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Influence of Electromagnetic Fields on Endochondral Bone Formation

Deborah McK. Ciombor and Roy K. Aaron

Department of Orthopaedics, Brown University, and Orthopaedic Research Laboratory, Department of Surgery, Roger Williams Medical Center, Providence, Rhode Island 00928

Abstract Endochondral ossification is a basic physiological process in limb development and is central to bone repair and linear growth. Factors which regulate endochondral ossification include several biophysical and biochemical agents and are of interest from clinical and biological perspectives. One of these agents, electric stimulation, has been shown to result in enhanced synthesis of extracellular matrix, calcification, and bone formation in a number of experimental systems and is the subject of this review. The effects of electric stimulation have been studied in embryonic limb rudiments, growth plates, and experimental endochondral ossification induced with decalcified bone matrix and, in all these models, endochondral ossification has been enhanced. It is not known definitively whether electric fields stimulate cell differentiation or modulate an increased number of molecules synthesized by committed cell population and this is a fertile area of current study. © 1993 Wiley-Liss, Inc

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Endochondral ossification is the basic developmental process in skeletal embryogenesis, growth, and fracture repair. Although spatially and morphologically distinct, endochondral ossification in these physiological events bears similarities from the cellular point of view. The process consists of cell replication followed by sequential gene expression for, and synthesis of, characteristic extracellular matrix molecules culminating in bone formation [9]. Chondrogenic precursor cells are characterized by the expression of type I collagen and proteoglycan with long chondroitin sulfate chains (30,000 D) and little to no keratan sulfate. Mature chondrocytes express and synthesize type II collagen and proteoglycan containing keratan sulfate chains and chondroitin sulfate chains of shorter length. Terminally differentiated (hypertrophic) chondrocytes synthesize proteoglycan with even shorter chondroitin sulfate chains (15,000 D) and larger keratan sulfate chains (10,000 D) and type X collagen. Hypertrophic cartilage undergoes calcification, vascularization, and, ultimately, removal by chondroclasts and replacement by trabecular bone.

The synthesis of extracellular matrix in endochondral ossification has been shown to be regulated by a variety of biochemical and biophysical factors including cytokines and growth factors, hormones, oxygen tension, mechanical strain, and electric fields. Understanding regulatory events offers the opportunity for therapeutic modulation of skeletal morphogenesis, growth, and repair. This review discusses the ability of one biophysical factor, electromagnetic fields, to regulate endochondral ossification.

TECHNIQUES FOR ELECTRIC STIMULATION OF BIOLOGICAL TISSUE

Electric and magnetic stimulation of biological tissue can be accomplished by three methods. Each has its unique biophysical characteristics and is applicable, to varying degrees, to organs, tissues, and cells, in vivo and in vitro. Stimulation with direct electric current (DC) is achieved by the placement of electrodes into the experimental system with the cathode at the desired site of bone deposition. General signal parameters include a voltage of 1.0–1.5 V and current of 5–20 μA. The electric field is highly localized and rapidly falls off as a function of the distance from the electrode [6]. Biochemical re-

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Address reprint requests to Deborah McK. Ciombor, Orthopaedic Research Laboratory, Department of Surgery, Roger Williams Medical Center, 825 Chalkstone St., Providence, RI 00928.

actions occur at the cathode which may be detrimental to biological systems under some circumstances. These reactions include decreased local oxygen tension, hydroxyl radical production, corrosion, and electrolysis. Electric fields can be produced in biological systems without the introduction of electrodes by two techniques, capacitive or inductive coupling. Capacitive coupling (CC) requires that opposing electrodes be placed in contact with a conducting medium apposed to the biological system. Similar electric potentials are applied as with DC stimulation with frequencies of 20–200 kHz resulting in the induction of electric fields of 1–10 mV/cm. Electric stimulation by the CC technique distributes the induced current over the relatively large volume of tissue between the electrodes and avoids high local potentials. Electric fields can also be induced in biological systems by inductive coupling (IC) with a time-varying magnetic field. Since this technique does not require physical contact with the biological system it is quite useful for in vitro work. This technique employs a current-carrying coil or coils which elaborate a time-varying magnetic field and which induce an electric field in the biological system. The induced electric field is related to the characteristics of the applied magnetic field and the properties of the biological system. A variety of signal configurations have been utilized in experimental studies, including sine waves, single pulse, and pulse-train or burst wave forms. Configurations can be designed to produce voltage gradients of 1–10 mV in an inductive search coil.

It is not clear whether the biological effects of these three techniques are due to common properties such as the induced electric field. However, because all three modalities induce bone formation in tissue with an electric field of approximately 1–10 mV/cm it is often assumed that this range is stimulatory to osteogenesis. It is important to note, however, that other variables related to the signal such as amplitude, frequency, and exposure duration are probably of equal importance in producing a biological response.

STIMULATION OF ENDOCHONDRAL OSSIFICATION IN SKELETAL EMBRYOGENESIS

Early attention has been focused on the effects of electric and magnetic stimulation of endochondral ossification in limb development perhaps, in part, because of the observations of endogenous electric currents in developing and regenerating amphibian limbs [7,18]. Endogenous electric currents may be one factor influencing pattern formation of skeletal elements in limb buds. In regenerating amphibian limbs, current density of 20–100 mA/cm^2 are observed to flow from the intact to the severed portion of the limb. Inhibition of this current interferes with normal limb regeneration in lower amphibians. Amplification of the current flow results in augmentation of repair in amphibians which do not normally mount a regenerative process. Blastomas produced by the exogenous application of electric fields, while not normally organized, consist of multiple differentiated tissue types including muscle, cartilage, and bone.

The earliest studies of electrical effects on endochondral ossification examined skeletal morphogenesis in embryonic limb rudiments under IC or time-varying electromagnetic fields. These studies employed very low frequency fields (1–10 Hz) and demonstrated no increase in cartilage matrix synthesis. Inhibition of collagen synthesis was shown in 7-day-old embryonic chick tibial explants [4]. Explants of more advanced embryonic stages exhibited increases in protein synthesis, calcification, and growth. Other studies of chick tibial explants demonstrated the absence of sulfate incorporation into cartilage macromolecules and suppressed protein synthesis [5]. In another laboratory, an enhancement of calcium incorporation and calcification of cartilage matrix has been reported in the same experimental system [10]. The extent of calcification was found to be related to frequency and exposure time in a dose-dependent manner. Increases in cAMP levels were observed in calcifying cartilage after IC stimulation [14]. These studies were interpreted as indicating that the fields employed stimulated calcification of cartilage matrix rather than enhancing matrix protein synthesis.

Studies with other stimulation techniques or biological systems have demonstrated changes in proliferation or extracellular matrix synthesis induced by the electric field. In chick embryonic tibia, cartilage synthesis has been shown to be increased in a dose-related fashion under CC electrical stimulation [11]. Exposure to a voltage of 0.1 mV for 30 min per day for 72 h increased hydroxyproline incorporation 83–125% of control while continuous exposure for 72 h increased hydroxyproline incorporation by 29%. Evidence of increased proliferation and incorporation of thymidine of 0–150% was ob-

served in cultures treated for 30 min per day. A study was carried out to examine proliferation in a predominant chondroblast cell culture derived from chick embryo sterna under conditions of IC electrical stimulation. Under conditions promoting rapid growth to confluency, DNA content was increased in cultures treated with electric fields. The turnover of glycosaminoglycans, both synthesis and degradation, also appeared to be increased with a net loss of tissue glycosaminoglycans [17]. Studies with DC fields in 21-day-old fetal rat tibia demonstrated stimulation of osteogenesis related to field configurations [15,16]. Enhanced calcification was demonstrated by electron microscopy, energy dispersive X-ray micro analysis, micro auto radiography, calcium incorporation, and light microscopic morphology. Thicker trabeculae with proliferative periosteum and a greater number of osteoblasts were observed in limbs treated with DC fields.

These studies, taken as a group, have demonstrated a promotion of endochondral ossification and osteogenesis in embryonic long bone rudiments under conditions of IC and DC electrical stimulation. Some studies have demonstrated an increase in cell proliferation and extracellular matrix synthesis as well. In all likelihood, the precise effects observed reflect the in vitro culture conditions, stage of development of the tissues, and configuration of the electrical stimulation used in each laboratory.

STIMULATION OF GROWTH PLATE AND PHYSEAL CHONDROCYTES

The effects of CC electric fields upon growth plate elongation has been studied in vivo [8]. Only a brief, 48 h, stimulation was utilized and linear growth was measured by double tetracycline labeling. A 60 kHz sine wave stimulated growth plate elongation in a dose-dependent manner with the optimum voltages for stimulation being 5–10 V. Further studies of the effects of electric stimulation on growth plate have been done using IC, or time-varying, electric fields in vitro. No effects were observed with a specific pulse configuration applied at varying amplitudes of 6.3–50 Gauss and calculated induced fields of 0.57–6.11 mV/cm alone. However, significant elongation and linear growth was observed under conditions of IC stimulation of 37.5 and 50 Gauss applied for 48 h in the presence of thermal effects [13].

STIMULATION OF EXPERIMENTAL ENDOCHONDRAL OSSIFICATION

The introduction of decalcified bone matrix (DBM) particles into the subcutaneous space of immature rats has been shown to produce an ossicle comprised of trabecular bone at 3 weeks after DBM implantation [19]. The process follows the developmental sequences of endochondral ossification and is analogous to other forms of endochondral bone formation in cellular differentiation, elaboration of extracellular matrix, and trabecular formation. The system has been temporally well characterized both biochemically and morphologically and serves, therefore, as an excellent test system for factors which affect the rate and quality of endochondral ossification. In this system, proliferation of mesenchymal cells occurs on days 2–4. Differentiation of chondrocytes is apparent on day 6, with chondrogenesis becoming maximal on days 8–10. Endochondral calcification is observed on day 10. Progressive calcification occurs, with trabeculae appearing on days 12–14, maturing by days 18–20. This experimental system has been utilized to test the effects of IC electrical fields on endochondral ossification [1]. Significant increases in indices of chondrogenesis and bone formation have been observed. Twofold increases in sulfate incorporation into cartilage macromolecules and glycosaminoglycan content have been noted on day 8 of ossicle development. The volume of cartilage, measured histomorphometrically, was also elevated by a similar magnitude. Calcium content was significantly increased in ossicles exposed to electric fields on day 12 of development and these elevations persisted until the conclusion of ossicle development. The formation of trabecular bone, measured with quantitative histomorphometry, was markedly accelerated by day 12 of development. The rate of trabecular development in day 12 ossicles exposed to electric fields was equivalent to that of 16 day control ossicles, indicating a 20% acceleration of trabecular formation. At day 20 of ossicle development the amount of trabecular bone in ossicles exposed to electric stimulation was nearly double that in control ossicles. Indices of trabecular and ossicle maturation also demonstrated an acceleration in treated ossicles. This study demonstrated unequivocally that the IC field applied increased extracellular matrix formation and organization. It showed that the effects of the field were

not, as some supposed, solely to increase calcification of previously synthesized matrix. These studies were extended by exposing ossicles to electric stimulation during selected stages of their development: mesenchymal, chondrogenesis, calcification, and trabecular formation [2]. This study demonstrated that stimulation during days 1–8 of development (mesenchymal and chondrogenic stages) or days 1–3 of development (mesenchymal stage) produced trabecular bone of equivalent maturation and quantity to that in ossicles exposed to electric stimulation for the full 20 days of development. Stimulation during cell recruitment and proliferation (mesenchymal stage), therefore, apparently provided stimulation of the precursor cell pool sufficient to accelerate all subsequent stages of development. Both the synthesis of cartilage matrix and subsequent calcification and trabeculation were accelerated. Cell proliferation itself, as measured by thymidine incorporation, and overall cellularity, measured by DNA content, were not increased in stimulated ossicles. These studies suggested a stimulatory effect of the electric fields on cell differentiation.

Another study has been carried out to indicate that exposure of ossicles during chondrogenesis results in the increased synthesis of extracellular matrix molecules which are of normal size distribution and chemical composition [3]. Proteoglycans extracted from cartilage treated with electric stimulation eluted on Sepharose 2B in an identical position to those of control ossicles. However, a significantly greater fraction of the proteoglycan synthesized under conditions of electric stimulation eluted in the position characteristic of cartilage-specific molecules. Digestion of the pooled cartilage-specific peak and chromatography on Sepharose 6B revealed that glycosaminoglycans synthesized under conditions of electric stimulation were also of normal size distribution. Enzyme analysis of the chemical composition of glycosaminoglycans in treated ossicles reveal them to be similar to that of control ossicles and to be comprised largely of chondroitin sulfate. Finally, the increase in chondrogenesis seen under electric stimulation was accompanied by an earlier expression of mRNA for proteoglycan core protein and type II collagen.

Confirmation of these studies was provided by a study of the response of osteoprogenitor (marrow) cells to DBM stimulation under conditions of DC stimulation [12]. Marrow cells and DBM were placed into diffusion chambers implanted in rabbit muscle pockets and the amount of cartilage and subsequent calcification assessed histologically. To some chambers was added DC electrical stimulation with implanted cathodes delivering 5 μA current and approximately 1 V. Both chondrogenesis and calcification were accelerated in chambers with electrical stimulation.

CONCLUSIONS

Evidence from many experiments with three systems of endochondral ossification indicates that extracellular matrix synthesis and/or calcification can be increased by exposure to appropriately configured electric fields. The multiplicity of models and stimulation techniques and wave forms makes direct comparisons among the studies difficult. However, this difficulty should not obscure the generally observed enhancement of endochondral ossification by electric stimulation. Both chondrogenesis and osteogenesis can be enhanced. Differing sensitivities to electrical stimulation of the various phases of endochondral ossification are apparent with stimulation of the stem cell population being most sensitive. These studies, as a group, form the scientific basis for the clinically observed acceleration of endochondral ossification in the repair of fractures and non-unions.

While it is clear that the synthesis and organization of extracellular matrix molecules can be accelerated by exposure to electric fields, the mechanism by which cells respond is not so clear. Although certain systems do indicate the ability of some fields to stimulate cell proliferation, this does not appear to be a major mechanism of stimulation of organ cultures of endochondral ossification. Experimental data suggest that the fields may promote differentiation of precursor stem cells to chondrocytes, although the open-ended cell pool in in vivo models makes this difficult to establish with certainty. In vitro models are now being explored to test this hypothesis. Whether subsequent bone formation results simply from the increase in chondroid matrix or whether a separate bone precursor cell pool is being stimulated remains to be studied.

REFERENCES

1. Aaron RK, Ciombor D McK, Jolly G (1989): Stimulation of experimental endochondral ossification by low energy pulsing electromagnetic fields. J Bone Miner Res 4:227–233.

2 Aaron RK, Ciombor D McK (1987a) The effects of pulsing electromagnetic fields on tissue maturation in experimental endochondral ossification Trans Bioelectric Repair and Growth Society 737B
3 Aaron RK, Ciombor D McK (1987b) Stimulation of chondrogenesis in experimental endochondral ossification by pulsing electromagnetic fields Trans Bioelectric Repair and Growth Society 737A
4 Archer C, Ratcliffe A (1981) The effects of pulsed magnetic fields on bone and cartilage in vitro Trans Bioelectric Repair and Growth Society 1 1
5 Archer C, Ratcliffe A (1983) The effects of pulsed magnetic fields on chick embryo cartilaginous skeletal rudiments in vitro J Exp Zoo 225 243–256
6 Black J (1987) "Electrical Stimulation—Its Role in Growth, Repair and Remodeling of the Musculoskeletal System " New York Praeger
7 Borgens R (1982) The role of ionic currents in the regeneration and development of the amphibian limb In Kelley R, Goetinck P, MacCabe J (eds) "Limb Development and Regeneration " New York Alan R Liss, pp 597–608
8 Brighton C, Pfeffer G, Pollock S (1983) In vivo growth plate stimulation in various capacitively coupled electrical fields J Orthop Res 1 42–49
9 Caplan A (1991) Mesenchymal stem cells J Orthop Res 9 641–650
10 Fitton-Jackson S, Jones D, Murray D, Farndale R (1981) The response of connective and skeletal tissues to pulsed magnetic fields Trans Bioelectric Repair and Growth Society 1 85
11 Fitzsimmons R, Farley J, Adey W, Baylink D (1986) Embryonic bone matrix formation is increased after exposure to a low amplitude capacitively coupled electric field in vitro Biochem Biophys Acta 882 51–56
12 Friedenburg Z, Brighton C, Michelson J (1989) The effects of demineralized bone matrix and direct current on an in vivo culture of bone marrow cells J Orthop Res 7 22–27
13 Iannacone W, Pienkowski D, Pollock S, Brighton C (1988) Pulsing electromagnetic field stimulation of the in vitro growth plate J Orthop Res 6 239–247
14 Jones D (1982) The effect of pulsed magnetic fields on cAMP metabolism in chick embryo tibia Trans Bioelectric Repair and Growth Society 2 29
15 Noda M, Sato A (1985) Calcification of cartilaginous matrix in culture by constant direct current stimulation Clin Orthop 193 281–287
16 Noda M, Sato A (1985) Appearance of osteoclasts and osteoblasts in electrically stimulated bones cultured on chorioallantoic membranes Clin Orthop 193 288–297
17 Norton L (1982) Effects of a pulsed electromagnetic field on a mixed chondroblastic tissue culture Clin Orthop 167 280–290
18 Nuccitelli R (1988) Physiological electric fields can influence cell motility, growth, and polarity Advances in Cell Biology 2 213–233
19 Reddi H, Huggins C (1972) Biochemical sequences in the transformation of normal fibroblasts in adolescent rats Proc Natl Acad Sci USA 69 1601–1605