Healing of Chronic Arterial and Venous Leg Ulcers With Systemic Electromagnetic Fields

Luis Cañedo-Dorantes, a Rigoberto García-Cantú, b,c† Raúl Barrera, c Ignacio Méndez-Ramírez, d Víctor Hugo Navarro e and Gregorio Serrano b

a División de Investigación, Hospital Juárez de México, Secretaría de Salud (SSA), Mexico City, Mexico
b Departamento de Ingeniería Eléctrica, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico
c Departamento de Bioquímica, Instituto de Enfermedades Respiratorias, SSA, Mexico City, Mexico
d Departamento de Estadística, Instituto de Investigaciones en Matemáticas Aplicadas y Sistemas, Universidad Nacional Autónoma de México, Mexico City, Mexico
e Servicio de Angiología, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico

Received for publication March 27, 2001; accepted December 7, 2001 (01/043).

Background. Mitogen-activated autologous peripheral blood mononuclear cells applied locally on the ulcer surface promote healing of chronic arterial and venous leg ulcers. In vitro, extremely low frequency electromagnetic fields (ELF) interact with peripheral blood mononuclear cells (PBMC) via Ca ++ channels, activating signal transduction cascades, promoting cytokine synthesis, and changing cell proliferation patterns.

Methods. ELF frequencies were configured to interact in vitro with the proliferation patterns of PBMC obtained from normal human volunteers. These ELF were then applied peripherally as the sole treatment to 26 patients with 42 chronic leg ulcers of predominantly arterial or venous etiology unresponsive to previous medical and/or surgical treatments in a phase I before-after design.

Results. At admission, age of ulcers had a skewed distribution with a median of 639 days. Wound healing or deleterious effects began in all patients during the first 2 weeks after ELF exposure, permitting their previously unresponsive ulcers to function as internal controls. After ELF exposure, 69% of all lesions were cured or healed >50% in a period <4 months. Defective wound healing was observed in lesions associated with important arterial occlusion, uncontrolled arterial hypertension, severe lipodermatosclerosis, non-pitting edema, and obesity (body mass index >30). Lesions worsened in patients with autoimmune diseases.

Conclusions. Systemic effects are hypothetically explained by ELF activation of PBMC and their subsequent transportation to the ulcer site via humoral route. This therapy is effective in selected patients with chronic arterial and venous leg ulcers. © 2002 IMSS. Published by Elsevier Science Inc.

Key Words: Leg ulcers, Skin ulcers, Electromagnetic fields, Cell activation.

Introduction

Chronic arterial and venous leg ulcers can be thought of as dysregulated inflammatory processes produced by inadequate blood supply, tissue anoxia, edema, cell death, and infection, among other factors (1). These changes alter interaction among structural components of affected tissues and between these and immune cells in a manner that impedes wound healing. Existing hypotheses on the pathophysiology of chronic arterial and venous leg ulceration concentrate on local effects induced by hemodynamic alterations (2–8). Treatments at present focused on alleviating these local changes include hemodynamic preventive...
viability was always >90% determined on the basis of trypan blue exclusion.

**PBMC magnetic field exposure conditions.** Homogeneous SMF of 503 ± 45.9 SD Gauss were generated inside the exposure chamber with permanent magnets around the coil (Figure 1 A,a). ELF were produced inside a 4-cm length coil composed of four layers of 22 AWG wire (272 total turns). Measured inductance was 5.61 mH. (Inductimeter, Beckman model LM22A). An alternating current power source (120 V/60 Hz) was connected to a transformer and a rectifier bridge, which supplied 100 mA rms to the coil (Figure 1 A,b). ELF magnetic field strength was 8.02 Gauss. Cells were exposed inside Eppendorf tubes placed in the outer edge of an acrylic rack inside a cylindrical exposure chamber 7 cm in diameter and 8 cm in length (Figure 1A,c). Exposure chambers were shielded from the magnetic fields of the cell culture incubator using µ-metal. Magnetic fields ambient background levels were <0.44 Gauss.

**Patients.** A phase I before-after trial to document ELF’s systemic effects on chronic arterial and venous leg ulcers was approved by the Hospital Juárez Institutional Review Board/Ethics Committee (September 25, 1995). Pregnant women and patients with cancer were excluded. All recruited patients had chronic leg ulcers resistant to medical and surgical treatment and were under medical care prior to admission. After signing a voluntary consent form, 30 patients with 49 chronic leg ulcers and two paraplegic patients with decubitus ulcers below the spinal lesion were accepted into the protocol. During the study, three patients with chronic leg ulcers (five wounds) were excluded, one with factitious ulcers, one who employed unauthorized treatments, and one with secondary effects of amlodipine. The paraplegic patients and one patient with two ulcers diagnosed as pyoderma gangrenosum associated with chronic venous disease were analyzed separately. A total of 26 patients with 42 chronic leg ulcers of predominantly arterial or venous etiology unresponsive to medical and surgical treatments were studied. Standards recommended by the Society for Vascular Surgery/North American Chapter and the International Society for Cardiovascular Surgery were used as criteria to define the contribution of arterial and venous disease in chronic leg ulcer etiology (23,24). Patients were exposed to ELF alone (five patients) or to a combination of ELF and SMF (21 patients). Eight patients had 17 chronic leg ulcers of predominantly arterial etiology and 18 patients had 25 chronic leg ulcers of predominantly venous origin. Their characteristics are summarized in Table 1. Patients were allowed to continue systemic treatments for pain, rheumatoid arthritis, arterial hypertension, and diabetes. Other systemic medications and preventive measures were discontinued. Local treat-
ments were limited to cleaning the ulcer with soap and water and subsequently covering it.

Patient exposure conditions. Patients were exposed to magnetic fields by placing either arm inside a cylindrical exposure chamber 10 cm in diameter by 25 cm in length. Its internal structure was similar to the in vitro exposure system (Figure 1A). Average exposure time was 2–3 h/day three times weekly (Figure 1B). ELFs were generated inside a coil 25 cm in length with three layers of 22 AWG wire (1,059 total turns). Measured inductance was 43 mH (Inductimeter Beckman model LM22A). An alternating current power source (120 V/60 Hz) was connected to a transformer and to a rectifier bridge, which supplied 680 mA rms to the coil. Spectral frequencies distribution of ELF were measured as an induced voltage through a 4.7-kΩ shunt resistor, bridging the leads of a 1-cm probe made of 190 turns of number 42-gauge magnet copper wire placed perpendicularly to the ELF direction. The probe was connected to a digitizing oscilloscope (TDS 420 Tektronik) and to a spectral analyzer (model SR 7609, Stanford Research Systems) (Figure 1C). ELF magnetic field strength was 36.36 Gauss. Maxwell equations defined induced variable electric fields perpendicular to magnetic field flux lines with amplitude proportional to strength and time variation of magnetic fields (dB/dt). Homogeneous static magnetic field of 522 ± 93.6 SD. Gauss were generated inside the exposure chamber with permanent magnets around the coil (Figure 1D). SMF strength was measured with a calibrated Hall effect probe (RFL model 912) placed perpendicularly to uniform SMF. A map of homogeneous magnetic field generated by combined ELF and SMF inside the exposure chamber is depicted in Figure 1E. Magnetic fields ambient background levels were 1.0 Gauss (patent pending). Magnetic field ambient background levels were 1.0 Gauss (Figure 1B).

Statistics. Follow-up ulcer size and appearance were consecutively documented with photographs that in turn were digitized and processed with Internet Scion Image software (25). Areas were calculated by delineating ulcer contours, counting the number of pixels contained inside the area and comparing it with the number of pixels contained in 1 cm² measured above a ruler placed near the ulcer (Figures 3–5). JMP 3.2.1 statistical software from the SAS Institute (Cary, NC, USA) was used for descriptive statistics (mean ± SD, medians and ranges), χ², ANOVA F, t tests, and linear regression.

Results

In vitro Studies. PBMC exposure to ELF alone decreased the stimulation produced by phytohemagglutinin by 62.2% with statistical significance p <0.05 (Tukey test). This effect was inhibited when PBMC were exposed to a combination of ELF and SMF. Data were analyzed by two-way ANOVA, with patients as blocks and experimental conditions. Patients: p <0.04; conditions: p <0.0001 (F test), and Tukey test was done for conditions (Figure 2).

Patients. According to their response to treatment, patients were divided into two groups: responders when all wounds healed or with a >50% size reduction during the first 4 months and non-responders when at least one ulcer had a <50% size reduction or increased in size within the same time period (Figures 3 and 4) (Table 1). Age and initial size of chronic leg ulcers at admission had a skewed distribution. Age range was as follows: 30–4,745 days, median: 639 days; quartile 25%: 171 days, and quartile 75%: 2,281 days. Initial size range: 0.43–350.7 cm², median: 6.45 cm²; quartile 25%: 1.97 cm², and quartile 75%: 24.93 cm². Healing or deleterious effects began in all patients during the first 2 weeks after initiation of treatment (Figures 3–5). Negative secondary effects were absent during treatment and follow-up periods. ELF alone or associated with SMF produced similar healing or deleterious effects (Figures 3–5).

Responders. Twenty-nine ulcers of different size and age previously unresponsive to conventional treatments began to heal after ELF arm exposure. Healing velocity as percentage of area reduction/day was calculated for each chronic arterial and venous leg ulcer by linear regression with values of R² around 0.9 and p <0.01 for all cases. In 93% of chronic arterial and venous leg ulcers, this value ranged from 0.3 to 3.0%; the remaining 7% varied from 3.0 to 12.0%. No statistical differences in healing speed were observed between chronic arterial and venous leg ulcers (ANOVA for regression coefficients, p >0.05). Healed ulcers remained healed for at least 6 months and up to 2 years after the conclusion of treatment. In the affected legs of patients with chronic arterial leg ulcers, the superficial vascular network became visible and skin temperature increased after 4–8 weeks of treatment (Figure 3 a–c). In chronic leg ulcers of predominantly venous etiology, pain, edema, and weeping were reduced significantly or eliminated 3–6 weeks after the initiation of treatment (Figure 3 e–f).

Non-responders. Thirteen ulcers healed poorly or increased in size. Most were of predominantly venous etiology, ulcers were older and larger in size, and patients of this group had higher body mass index (BMI) (Table 1). In ulcers of predominantly arterial origin, deficient wound healing was associated with severe arterial occlusion (Figures 4 a–d) and uncontrolled arterial hypertension. In ulcers of predominantly venous etiology, non-healing was associated with obesity and/or non-pitting edema. Figures 4 e–g depict different responses to treatment in separate ulcers of the same patient. Two ulcers located in areas with non-pitting edema increased in size (only one is shown, top), while the third ul-
cer (bottom) located in an area without non-pitting edema healed by 87.5%. Poor healing response was observed in severe lipodermatosclerosis (Figures 4 h–i). Pain was only partially reduced 4–6 weeks after initiation of treatment. In one patient with pyoderma gangrenosum associated with chronic venous disease, ulcer size increased after magnetic field exposure (Figures 5 a–c).

To ascertain whether systemic effects were caused by magnetic field interaction with action potentials, we measured differences in amplitude or latency of radial nerve somatosensory-evoked potentials before, during, and after magnetic field exposure. No changes in nerve conduction parameters associated with magnetic fields exposure were found (unpublished results). The healing response of two paraplegic women with chronic skin wounds resistant to medical and surgical treatments below the spinal lesion was studied in the patients’ homes. Activation of the wound repair process began the second week after ELF exposure; healing speed was similar to that of patients with chronic wounds, intact nervous systems, and under the same treatment conditions (Figures 5 d–g).

Discussion

It has been reported that ELF (15–19) and SMF (26) elicit changes in cells of the immune system through Ca++ signaling. The in vitro studies were intended to configure optimal ELF frequencies that could interact with PBMC proliferation and to investigate SMF and ELF/SMF effects on PBMC proliferation. PBMC were obtained from healthy human volunteers to exclude interference of a diseased status. These experiments showed that ELF exposure decreases PBMC proliferation and ELF combined with SMF inhibited the in vitro ELF effect (Figure 2). In both cases, cell viability was maintained during the incubation period, indicating that the magnetic fields used interacted with phytohemagglutinin-induced PBMC proliferation. Hypothetically, it was possible for in vitro interactions between

![Figure 2. ELF modifies PBMC proliferation patterns without reducing cell viability. Each column represents the results of three independent experiments done in triplicate. A) PBMC without phytohemagglutinin. Mean 1,775 ± 675 SD. B) PBMC proliferation increased 21 times with phytohemagglutinin. Mean 37,394 ± 12,674 SD. C) PBMC with phytohemagglutinin exposed to ELF. Mean 14,514 ± 10,528 SD. Proliferation was reduced by 62.2% with statistical difference p <0.05 (Tukey test) with (B) and (D), and D) PBMC with phytohemagglutinin exposed to ELF combined with SMF. Mean 41,514 ± 14,717 SD. No statistical difference was found between (D) and (B): p >0.05 (Tukey test). Error bar = mean ± SD.]

---

Table 1. Chronic leg ulcers of predominantly arterial etiology (CALU) and chronic leg ulcers of predominantly venous etiology (CVLU)

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Non-responders</th>
<th>All patients</th>
<th>Test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td># patients</td>
<td>CALU</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>Fisher exact</td>
</tr>
<tr>
<td></td>
<td>CVLU</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>Student t</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16</td>
<td>10</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Patient (age/years)</td>
<td>Range</td>
<td>33–85</td>
<td>46–80</td>
<td>33–85</td>
<td>Student t</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>59.4</td>
<td>62.1</td>
<td>60.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>17.0</td>
<td>10.43</td>
<td>14.6</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>Fisher exact</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>14</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>Range</td>
<td>15.8–32.5</td>
<td>23–45.32</td>
<td>15.8–45.32</td>
<td>Student t</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>25.85</td>
<td>31.4</td>
<td>27.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>4.94</td>
<td>6.97</td>
<td>6.31</td>
<td></td>
</tr>
<tr>
<td># ulcers</td>
<td>CALU</td>
<td>15</td>
<td>2</td>
<td>17</td>
<td>Chi square</td>
</tr>
<tr>
<td></td>
<td>CVLU</td>
<td>14</td>
<td>11</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>29</td>
<td>13</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Ulcer age at admission</td>
<td>Range</td>
<td>30–2,550</td>
<td>91–4,745</td>
<td>30–4,745</td>
<td>Wilcoxon</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>274</td>
<td>2,190</td>
<td>639</td>
<td></td>
</tr>
<tr>
<td>Ulcer size at admission</td>
<td>Range</td>
<td>0.43–116.05</td>
<td>2.19–350.7</td>
<td>0.43–350.7</td>
<td>Wilcoxon</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.92</td>
<td>32.3</td>
<td>6.45</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Exposure conditions and magnetic field parameters (See Materials and Methods).

Figure 3. Chronic leg ulcer evolution in responder patients. (A) Predominantly arterial etiology. (B) Predominantly venous etiology. Graphs in Figures 3–5 depict ulcer-size evolution as change in percentage over time of magnetic field exposure. Dotted lines (ELF alone). Solid lines (ELF + SMF). Solid squares (photographic examples). Color picture numbers: days of exposure; black and white pictures numbers: left (area in cm$^2$), right (area as percentage). See Results.
Figure 4. Evolution of chronic leg ulcers in non-responder patients. (A) Predominantly arterial etiology. (B) Predominantly venous etiology. See Results.

Figure 5. (A) Pyoderma gangrenosum associated to chronic venous disease. (B) Decubitus ulcer in sacral region of paraplegic patient. Arrow indicates suture from previous unsuccessful surgery. See Results.
ELF and ELF/SMF with PBMC to occur in vivo because the human body is transparent to magnetic fields. Under this assumption, ELF alone or combined with SMF was applied to patients with chronic leg ulcers in a clinical phase 1 study. In the responder group, ulcers had been under medical treatment prior to admission and their median age was 639 days. Wound healing or deleterious effects began in all patients during the first 2 weeks after ELF or ELF/SMF exposure. Healing time was comparable to other present treatments (1,8–14,27) and healing was independent of lesion etiology or environmental location of patients (Figures 3 and 5 d–g). These responses were partially explained in chronic arterial leg ulcer patients by the increased vascular network associated with ulcer healing (Figures 3 a–c), and in chronic venous leg ulcers by edema reduction during the first weeks after magnetic fields exposure (Figures 3 e–h). These changes indicated that interaction between PBMC and other wound-repair active molecules with local tissues (28) had been restored. In the non-responder group, poor healing was partially explained in chronic leg ulcers of predominantly arterial etiology by severely reduced blood supply, as in critical leg ischemia (8) (Figures 4 a–d), increased sympathetic activity in uncontrolled arterial hypertension (29), and endocrine alterations in obesity (30). In chronic leg ulcers of predominantly venous etiology, ulcers located in different skin areas of the same patient responded differently to treatment. In areas with normal skin structure, edema reduction activated ulcer healing; in areas with non-pitting edema, reduction of extracellular fluid caused altered skin structures to retract, increasing ulcerated areas (Figures 4 e–g). In lipodermatosclerosis (2), destruction of skin structures impeded the normal wound healing process from taking place (Figure 4 h and i), and in pyoderma gangrenosum reactivation of the disease process could be attributed to activated immune cells (31) (Figures 5 a–c). Healing of chronic leg ulcers and some deleterious effects appear associated with magnetic fields exposure and argue against a placebo effect of this treatment.

SMF interaction in vitro with human leukocytes (26) has been reported. How could magnetic fields systemic effects be explained? Exposure time and magnitude of the SMF used in these experiments do not produce measurable effects in humans (32). Transmission of nerve impulses was not altered by SMF exposure and a hydrodynamic effect is excluded by the magnitude of SMF (32). Therefore, a mechanism based on changes induced by SMF in exposed arm tissues appears unlikely.

Propagation of electric potential differences produced by ELF through the skin and other tissues is theoretically implausible because according to the Faraday law of induction, electric potential differences are confined within the exposure chamber. Alteration of blood-transported molecules was eliminated due to the low energy of ELF. A mechanism based on molecules secreted by exposed arm tissues and acting at a distance appeared improbable because most molecules that activate wound healing act in an autocrine or paracrine manner. Absence of magnetic field effects on somatosensory-evoked potentials and the wound healing response observed in paraplegic patients suggested a humoral route. Although it is not possible to extrapolate to patients with leg ulcers in vivo results obtained with cells harvested from healthy human donors, clinical results suggested as a plausible framework for consideration the peripheral activation of PBMC followed by their transportation to the ulcer site via humoral route.

This phase I before-after study defined the conditions under which to conduct a randomized, placebo-controlled study. In patients, similar healing effects were observed with ELF alone or combined with static magnetic fields (Figure 3). These data, preliminary in nature, suggested that PBMC activation was necessary for wound repair and not a specific pattern of cell proliferation. To define a biological correlation between ELF and PBMC activation, the clinically controlled study should be compared with in vitro ELF or ELF/SMF activation of PBMC obtained from the same patients. Cellular activation should be documented with quantitative experiments of gene activation such as cytokine expression using RNA messenger identification by reverse transcriptase/polymerase chain reaction. In addition, PBMC harvested from patients with chronic leg ulcer should be exposed to ELF or ELF/SMF in vitro and reintroduced into the blood or applied on the surface of the ulcer (14) of donor patients to observe whether healing is stimulated. It has been reported that activated PBMC, in particular memory/effector T cells, concentrate in sites of chronic inflammation (33) where they participate as a source of cytokines (34,35). CLA lymphocytes (33) can be collected from ulcer fluid before and after ELF- or ELF/SMF-induced healing effects are observed, their status of cellular activation determined and compared with similar data from CLA lymphocytes before and after in vitro ELF or ELF/SMF exposure.

Therapeutic applications of magnetic fields have grown over the last three decades, gaining acceptance in some medical specialties. However, the majority of the medical community remains unconvinced. This could be attributed to a) difficulty in reproducing clinical results under the same experimental conditions, b) fear of undesirable side effects, and c) the broad spectrum of interaction mechanisms between magnetic fields and living tissues (36). The following solutions to these problems have been offered: Standards for reporting work with magnetic fields are available (37,38); the controversy over its undesirable health effects is under control (39,40), and some cellular effects have been unveiled (16–22). These advances will progressively encourage physician interest in understanding the therapeutic applications of magnetic fields.
Acknowledgments
We thank M. Amieva and J.J. Godina for their valuable suggestions and critical review of the manuscript, I. Trigos for providing paraplegic patients and D. Delgado for isolating PBMC. L.C. is grateful to Promotora Servia, S.A. de C.V. for financial support.

References

15. Liburdy RP, Callahan DE, Harland J, Dunham E, Sloma TR, Yaswen P.