

Nitric Oxide

= Abstract =

Nitric Oxide Production of Rat Osteoblast Cells by Pulsed Electromagnetic Field Radiation

Seong-Hwan Moon, M.D., Jin-Woo Lee, M.D., Jun-Seop Jahng, M.D.

Department of Orthopaedic Surgery Yonsei University College of Medicine, Seoul, Korea

Experimental study was conducted to prove the effect of pulsed electromagnetic field(PEMF) on the production of Nitric oxide(NO) from the cultured rat osteoblast-like cells. Calvarium of thirty Sprague-Dawley rats was digested by sequential collagenase and cultured in-vitro. The osteoblast cell phenotype was confirmed by expression of osteocalcin by immunohistochemistry. PEMF was generated and applied to cultured osteoblast cells. Production of NO was measured by Greiss reaction. Ten minute exposure of PEMF to osteoblast cell showed increased NO content at 24 and 48 hours(p<0.05). Cultures with different duration of PEMF exposure(10, 20, 30 60 minutes) demonstrated similar responses. In conclusion, this study proved that NO can be generated with PEMF which support the notion that NO can be a possible mediator of PEMF on bone metabolism.

Key Words : Osteoblast, Pulsed electromagnetic field, Nitric oxide

Nitric Oxide(NO) Free radical
가 NO가

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Tel : 02) 361-5649, Fax : 02) 363-1139

E-mail : shmoon@yumc.yonsei.ac.kr

* 1996

4)
 (osteoblast) inter-
 leukin-1, TNF- α , interferon-
 NO²¹⁾, dexamethasone, 1.
 cyclohexamide, PTH, Vitamine D, TGF- β
 가^{17,21)}. Estrogen 1 Sprague-Dawley rat 30
 NO²⁶⁾
 calcitonin cAMP 가가 inducible Phosphate buffered
 nitric oxide synthase(NOS) mRNA saline(PBS) 1-2mm³
 가 NO 가 가 PBS
 NO¹⁷⁾ . 0.1% collagenase, 0.05%
 NO²²⁾ trypsin, 136mM NaCl, 2.6mM KCl,
 0.36mM NaH₂PO₄, 5mM EDTA
 37° 20
 7) . 4 . 3, 4
 nylon mesh
 6) Dulbeccó's
 modified eagle's medium (DMEM) 10cc
 2 .
 10% Fetal calf serum(FCS)
 1,2,9,11,12) DMEM 가 5×10⁶/M ϕ
 tissue culture flask(25cm²)
 CO₂ incubator (37°C, 5%
 4 . 24 , 3 , 7
 3), BMP-2, BMP- CO₂). 7 100%
 lysozyme
 19), 13), DNA
 가¹⁸⁾ .
 NO가¹⁶⁾ 2. (Osteocalcin)
 NO 7 4% paraformaldehyde
 NO 4 °C protein blocking agent
 가 5 . Rat osteocalcin anti-
 가 NO serum(1:1000) 4 °C
 NO . biotin-strepta
 NO 가 . vidin-peroxidase (Lipshaw, Pittsburgh,
 NO . PA) immunoreactivity
 가 NO 3.
 McLeod¹⁵⁾

30cm polyethylene tube 12cm NO (normalization)

3 650 NO 3

1.8millitelsa(mT) magnetic flux density (Walker Mode MG2A gaussmeter) 30Hz sinusoidal 1.8mT NO t-test

30Hz sinusoidal 1.8mT NO (ANOVA) p<0.05

4.

1) (10) NO 1.

(50μl)

10 CO₂ incubator 6 osteoclastin 90%

48 -80 (Fig. 1).

2) NO 2. (10) NO

10, 20, 30, 60 CO₂ incubator 가 10 6

48 80.

5. NO

NO NO Nitrite spectrophotometric assay

Greiss¹⁰⁾ NO₂- NO₃- NO₂-²⁴⁾

NO Greiss NO

3

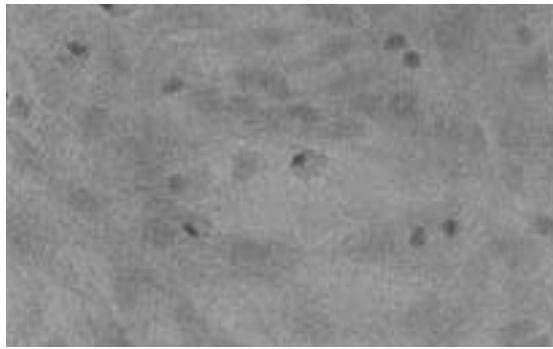


Fig. 1. Immunohistochemical staining for osteocalcin in cultured osteoblast-like cells from calvarium of Sprague-Dawley rat(X 200)

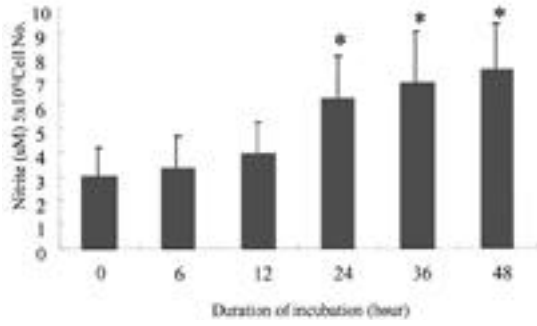


Fig. 2. Production of NO (mean ± standard deviation) over time (0, 6, 12, 24, 36, 48 hour) from cultured rat osteoblast under the stimulation of pulsed electromagnetic field with 10 minute stimulation *: p<0.05

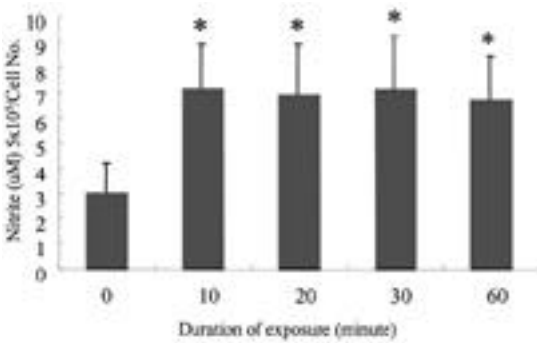


Fig. 3. Production of NO (mean ± standard deviation) at 48 hour incubation from cultured rat osteoblast under the stimulation of pulsed electromagnetic field with various duration (10, 20, 30, 60 minute). *: p<0.05

NO 24 48 NO 가 NO (p<0.05)
 (Fig. 2).

3. NO 10, 20, 30, 60 가 NO 가 NO (p<0.05).
 (Fig. 3).

NO가 가
 NO
 가 NO 가
 cytokine NO 가
 가 inducible NOS NO
 NO 가 NO 2.5 cytokine
 NO inducible NOS 가 NO가
 NO NO
 NO cytokine
 가 가

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