th days with a duration of 8 hrs/ day. The proliferation of osteoblasts was assessed by colorimetric MTT assay, and the synthesis and secretion of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) by osteoblasts were measured. The differentiation was analyzed by ALP staining and von-Kossa staining; while the gene expression during PEMF stimulation was also analyzed. The cellular density in the PEMF group increased faster than in the control group until the cells became confluent and started to form multilayers; thereafter, the optic absorption was indiscernible in both groups.

The ALP concentrations in the PEMF stimulated group decreased over the control group at 3, 5 and 7 days of culture, respectively. Similar result was observed in the von-Kossa staining. In the presence of on PEMF stimulation, type I collagen, osteocalcin and osteopontin mRNA expression were quite similar to that of control group at the 14 days’ experimental period, while the OPG mRNA expression was up-regulated and the RANKL mRNA expression were down-regulated than that of the control. Our result demonstrated that PEMF treatment on osteoblasts may accelerate cellular proliferation, but did not affect cellular differentiation, maturation and mineralization nodules formation. The PEMF stimulated increase in the bone tissue-like formation was most likely associated with the increase in the number of cells, but not with the enhancement of the osteoblasts differentiation.

Keywords: Osteoblasts, PEMF, collagen, osteocalcin, RANKL

Introduction

Life on earth has evolved in a sea of natural electromagnetic fields. Over the past century, this natural environment has sharply changed with introduction of a vast and growing spectrum of man-made electromagnetic fields [Adey WR 1993]. The use of electricity in the medicine was accelerated in 1953 when Yasuda induced osteogenesis by electrical stimulation [Kubota K et al. 1995]. Increased osteogenesis has been reported using all major forms of electrical stimulation; direct current, capacitively coupled and inductively coupled electromagnetic fields [Kubota K
et al. 1995]. Later, electrically induced osteogenesis has been studied intensely both in vivo and in vitro [Fredericks et al. 2000]. Despite the clinical success, negative reports on the in vitro effects of electric stimulation on the cellular proliferation, differentiation, and bone formation were reported [Elliott et al. 1988]. The manner in which electric fields influence the behavior of cells remains poorly understood. The purposes of this research were to assess the stimulatory effect of pulsed electromagnetic field (PEMF) on bone cells and to validate that bone tissue-like formation was associated with the increase in the number of cells and/or with the enhancement of cellular differentiation.

Materials and Methods
The osteoblast-like cells were isolated from sequential digestion of newborn ICR mice. The cells were cultured in α-MEM supplemented with antibiotics, 10% FBS, ascorbic acid, and β-glycerophosphate; fresh medium was replaced every 2 days. The construction of the generator used to stimulate the cells has been manufactured and calibrated by the Department of Mechanical Engineering and Department of Electrical Engineering, National Taiwan University. Briefly, pulsed electromagnetic field (PEMF) stimulation was generated to produce a pulsed electromagnetic field with a frequency 15 Hz (pulse width T1: 5 msec., burst width T2: 0.2 msec., burst wait T3: 0.028 msec., pulse wait: 61.6 msec.), and magnetic field strength of 1 gauss (electric field strength: 2 mv/cm). In order to assess the effects of PEMF stimulation on the osteoblasts, a series of osteoblast cell populations were cultured for 2 days without treatment to facilitate the attachment of osteoblasts, then were stimulated from the 3rd to 14th days with a duration of 8 hrs/ day. The proliferation of osteoblasts was assessed by colorimetric MTT assay, and the synthesis and secretion of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) by osteoblasts were measured. The differentiation was analyzed by ALP staining and von-Kossa staining; while the gene expression during PEMF stimulation was also analyzed.

Results
The cellular density in the PEMF group increased faster than in the control group until the cells became confluent and started to form multilayers; thereafter, the optic absorption was indiscernible in both groups. In both control and PEMF stimulated groups, there is no detectable LDH secretion to the medium noted during the experimental period. The ALP concentrations in the PEMF stimulated group decreased 11.4, 20.1 and 32.0% over the control group at 3, 5 and 7 days of culture, respectively. At the 14 day of culture, the ALP concentration of PEMF group still decreased 12.4% over the control group. The ALP positive staining increased gradually as the culture period passed; however, did not attain a grossly-observable significant degree at the 14th day’s culture. Similar result was observed in the groups cultured with PEMF stimulation. Similar result was observed in the von-Kossa staining. In the presence of on PEMF stimulation, type I collagen, osteocalcin and osteopontin mRNA expression were quite similar to that of control group at the 14 days’ experimental period, while the OPG mRNA expression was up-regulated and the RANKL mRNA expression were down-regulated than that of the control.

Discussion
The present research was undertaken to assess the effects of PEMF stimulation on the neonatal osteoblasts. Our result demonstrated that PEMF treatment on osteoblasts may accelerate cellular proliferation, but did not affect cellular differentiation, maturation and mineralization nodules formation. The PEMF stimulated increase in the bone tissue-like formation was most likely
associated with the increase in the number of cells, but not with the enhancement of the osteoblasts differentiation.

References