

The Effects of Extremely Low Frequency Pulsed Electromagnetic Field on Collagen Synthesis of Rat Skin: a Biochemical and Histological Approach

Shahin Ahmadian^{*1}, Saeed Rezaei Zarchi¹ and Bahram Bolouri²

¹Dept. of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran; ²Dept. of Biophysics, Iran University of Medical Sciences, Tehran, Iran

Received 11 December 2004; revised 13 July 2005; accepted 14 December 2005

ABSTRACT

The efficacious effects of pulsed electromagnetic field (PEMF) under the certain field parameters like frequency and the field intensity have been reported for various tissue and molecules. Since collagen is found abundantly in most tissue structures, this research was designed to further investigate the effects of extremely low frequency (ELF) PEMF on the synthesis of the epidermal collagen. To do the task, six groups of animals each consisting of eight mature male rats were selected randomly as one group for the control and five for the test. The field was generated by using a parallel set of Helmholtz coil. The first set of experiments was carried out at the peak intensity of 2 mT (milli Tesla) for different frequencies of 25, 50 and 100 Hz. Since the most effective frequency turned out to be 25 Hz, another set of experiment was conducted using this frequency and two different field intensities of 1 and 4 mT. The field was applied for 2.5 h/day lasting for 8 days, keeping the same procedure for the control group except for the field turned off. On the ninth day, the rats were sacrificed and the skin samples from the dorsal region were taken for biochemical assessment of collagen by measuring hydroxyproline content using Stegeman-Stalder method and histological assessment. The data indicated that pulsed electromagnetic field of 2 mT at 25 Hz increased the collagen synthesis ($P<0.05$). The other intensities and frequency setting did not have much distinguishable effect, however, at the frequency of 25 Hz and 4 mT, the field effect on the collagen increase was also noticeable. It was concluded that applying the field parameters of 25 Hz and 2 mT peak intensity for 2.5 h/day during eight days rendered a significant increase in collagen synthesis in rat skin. Histological observations were consistent with the biochemical findings. *Iran. Biomed. J. 10 (1): 33-38, 2006*

Keywords: Extremely low frequency pulsed electromagnetic field (ELF-PEMF), Collagen synthesis, Rat skin

INTRODUCTION

Collagen, the most important component of animal connective tissue comprises about one-third of body protein. It is not only the most abundant, but also one of the most unusual animal proteins, since it is devoid of cysteine and tryptophan and contains more than 30 percent glycine and such unusual amino acids as hydroxyproline and hydroxylysine. Hydroxyproline is found almost exclusively in collagen, constituting about 14% of the dry weight of the protein, and the amounts of this imino acid are relatively constant in collagens from various tissues in humans. The rate of hydroxyproline formation is therefore considered

to be a good indication of the rate of collagen biosynthesis. The collagen content of a tissue is determined by measuring the content of protein bound hydroxyproline [1, 2]. In the last 20 years, there has been increasing interest in investigating the possible effects of extremely low frequency electric and magnetic fields [3-7]. The experimental approach to the effects of electromagnetic fields (EMF) on living systems is complicated by reported non-linearities (intensity, frequency and time windows of the fields) and other variables (cell type, age and treatment) so that extrapolation or replications among laboratories is difficult. The possible mechanisms of the induced effects are not known, although a number of theoretical models

*Corresponding Author; Tel. (+98-21) 6695 6983; Fax: (+98-21) 6640 4680; E-mail: ahmadian@ibb.ut.ac.ir

have been proposed [8-11]. This may be partially due to difficulties in demonstrating reproducible results. As collagen constitutes almost one third of body protein and plays an important role in different tissue structures, the aim of this research was to study the effects of pulsed EMF at different frequency and field intensity on synthesis of the epidermal collagen.

MATERIALS AND METHODS

Chemicals and animals. Chemicals were purchased from Sigma (St. Louis, Mo). Adult male Sprague-Dawley rats weighing approximately 175 ± 25 g (8 weeks of age) were obtained from the Pasteur Institute of Iran (Tehran) and acclimatized for 5 days in one of the animal rooms in the Department before being used for the EMF experiment. They were maintained under controlled temperature of $21 \pm 1^\circ\text{C}$ in 12 h light/12 h darkness schedule. Food and tap water were freely available in the cage.

Experimental set up. The PEMF was generated by using a set of parallel Helmholtz coil having 75 cm diameter. The coils were separated 35 cm a part and housed in wooden frame support. The animals, residing in plexi glass housing, were placed in the coil center to assure receiving a uniform field. No metal components were included in the experimental set up. Six groups of animals each consisting of eight mature male rats were selected randomly as one group for the control and five groups for the test. A field intensity of 2 mT (milli Tesla) for different frequencies of 25, 50 and 100 Hz yielded the most effective frequency to be 25 Hz. Then, at this frequency, two different field intensities of 1 and 4 mT were applied. The treatment time of 2.5 h/day lasted for 8 days, keeping the same procedure for the control group except with the field turned off. On the ninth day, the rats were sacrificed by using anesthetic, and skin samples from the posterior dorsal region were taken for collagen assessment.

Determination of tissue hydroxyproline content. At the end of the application time, a piece of dorsal skin (1 g) was excised and hydroxyproline contents of samples were determined with Stegmann's method [12] briefly small sample of skins was hydrolyzed without any preliminary purification by

adding 6 N HCl for 16 h at 107°C . The pH of sample solutions was adjusted to 6-7 with dilute HCl and 2.5 N NaOH. Two ml of each sample solution was taken to tubes and their optical densities were determined spectrophotometrically at 557 nm. The hydroxyproline concentrations were determined directly from the standard curves obtained fresh daily with freshly prepared standard solutions.

Histological investigation. For light microscopy, skin tissue samples (about 2 mm thick) were fixed in 10% formalin and embedded in paraffin according to the standard procedures. The transverse and longitudinal sections (5 μm thick) were cut and stained using Masson's Trichrom stain, to check for bluish color associated with the presence of collagen fibers [13].

Statistical analysis. For each group, the amount of hydroxyproline content was compared using student's *t*-test. The comparison of each group with the control with respect to the different intensity was made with one way analysis of variance (ANOVA); $P < 0.05$ was considered significant.

RESULTS

Effects of 25, 50 and 100 Hz with 2 mT field intensity on the hydroxyproline contents of rat skin. There were no significant differences in food or water consumption and no visible clinical signs were observed during the study between the test groups and the control animals. The average skin hydroxyproline amounts of control group were found to be 12.24 ± 1.86 mg/g wet wt. The average hydroxyproline contents group 1 (25 Hz, 2 mT) were found to be 14.61 ± 1.33 mg/g wet wt. Figure 1A shows the effects of 25 Hz magnetic field on collagen synthesis of rat skin. As can be seen, after 8 days, 2.5 h/day of exposure, there is a statistically significant increase in hydroxyproline in 25 Hz group as compared with the control group ($P < 0.02$). The average skin hydroxyproline amounts of groups 2 (50 Hz, 2 mT) and 3 (100 Hz, 2 mT) were found to be 12.27 ± 1.77 mg/g wet wt and 12.55 ± 1.79 mg/g wet wt, respectively. The changes in hydroxyproline contents of these groups are given in Figure 1B and C. The results of statistical analysis found for each group show that the increase of hydroxyproline at these frequencies were not statistically significant ($P > 0.05$).

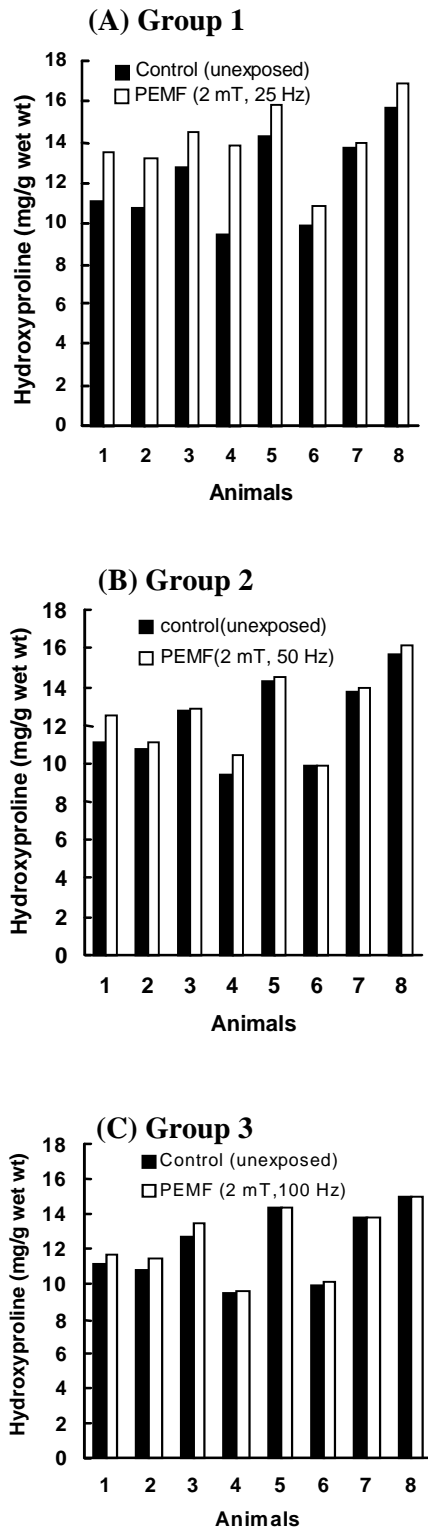


Fig. 1. The hydroxyproline contents of rat skin treated with field intensity of 2 mili Tesla with frequencies of (A, 25; B, 50 and C, 100 Hz). Statistical comparisons were made with paired student's *t*-test. There is statistically significant increase in hydroxyproline contents for 25 Hz, $P < 0.02$. PEMF, pulsed electromagnetic field.

Effects of 25 Hz magnetic field with 1 and 4 mT intensities on the hydroxyproline contents of rat skin. As the results indicated in Figure 1A, 25 Hz yielded the most effective frequency. At this frequency, two different field intensities of 1 and 4 mT (below and above intensities of 2 mT) were applied. The treatment time again was 2.5 h/day lasted for 8 days. The results of statistical analysis found for each group are as below:

a) 25 Hz, 1 mT: field intensity of 1 mT increased skin hydroxyproline for the 8 days duration of field application, however, the increase was not found statistically significant ($P > 0.05$, Fig. 2A).

b) 25 Hz, 4 mT: field intensity of 4 mT increased rat skin hydroxyproline much more than 1 mT and the increase was statistically significant ($P < 0.05$, Fig. 2B). The mean of hydroxyproline contents of the groups is given in Figure 3.

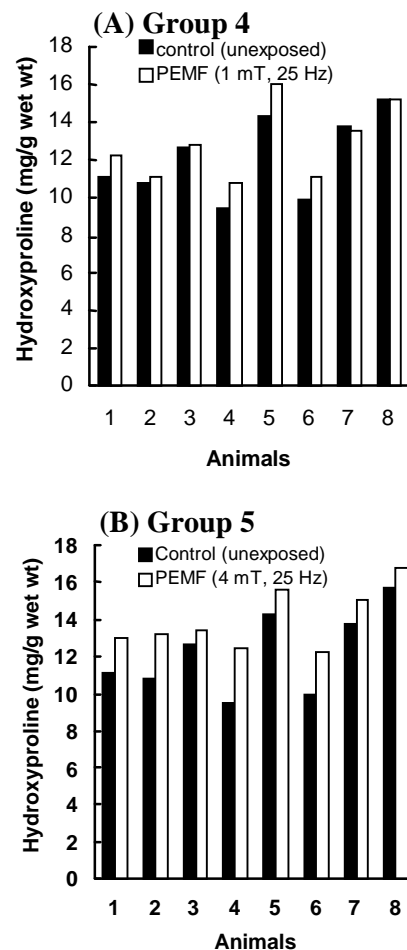


Fig. 2. The hydroxyproline contents of rat skin treated with 25 Hz and field intensity of (A, 1 and B, 4 mili Tesla). There is statistically significant increase in hydroxyproline contents at 4 mili Tesla, 25 Hz, $P < 0.04$. PEMF, pulsed electromagnetic field.

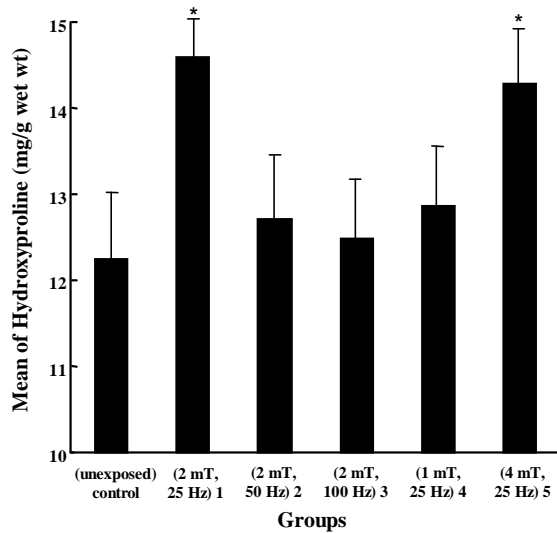


Fig. 3. The mean of hydroxyproline contents (mg/1g wet wt) of exposed groups with the control (sham). *Significantly different from control $P < 0.05$. mT, mili Tesla.

Histological studies. The tissue samples from each test and control group were taken for histological examinations. The results of Masson's Trichrom staining of group 1 (25 Hz, 2 mT) as compared to the control rendered more blue colors as blue stain was associated with the collagen (Fig. 4A and B). However, the difference of collagen content in the other groups were not significant for

the group 5 (25 Hz, 4 mT) the difference was markedly noticeable.

DISCUSSION

In this work, we investigated the effects of PEMF on the synthesis of collagen of rat's skin at three different field intensities (1, 2 and 4 mT) and three different frequencies (25, 50 and 100 Hz) setting. The findings indicate that PEMF does have effect on collagen synthesis of the rat's skin. Yet, out of these different frequencies used in the experiments, the 25 Hz at field intensity of 2 and 4 mT was the most effective. This means that the process in a way observed some kind of "window effect" with regard to the employed frequency range. The window effect is in consistent with the work of others [14-16]. These results are also consistent with the enhancement of differentiation of rat osteoblastic cells and human fibroblasts in response to PEMF exposure [17,18]. Differentiation-modulating activity of EMF includes effects on transcriptional activity of human cells and phenotypic differentiation of fibroblasts [19]. In animal cell culture, EMF treatment induced differentiation of chondrocytes [20]. In contrast, EMF treatment has also been shown to inhibit retinoic acid-induced differentiation of embryonal carcinoma cells [21].

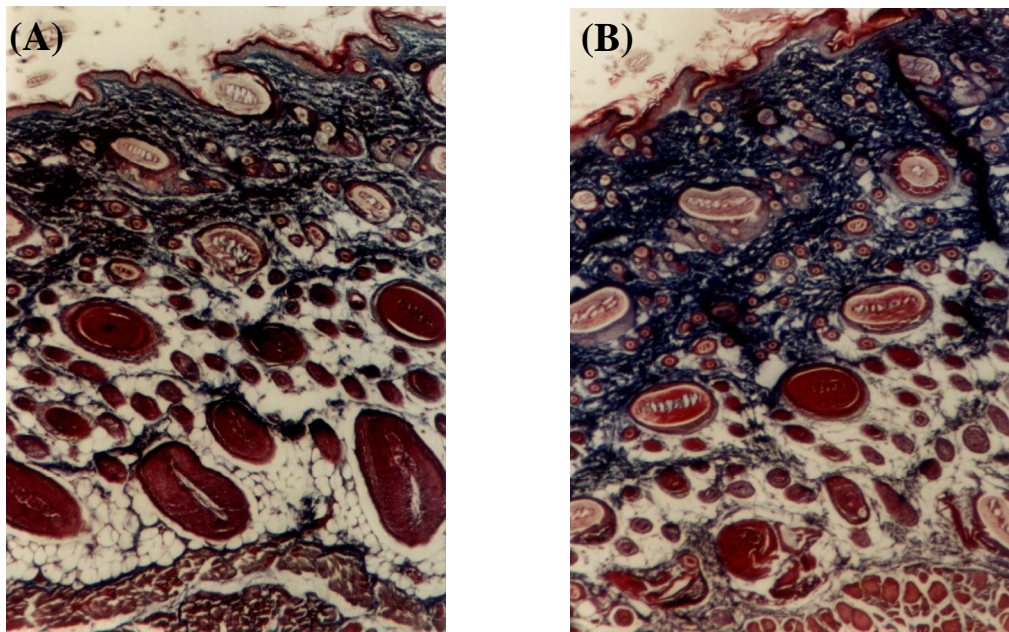


Fig. 4. Histological sections of rat skin. (A), Control (unexposed) group; (B), Pulsed electromagnetic field (2 mili Tesla, 25 Hz)-treated group. The blue colors indicate the presence of collagen in tissue. A noticeable increase in the amount of collagen is seen in treated group (Masson's Trichrome stain, $\times 160$).

The EMF effects on differentiation and collagen synthesis depend on a number of factors including the frequency, the contribution of electrical field components generated by the coil [17], the level of magnetic induction, the cell density, and the cell type and the assay system investigated, and finally, the effects of EMF are limited to narrow biologically active windows in frequency and intensity [22, 23]. Histological observation of the collagen synthesis of treated-rat skin revealed more intense staining with Masson's Trichrom stain which is specific for collagen in compare to untreated skin. Considering the histological and biochemical results, different electromagnetic frequencies seem to exert a different effect on collagen synthesis.

It was concluded that PEMF of 25 Hz with peak intensity of 2 mT can significantly enhance the collagen synthesis in rat skin when applied for 2.5 h/day for duration of 8 days.

ACKNOWLEDGMENTS

The authors wish to thank Dr. S. Yousefi and the colleagues at Pathology Department, Ali Asghar Medical Teaching Hospital for the expert histological comments and Mrs. M. Shafiezhadeh for her assistance in the preparation of this manuscript. This work was supported by Research Council of the University of Tehran (No.521/3/603).

REFERENCES

1. Kelley Carneiro, J. (1992) Basic Histology, Appleton & Lang Company. pp. 137-147.
2. Prockop, D.J. (1979) The biosynthesis of collagen and its disorders. *N. Engl. J. Med.* 301: 13-23.
3. Tenforde, T.S. (1995) Interaction of extremely low frequency electric and magnetic fields with humans. In: *Handbook of Biological Effects of Electromagnetic Fields*. (Polk, C. and Postow, E. eds.), 2nd ed., Boca Raton, CRC press. pp: 185-230.
4. Obo, M., Konishi, S., Otaka, Y. and Kitamura, S. (2002) Effects of magnetic field exposure on calcium channel currents using patch clamp technique. *Bioelectromagnetics* 23: 306-314.
5. Marino, A.A., Kolomytkin, O.V. and Frilot, C. (2003) Extra cellular currents alter gap junction intercellular communication in synovial fibroblasts. *Bioelectromagnetics* 24 (3): 199-205.
6. Chang, W.H., Chen, L.T., Sun, J.S. and Lin, F.H. (2004) Effect of pulse-burst electromagnetic field stimulation on osteoblast cell activities. *Bioelectromagnetics* 25 (6): 457-465.
7. De Mattei, M., Caylino, N., Moscheni, C., Dellavia, C., Calastrini, C., Pellati, A., Gioia, M., Caruso, A. and Stabellini, G. (2005) Changes in polyamines, c-myc and c-fos expression in osteoblast-like cells exposed electromagnetic fields. *Bioelectromagnetics* 26 (3): 207-214.
8. Barnes, P.S. (1996) Effect of electromagnetic field on the rate of chemical reactions. *Biophysics* 41: 801-808.
9. Loschinger, M., Thumm, S., Hammerle, H. and Rodemann, H.P. (1999) Induction of intracellular calcium oscillation in human skin fibroblast populations by sinusoidal extremely low frequency magnetic fields (20 Hz, 8 mT) is dependent on the differentiation state of the single cell. *Radiat. Res.* 151 (2): 195-200.
10. Hair, P.S., Schoebach, K.H. and Buescher, E.S. (2003) Sub-microsecond intense pulsed electric field applications to cells show specificity of effects. *Bioelectrochemistry* 61 (1-2): 65-72.
11. Aaran, R.K., Boyan, B.D., Ciombor, D.M., Chwartz, Z. and Simon, B.J. (2004), Stimulation of growth factor synthesis by electric and electromagnetic fields. *Clin. Orthop. Relat. Res.* 419: 30-37.
12. Stegemann, H. and Stalder, K. (1967) Determination of hydroxyproline. *Clin. Chim. Acta* 8: 267-273.
13. Bancroft, J. and Stevens, A. (1982) Theory and practice of Histological Techniques. 2nd ed., pp. 131-135.
14. Good, R. and Blank, M. (2002) Insights into electromagnetic interaction mechanisms. *J. Cell Physiol.* 192 (1): 16-22.
15. Binhi, V.N. and Goldman, R.J. (2000) Ion-protein dissociation predicts "windows" in electric field-induced wound-cell proliferation. *Biochem. Biophys. Acta* 1474 (2): 147-156.
16. Blank, M. (1995) Biological effects on environmental electromagnetic fields: Molecular mechanisms. *Biosystems* 35: 175-178.
17. Katsir, G. and Parolo, A.H. (1998) Enhancement proliferation caused by a low frequency weak magnetic field in chick embryo fibroblasts is suppressed by radical scavengers. *Biochem. Biophys. Res. Commun.* 252 (3): 753-756.
18. Sun, S., Wise, J. and Cho, M. (2004) Human fibroblast migration in three-dimensional collagen gel in response to noninvasive electrical stimulus. Characterization of induced three-dimensional cell movement. *Tissue Eng.* 10 (9): 1548-1557.
19. Rodemann, H.P., Bayreuther, K. and Pflleiderer, G. (1989) The differentiation of normal and transformed human fibroblasts *in vitro* is influenced by electromagnetic fields. *Exp. Cell Res.* 182: 610-621.
20. Hiraki, Y., Endo, N., Takigawa, M., Asada, A., Takashi, H. and Suzuki, F. (1987) Enhancement responsiveness to parathyroid hormone and induction of functional differentiation of cultured rabbit costal chondrocytes by a pulsed electro-

- magnetic field. *Biochim. Biophys. Acta* 93: 94-100.
21. Akamine, T., Muramatsu, H., Hamada, H. and Sakou, T. (1985) Effects of pulsed electromagnetic field on growth and differentiation of embryonal carcinoma cells. *J. Cell Physiol.* 124: 247-254.
 22. Takahashi, K., Kaneko, I., Date, M. and Fukada, E. (1986) Effect of pulsing electromagnetic fields on DNA synthesis in mammalian cells in culture. *Experientia* 42:185-186.
 23. Ivancsits, S., Pilger, A., Diem, E., Jahn, O., Rudiger, H.W. (2005) Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields. *Mutat. Res.* 583 (2):184-188.