

Acute Effects of Low-frequency Electromagnetic Fields on Leukocyte-endothelial Interactions *In Vivo*

AKIRA USHIYAMA and CHIYOJI OHKUBO

Department of Environmental Health, National Institute of Public Health, Tokyo, Japan

Abstract. *Background:* To clarify the effect of extremely low frequency electromagnetic field (ELF-EMF) on the microcirculatory system, acute effects on leukocyte-endothelium interactions resulting from ELF-EMF exposure were examined with conscious BALB/c mice by means of a dorsal skinfold chamber. *Materials and Methods:* The fluorescent dye, rhodamine 6G, was injected into the caudal vein to visualize leukocytes *in vivo* and to analyze leukocyte-endothelium interactions. Mice were exposed *in vivo* to 50 Hz linear sinusoidal wave EMF at magnetic flux densities of 3, 10 or 30 mT, for 30 minutes. *Results:* The mean velocity of free flowing leukocytes in the center stream of venules revealed no distinct changes following exposure. However, at a flux density of 30 mT, the number of adherent leukocytes, defined as the total number of rolling and endothelium-adhering leukocytes, increased significantly following ELF-EMF exposure ($p < 0.05$); no significant differences in adherent cell numbers were found in the 3 mT and 10 mT exposure groups or sham controls comparing pre- and post-exposure periods. *Conclusion:* These results demonstrated that high magnetic flux densities of ELF-EMF had an effect on leukocyte-endothelium interactions and indicated that a threshold level for this phenomenon exists in the range of 10-30 mT under these experimental conditions.

Exposure to extremely low frequency electromagnetic fields (ELF-EMF) is widespread in urban societies, but how electromagnetic fields (EMF) may effect health remains ambiguous. Several epidemiological reports on adults (1) and children (2, 3) have suggested EMF are associated with increased health risks, particularly cancer. However, the potential adverse effects of EMF are still controversial

Correspondence to: Akira Ushiyama Ph.D., Department of Environmental Health, National Institute of Public Health, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8638, Japan. Tel: +81-3-3441-7174, Fax: +81-3-3446-4635, e-mail: ushiyama@niph.go.jp

Key Words: Microcirculation, low-frequency electromagnetic fields, leukocyte-endothelium interactions, immunological response.

because few reports have been able to correlate such epidemiological data with pathophysiological findings. Furthermore, the elevations in relative risk reported previously have generally been minor. Thus, more conclusive research is warranted for a better understanding of the relative health risks posed by ELF-EMF.

Numerous *in vitro* and *in vivo* experimental studies elucidating the biological effects induced by ELF-EMF are reported. For example, it is known that *in vitro* exposure to ELF-EMF influences some biological processes: the enzymatic activity of ornithine decarboxylase (4), the signal transduction relating protein kinase C (5) and the expression of several genes such as heat-shock proteins (6, 7).

Although ELF-EMF are believed to affect biological immune systems, thereby increasing the likelihood or promotion of tumors and other disease, direct evidence supporting this hypothesis is still insufficient. Since the immune system plays an essential role in the pathophysiology and development of disease, it is important to clearly determine how immunological functions can be influenced by ELF-EMF exposure. Studying immune response to 50 Hz ELF-EMF exposure *in vitro* by assaying cytokines produced by human peripheral blood mononuclear cells (PBMCs) showed that some cytokine levels changed in response to exposure (8). Specifically, a significant decrease in tumor necrosis factor α (TNF- α) and a significant increase in interleukin 1 β (IL-1 β) levels were observed. It is well established that changes in cytokine production induce specific cellular and molecular responses in target cells. In the case of leukocytes, some cytokines (IL-1 β , TNF- α) can stimulate the up-regulation of E-selectin and L-selectin ligands, which mediate subsequent leukocyte rolling (9). In addition, TNF- α , IL-1 β and interferon γ (IFN- γ) enhance the expression of ICAM-1 and VCAM-1 in endothelial cells, which mediate leukocyte adhesion to the endothelium (10-12).

Taking these findings into consideration, we have hypothesized that if cytokine production is influenced by *in vivo* exposure to ELF-EMF, then quantitative changes in leukocyte-endothelium interactions should be observable by intravital microscopy. To evaluate our hypothesis, we

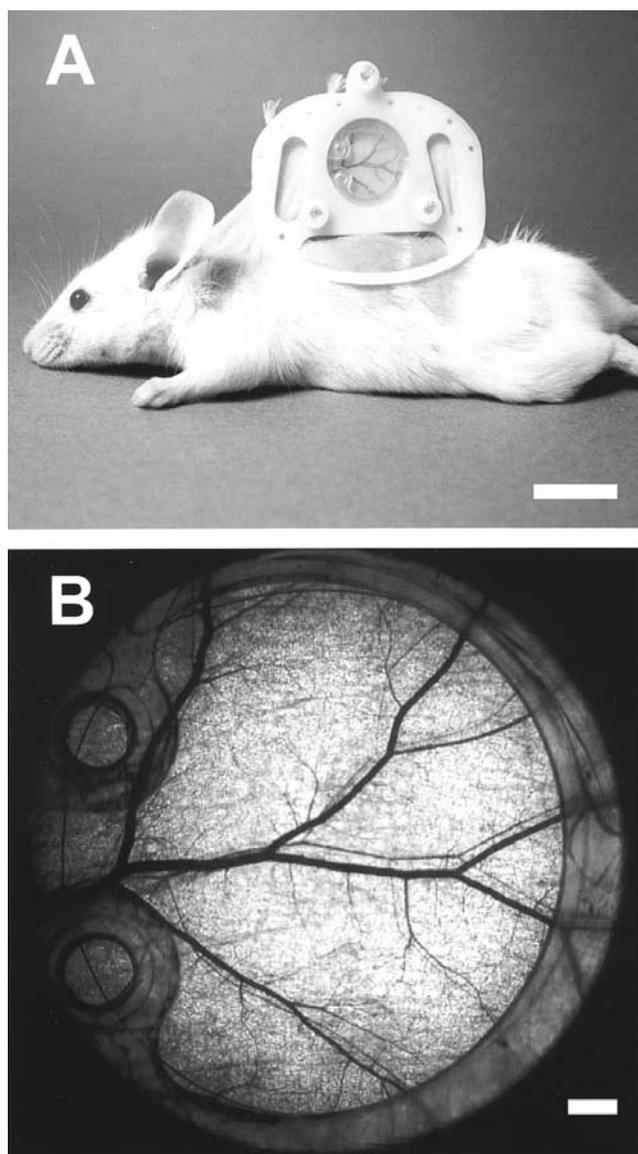


Figure 1. Overview of mouse dorsal skinfold chamber. (A) Whole body image of a mouse bearing a DSC 10 days after implantation (bar indicates 1 cm). (B) Intravital microscopic image of subcutaneous vessels using a DSC (bar indicates 1mm).

employed a dorsal skinfold chamber (DSC), to visually monitor the vascular system and blood circulation of mice *in vivo*, using a modified experimental method described earlier (13). Combining the use of a uniquely designed DSC and intravital fluorescence microscopy, we examined the effects of ELF-EMF on the mouse microcirculatory system and observed that leukocyte-endothelium interactions are specifically affected by 30 mT EMF.

Materials and Methods

Animal models. The dorsal skinfold chamber (DSC) model for the mouse was originally designed by Algire and Legallais (14) and modified chambers have since been applied to various experimental animal models (15). The most commonly used DSC is made of titanium. In the present study, we utilized a newly developed non-metal chamber to prevent heat production. The chamber frame used was made of Duracon™ resin, a non-metallic material that is not affected by magnetic fields and which we specifically designed for this purpose. As shown in Figure 1, DSCs were implanted in 8-week-old male BALB/c mice (obtained from Tokyo Zikken Doubutsu Co., Tokyo, Japan). The surgical procedure was essentially identical to that previously described (16). Briefly, mice were anesthetized by *i.m.* injection of a cocktail of ketamine hydrochloride (90 mg/kg body weight, Sigma Chemical Co. St. Louis, MO, USA) and xylazine hydrochloride (10 mg/kg body weight, Sigma Chemical Co.). Then the chamber frames were implanted so as to sandwich the extended double layer of the dorsal skin. Using an operating microscope and microsurgical instruments, one layer of skin was removed in a circular area of 15 mm in diameter and the remaining layer was covered with a coverslip incorporated into one of the chamber frames. The operation was performed at least 5 days before EMF exposure to allow for recovery from surgery and any related inflammation.

All animals were fed a standard pellet diet and given water *ad libitum*. They were maintained in individual cages with a 12-h light/dark cycle and at a temperature of $23.0 \pm 1.0^\circ\text{C}$. All procedures were conducted in accordance with the guidelines for animal experiments at the National Institute of Public Health.

Intravital fluorescence microscopy. Intravital fluorescence microscopy was used to evaluate leukocyte-endothelium interactions with the mouse DSC model. Observation of subcutaneous microcirculation was carried out by fluoromicroscopy (Optiphot XF-EFD2, Nikon, Tokyo, Japan) equipped with a 20 x long-working distance objective lens (Fluor 20, Nikon). Microscopic images were televised by CCD camera (WV-KS152, Matsushita Electric Industrial Co., Ltd. Osaka, Japan) and recorded on videotape using a video recorder (VC-BSS0, Sharp Co., Osaka, Japan) for subsequent off-line analysis. To evaluate dynamic video images, the time code generator signal (VTG33: For.A Co., Tokyo, Japan) was superimposed on video screen. Images were observed on a video monitor (AV-MT21, Victor JVC, Yokohama, Japan). Venular segments were chosen for examination because they are considered the major site of interaction between leukocytes and endothelium in response to noxious stimuli.

To visualize leukocytes *in vivo* the fluorescent marker rhodamine 6G (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) was used (17, 18). Rhodamine 6G was dissolved in physiological saline. Each solution was freshly prepared on the day of experiment and filtered through a $0.22 \mu\text{m}$ membrane filter (Syndring-driven filter unit Millex-GN, Millipore Co., Billerica, MA, USA) before each experiment. The prepared solution was injected into the caudal vein as a bolus of 0.3 mg/kg body weight 5 min before image recording. A Nikon G-filter block (excitation: 545 nm, emission: $>590 \text{ nm}$) was employed to view stained leukocytes. For microscopic observation during ELF-EMF exposure, animals were placed in an acrylic resin tube of 26 mm in inner diameter. The tube was fixed to an acrylic resin plate that was then set on a non-metallic microscope stage.

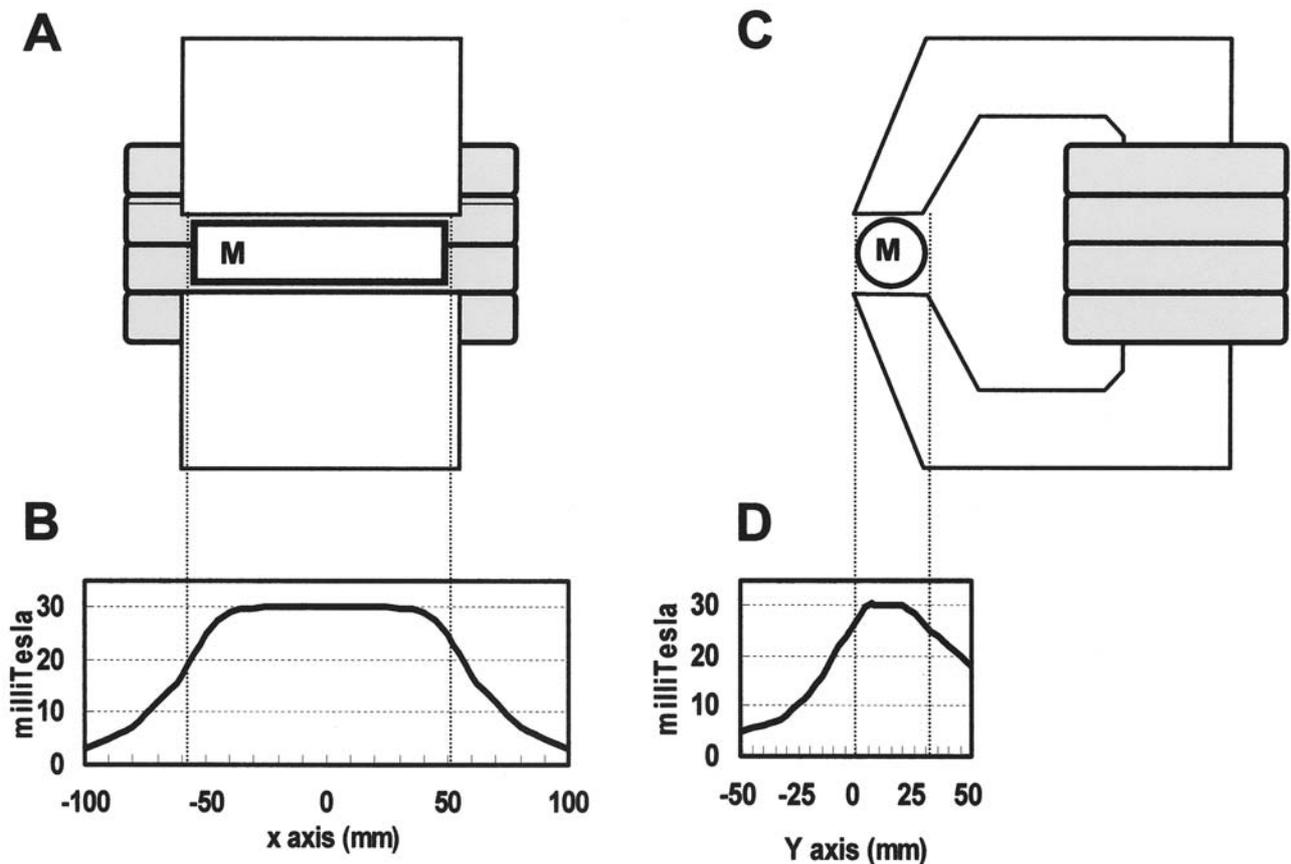


Figure 2. Schematic diagrams of electromagnet and magnetic field density distribution at the center of the C-shaped magnet. Diagrams illustrate front (A) and side (C) views. Gray sections depict the four blocks of coils and M represents the position of the mouse during exposure and observation. Distribution of magnetic field densities from front and side views are shown in graphs B and D. Density was measured along the center line of each axis with an output power set to 30 mT.

ELF-EMF exposure. The 50 Hz ELF-EMF was generated by an electromagnet (AMG1500A, Takano Giken Co., Kanagawa, Japan) shown in Figure 2 and an electricity supply (SMP-120MBA, Takano Giken Co.), as described previously (19). Briefly, the electromagnet comprised a C-shaped steel bar with four blocks of exposure coils. These coils were directly connected to the electricity supply. The electromagnet was made of a silica-iron laminated alloy to prevent heat transfer from the coils (dimensions: 120 mm in width, 30 mm in depth and 40 mm in height) and provided sufficient exposure area to cover the entire body of the mouse. The electromagnet was movable on a small railway connected to the acrylic stage of the microscope on which the mouse was set. Animals were subjected to magnetic flux densities of 3, 10 or 30 milliTesla (mT), controlled from the center of the electromagnet, or unexposed (sham, control group). Figure 2 also shows the distribution of magnet flux density along x and y axes when the output intensity was set to 30 mT. The distribution of magnetic intensity was measured by a Tesla meter (F.W. Bell Model 4048, Sypris Test & Measurement, Orlando, FL, USA).

Experimental procedures. We prepared 10 to 11 animals per exposure or sham group. DSC implanted mice were fixed onto the microscope stage and then placed within the C-shaped electromagnet. Rhodamine 6G was injected and leukocyte behavior was recorded in the pre-exposure state. As shown in Figure 3, leukocytes flowing inside venules were detected as red fluorescent cells under a G excitation lamp. Next, the mice were exposed to ELF-EMF for 30 min at each magnetic flux density applied and leukocyte behavior was recorded in the post-exposure state. The control experiment was also performed according to the same time course without exposure.

Analysis of leukocyte-endothelium interactions (leukocyte rolling or adhesion) was made in venules (inner diameter of 70-120 μm) during stable resting blood flow. For imaging analysis three parameters were measured: leukocyte-endothelium interaction, velocity of free flowing leukocytes and vessel diameter. Leukocyte-endothelium interactions were determined by counting the numbers of rolling or adhering leukocytes during a period of 10 sec within the defined area (20 x 20 μm) of a

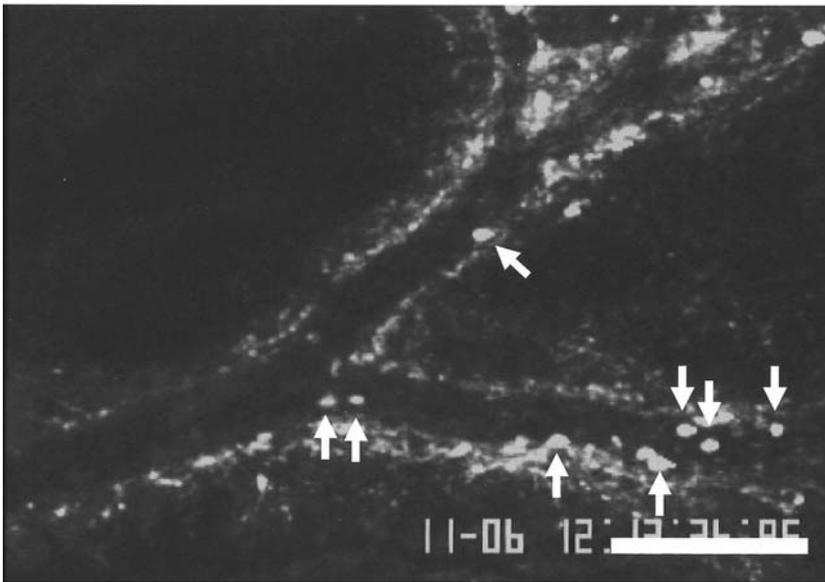


Figure 3. Video image of intramicrovascular leukocytes in vivo. Leukocytes (arrows) were stained with rhodamine 6G and observed by intravital fluorescence microscopy. Some cells showed slow flow compared to blood flow resulting from weak endothelial interactions or adhesion to vessel walls due to robust interaction. Scale bar indicates 100 μ m.

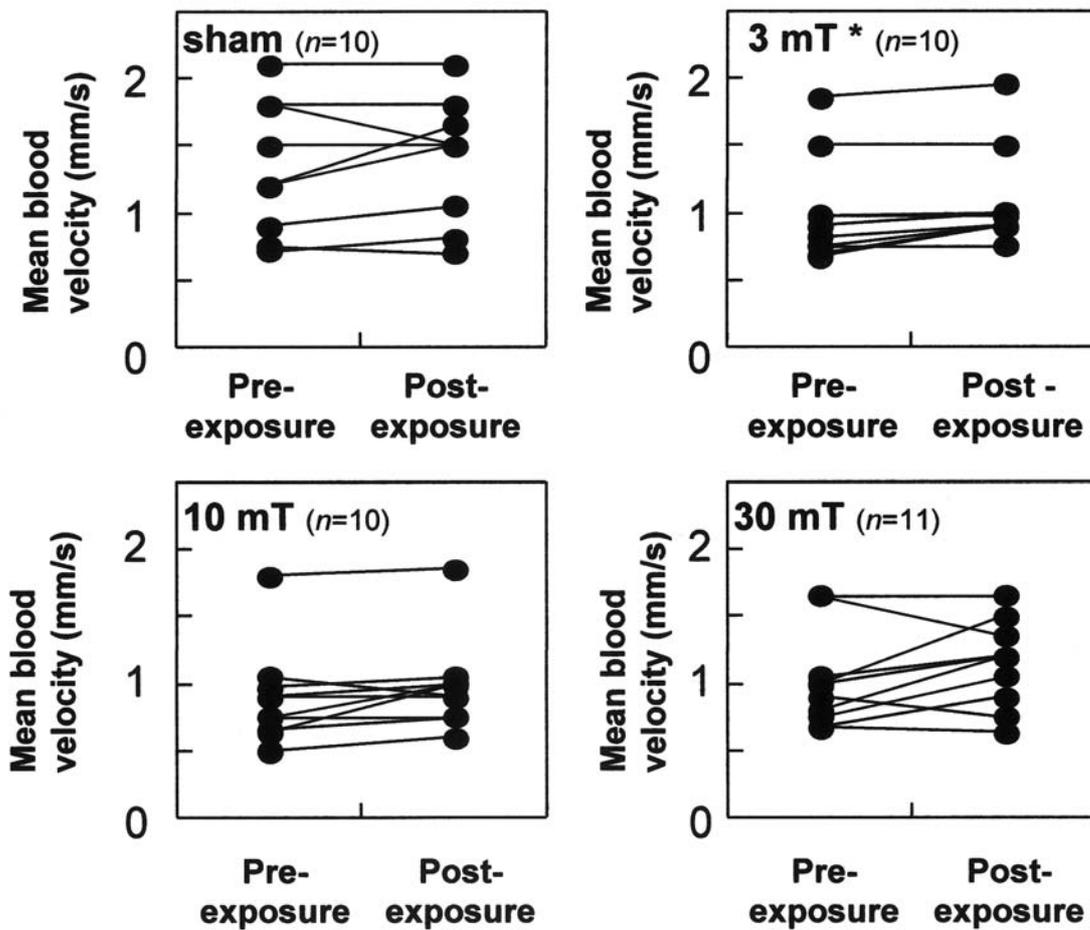


Figure 4. Changes in mean blood velocity of venules observed by DSC following acute exposure (30 min) to 50 Hz ELF-EMF at magnetic densities of 0, 3, 10 and 30 mT. The two connected closed circles in each graph show changes in the same animal before and after exposure to ELF-EMF. Each pair of closed circles represents the mean velocity of 10 free flowing cells. Mean blood velocity in the 3 mT group showed a significant difference between pre- and post-exposure $(p < 0.05)$. The number of animals in each sample group is represented by n.

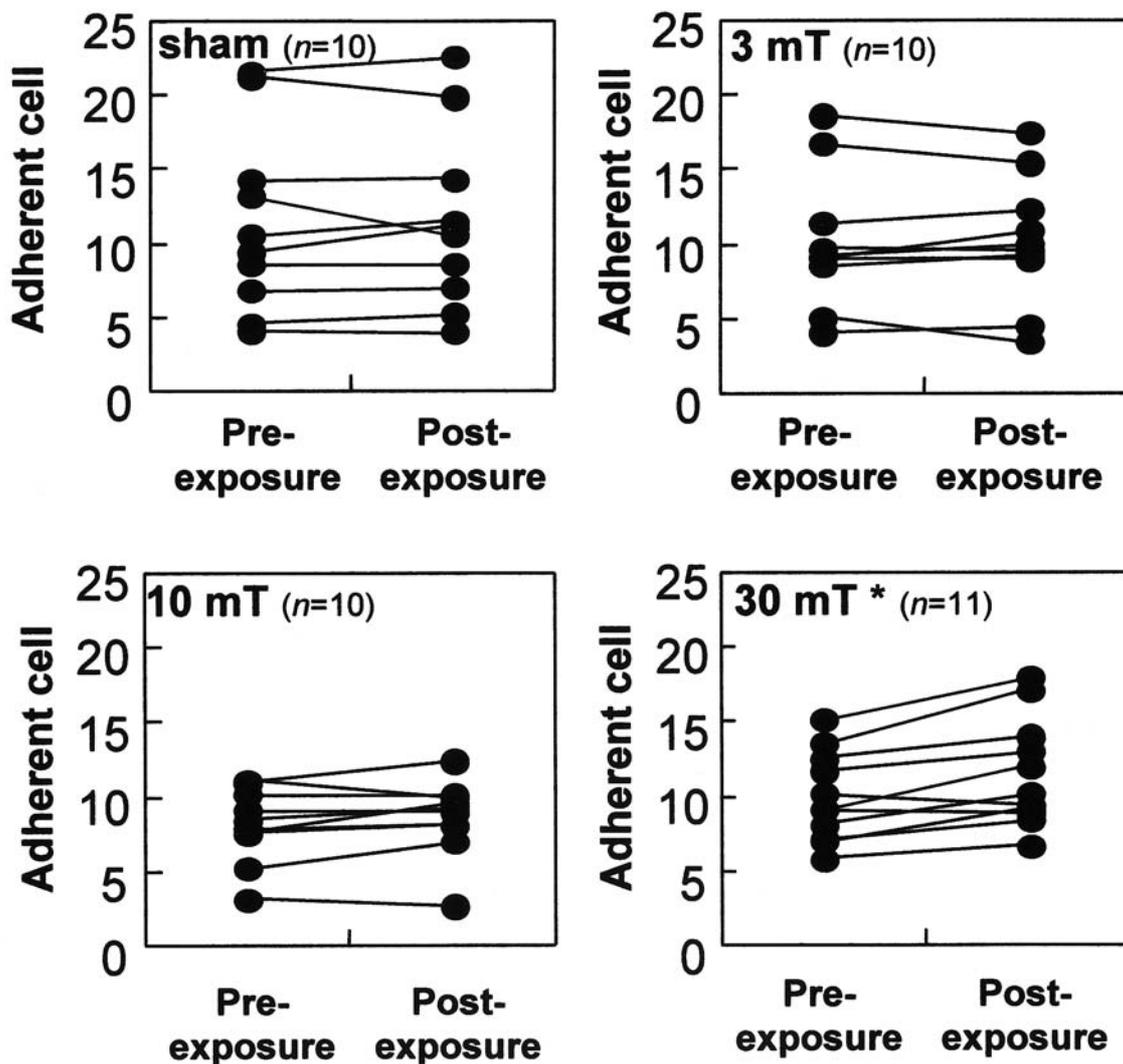


Figure 5. Changes in rolling and adherent leukocyte numbers in venules observed by DSC following acute exposure (30 min) to 50 Hz ELF-EMF with magnetic densities of 0, 3, 10 and 30 mT. The two connected closed circles in each graph show variations in the same animal before and after exposure to ELF-EMF. Each pair of closed circles represents the mean of 10 repeated observations. Adherent leukocytes in the 30 mT group showed a significant difference between pre- and post-exposure cell counts ($p < 0.05$). The number of animals in each sample group is represented by n .

venule. A rolling or adhering leukocyte was identified when its velocity was less than $150 \mu\text{m}/\text{sec}$, calculated by frame-by-frame analysis. The velocity of free flowing leukocytes in the center stream of venules was determined on a monitoring screen by measuring the distance a single leukocyte traveled per unit time. Ten cells were measured in each image, the fastest measurement being considered to be the velocity of free flowing leukocytes. The vessel diameter was measured with calipers in standstill frames of the video-recorded images.

Statistical analysis. Within group comparisons were performed using the Wilcoxon signed-rank test for paired analysis. Statistical significance was set at $p < 0.05$. Unless stated otherwise, n represents the number of sample group animals.

Results

Animal model and intravital fluorescence microscopy. For this study we used a mouse DSC made of Duracon™ resin, which is not affected by electromagnetic fields (e.g., EMF may produce thermal effects on commonly used metal frame chambers). Recent studies in our laboratory have shown that mice tolerate this dorsal skinfold chamber well and exhibit no adverse physiological effects (unpublished data). Experiments were conducted on mice at least 5 days after DSC implantation. Any animals showing signs of impaired microvascular blood flow or inflammation were

excluded from the experiments. Fluoromicroscopy with the leukocyte-specific fluorescent dye rhodamine 6G provided good resolution for detecting flowing and rolling leukocytes, as depicted in the magnified image in Figure 3.

Leukocyte velocity. The velocity of leukocytes flowing in the center streams of venules was determined in each animal. As shown in Figure 4, leukocyte velocities did not change markedly between pre- and post-exposure measurements. While velocity flow showed a significant difference in the 3 mT exposure group, it was only slightly faster in 7 out of 10 animals. Mice exposed to 30 mT ELF-EMF demonstrated accelerated velocities in 6 cases and decelerated velocities in 4 cases, among a total of 11 animals. The range of velocity change between pre- and post-exposure measurements in the 30 mT group was greater compared to the other groups, but it was not significant. Consequently, ELF-EMF exposure at the flux densities examined had negligible effects on leukocyte velocity.

Leukocyte endothelium interaction. Rolling and endothelium-adherent leukocyte numbers were determined by off-line image analysis. Cell counts were repeated 10 times for each animal at each exposure and non-exposure period. Data points shown in Figure 5 indicate mean values of total observed counts. The results varied slightly among mice, probably due to inherent individual behavioral differences. The control group revealed the highest number of pre-exposure adherent cells, 21.5 ± 3.4 (mean \pm S.D. of 10 repeated counts), while the lowest number observed was 3.2 ± 0.63 in the 10 mT exposure group. The average number of pre-exposure adherent cells, among a total of 41 test animals, was 9.9 ± 4.7 . After continuous exposure for 30 minutes, adherent cell numbers were recounted, showing that cell numbers increased in some mice. When the average cell number for the pre-exposure state was assumed to be 1, the largest increase was 1.34 (detected in the 10 mT group) and the largest decrease was 0.70 (detected in the 3 mT group). Comparisons of pre- and post-exposure results did not show any significant differences in the 3 mT or 10 mT EMF exposure groups, as was the case with the sham group. However, adherent cell numbers in the 30 mT group significantly increased following EMF exposure. This result indicated that whole body ELF-EMF exposure induced leukocyte adhesion at magnetic densities that are extremely high relative to normal urban environmental levels and that a threshold for this effect lay in the range of 10 mT to 30 mT under this set of experimental conditions.

Discussion

Epidemiological data indicate a relationship between extremely low frequency electromagnetic field (ELF-EMF) exposure and increased incidence of leukemia (20, 21).

While detailed mechanisms are still not well understood, these studies indicate that EMF-ELF probably alter host immune response. To date, numerous *in vivo* and *in vitro* investigations have been conducted, however, the association between ELF-EMF exposure and immune response remains ambiguous. *In vivo* studies by House *et al.* demonstrated the suppression of natural killer (NK) cell activity in mice after subchronic and chronic exposure to 60 Hz magnetic fields at 1.0 mT (22, 23). Tremblay *et al.* reported that *in vivo* exposure of rats to 60 Hz EMF for 6 weeks induced immunological perturbation such as decreased numbers of CD5⁺, CD4⁺ and CD8⁺ cell populations and increased NK cell activity (24), although the specific mechanisms related to these phenomena were unclear. Moreover, it is uncertain whether the same effects occur under normal physiological conditions. To better understand EMF interactions with *in vivo* systems, we investigated the pathophysiological effects of ELF-EMF exposure in mice. By adopting the dorsal skinfold chamber (DSC), we were able to clearly visualize subcutaneous microcirculation in mice by intravital microscopy, a technique widely applied to experiments with mice, rats and hamsters (15). Also, methodologies in fluorescence microscopy are well established for observing several physiological parameters (25). In this study, we focused on free flowing leukocytes in venules, to investigate the effects of short ELF-EMF exposure periods on the interaction between leukocytes and endothelium. Leukocyte-endothelium interactions mainly occur under conditions of inflammation, in regions where leukocytes secrete the cytokines IL-1 and TNF, which induce expression of the cell adhesion molecules ICAM-1 and VCAM-1 on endothelial cell surfaces. Under inflammatory conditions this process permits leukocytes to adhere to tissues (11).

Several *in vivo* investigations into the effects of ELF-EMF exposure on cytokine production have been published. Cossarizza *et al.* (26) demonstrated that the exposure of human peripheral blood mononuclear cells (PBMCs) to extremely low frequency pulsed electromagnetic fields (PEMFs) increased both spontaneous and phytohemagglutinin (PHA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) induced production of interleukin-1 β (IL-1 β) and IL-6. Jonai *et al.* (8) reported decreases in the spontaneous production of TNF- α in the intensity range of 1 mT to 30 mT, and in that of Interferon γ (IFN- γ) at 10 mT in human PBMCs. Petrini *et al.* (27) reported that sinusoidal 50 Hz EMF at 1 mT suppressed TNF- α production in human PBMCs. In contrast, Ikeda *et al.* (28) reported no effects from 50 and 60 Hz EMF exposure either in cytotoxic activity or cytokine production in human PBMCs. Taken together, this body of research findings led us to hypothesize that EMFs potentially modulate leukocyte-endothelium interactions in microcirculatory systems, with the basic assumption that the effects of EMF on *in vitro* and *in vivo* systems are essentially the same.

It is well established that leukocyte-endothelium interactions are affected by blood velocity in the microvasculature (29), therefore we undertook to measure mean blood velocities in venule segments of mice subjected to whole body EMF exposure. Our results found no discernable differences in mean blood velocity following EMF exposure. Xu *et al.* (19), on the other hand, reported that mice exposed to 50 Hz EMF at 1 mT for 10 min exhibit significantly increased peak blood velocity and that 1 mT was a threshold level for this effect. Although they used the same electromagnet that we did, they measured peak blood velocity of muscle capillaries in anesthetized mice, while our experiment measured mean blood velocity in venules of conscious animals. Further, our experimental exposure period of 30 min differed from their exposure times. Any of these factors, individually or in combination, may explain why we found only negligible differences in EMF effects on blood velocity.

A significant increase in endothelium-adhering and rolling leukocytes was detected in venules following EMF exposure (Figure 5). Under intravital microscopy these vessels showed no abnormal pathophysiology or inflammation. Since the velocities of free flowing leukocytes were unchanged, as determined by mean blood velocity results, the increased leukocyte-endothelium interaction observed may be attributed to EMF exposure, subsequently indicating that electromagnetic fields trigger the modulation of endothelial cell adhesion. Additionally, this result was found only in the 30 mT exposure group, suggesting that a threshold level for this effect exists between a flux density of 10 mT and 30 mT.

This study examined acute ELF-EMF effects on subcutaneous microcirculation using a DSC and a C-shaped electromagnet for exposure. The electromagnet was suitable for whole body exposure of the mouse. However, the spatial area ensuring uniform magnet intensity was limited and the mouse had to be fixed to the center of the electromagnet during exposure. Therefore, an exposure time of 30 min was applied in order to reduce stress caused by long restriction periods. To better characterize the differential effects in cases of chronic and subchronic EMF exposure, more study is needed for the design of better chronic exposure models. Microcirculatory vessels can be observed up to 4 weeks using this dorsal skin chamber, under optimal conditions, however, for several weeks of continuous ELF-EMF exposure, we are currently investigating an experimental exposure model employing Helmholtz coils.

The present experimental data showed that the continuous and whole body exposure of 50 Hz EMF at 30 mT for 30 min significantly influenced cell to cell interaction between venular endothelial cells and leukocytes in the mouse subcutaneous microvasculature although 3mT or 10 mT exposure did not affect leukocyte adhesiveness. While

only negligible differences in blood velocity were determined following EMF exposure, the increased leukocyte adhesion observed at 30 mT is in agreement with earlier reports showing that EMF modulates endothelium cell adhesion and increases leukocyte-endothelium interactions. Moreover, this acute EMF effect was shown only at a high flux density of 30 mT, indicating that a threshold level exists between 10 mT and 30 mT. The increased leukocyte-endothelium interactions seen with 30 mT exposure appears to result from altered immunological responses and subsequently confirm that ELF-EMF at very high flux densities evoke pathophysiological effects on biological systems *in vivo*.

Acknowledgements

We thank Dr. G. F. Lawlor for helpful suggestions and Ms. R. Sasaki for technical assistance.

References

- 1 Wertheimer N and Leeper E: Adult cancer related to electrical wires near the home. *Int J Epidemiol* 11: 345-355, 1982.
- 2 Savitz DA and Kaune WT: Childhood cancer in relation to a modified residential wire code. *Environ Health Perspect* 101: 76-80, 1993.
- 3 Wertheimer N, Savitz DA and Leeper E: Childhood cancer in relation to indicators of magnetic fields from ground current sources. *Bioelectromagnetics* 16: 86-96, 1995.
- 4 Litovitz TA, Krause D and Mullins JM: Effect of coherence time of the applied magnetic field on ornithine decarboxylase activity. *Biochem Biophys Res Commun* 178: 862-865, 1991.
- 5 Ohtsu S, Miyakoshi J, Tsukada T, Hiraoka M, Abe M and Takebe H: Enhancement of beta-galactosidase gene expression in rat pheochromocytoma cells by exposure to extremely low frequency magnetic fields. *Biochem Biophys Res Commun* 212: 104-109, 1995.
- 6 Goodman R, Blank M, Lin H, Dai R, Khorkova O, Soo L, Weisbrot D and Henderson A: Increased levels of hsp70 transcripts induced when cells are exposed to low frequency electromagnetic fields. *Bioelectrochem Bioenerget* 33: 115-120, 1994.
- 7 Miyakoshi J, Mori Y, Yaguchi H, Ding G and Fujimori A: Suppression of heat-induced HSP-70 by simultaneous exposure to 50 mT magnetic field. *Life Sci* 66: 1187-1196, 2000.
- 8 Jonai H, Villanueva MB and Yasuda A: Cytokine profile of human peripheral blood mononuclear cells exposed to 50 Hz EMF. *Ind Health* 34: 359-368, 1996.
- 9 Spertini O, Luscinskas FW, Kansas GS, Munro JM, Griffin JD, Gimbrone MA Jr and Tedder TF: Leukocyte adhesion molecule-1 (LAM-1, L-selectin) interacts with an inducible endothelial cell ligand to support leukocyte adhesion. *J Immunol* 147: 2565-2573, 1991.
- 10 Haraldsen G, Kvale D, Lien B, Farstad IN and Brandtzaeg P: Cytokine-regulated expression of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in human microvascular endothelial cells. *J Immunol* 156: 2558-2565, 1996.

- 11 Waldman WJ and Knight DA: Cytokine-mediated induction of endothelial adhesion molecule and histocompatibility leukocyte antigen expression by cytomegalovirus- activated T cells. *Am J Pathol* 148: 105-119, 1996.
- 12 Silverman MD, Zamora DO, Pan Y, Texeira PV, Planck SR and Rosenbaum JT: Cell adhesion molecule expression in cultured human iris endothelial cells. *Invest Ophthalmol Vis Sci* 42: 2861-2866, 2001.
- 13 Jain RK: The Eugene M. Landis Award Lecture 1996. Delivery of molecular and cellular medicine to solid tumors. *Microcirculation* 4: 1-23, 1997.
- 14 Algire GH and Legallais FY: Recent developments in the transparent-chamber technique as adopted to the mouse. *J Nat Cancer Inst* 10: 225-253, 1949.
- 15 Jain RK: The next frontier of molecular medicine: delivery of therapeutics. *Nat Med* 4: 655-657, 1998.
- 16 Leunig M, Yuan F, Menger MD, Boucher Y, Goetz AE, Messmer K and Jain RK: Angiogenesis, microvascular architecture, microhemodynamics, and interstitial fluid pressure during early growth of human adenocarcinoma LS174T in SCID mice. *Cancer Res* 52: 6553-6560, 1992.
- 17 Baatz H, Pleyer U, Thiel HJ and Hammer C: *In vivo* study of leukocyte-endothelium interaction in endotoxin-induced uveitis. *Invest Ophthalmol Vis Sci* 36: 1960-1967, 1995.
- 18 Baatz H, Steinbauer M, Harris AG and Krombach F: Kinetics of white blood cell staining by intravascular administration of rhodamine 6G. *Int J Microcirc Clin Exp* 15: 85-91, 1995.
- 19 Xu S, Okano H and Ohkubo C: Acute effects of whole-body exposure to static magnetic fields and 50-Hz electromagnetic fields on muscle microcirculation in anesthetized mice. *Bioelectrochemistry* 53: 127-135, 2001.
- 20 Savitz DA and Calle EE: Leukemia and occupational exposure to electromagnetic fields: review of epidemiologic surveys. *J Occup Med* 29: 47-51, 1987.
- 21 Savitz DA, Wachtel H, Barnes FA, John EM and Tvrdik JG: Case-control study of childhood cancer and exposure to 60-Hz magnetic fields. *Am J Epidemiol* 128: 21-38, 1988.
- 22 House RV, Ratajczak HV, Gauger JR, Johnson TR, Thomas PT and McCormick DL: Immune function and host defense in rodents exposed to 60-Hz magnetic fields. *Fundam Appl Toxicol* 34: 228-239, 1996.
- 23 House RV and McCormick DL: Modulation of natural killer cell function after exposure to 60 Hz magnetic fields: confirmation of the effect in mature B6C3F1 mice. *Radiat Res* 153: 722-724, 2000.
- 24 Tremblay L, Houde M, Mercier G, Gagnon J and Mandeville R: Differential modulation of natural and adaptive immunity in Fischer rats exposed for 6 weeks to 60 Hz linear sinusoidal continuous-wave magnetic fields. *Bioelectromagnetics* 17: 373-383, 1996.
- 25 Suzuki T, Yanagi K, Ookawa K, Hatakeyama K and Ohshima N: Flow visualization of microcirculation in solid tumor tissues: intravital microscopic observation of blood circulation by use of a confocal laser scanning microscope. *Front Med Biol Eng* 7: 253-263, 1996.
- 26 Cossarizza A, Angioni S, Petraglia F, Genazzani AR, Monti D, Capri M, Bersani F, Cadossi R and Franceschi C: Exposure to low frequency pulsed electromagnetic fields increases interleukin-1 and interleukin-6 production by human peripheral blood mononuclear cells. *Exp Cell Res* 204: 385-387, 1993.
- 27 Perini C, Dupuis M, Polichetti A, Romani C and Vecchia P: Tumor necrosis factor •and interferon •production by human peripheral blood mononuclear cells exposed *in vitro* to sinusoidal 50 Hz magnetic fields. *Bioelectrochemistry and Bioenergetics* 44: 121-125, 1997.
- 28 Ikeda K, Shinmura Y, Mizoe H, Yoshizawa H, Yoshida A, Kanao S, Sumitani H, Hasebe S, Motomura T, Yamakawa T, Mizuno F, Otaka Y and Hirose H: No effects of extremely low frequency magnetic fields found on cytotoxic activities and cytokine production of human peripheral blood mononuclear cells *in vitro*. *Bioelectromagnetics* 24: 21-31, 2003.
- 29 Mayrovitz HN, Wiedman MP and Tuma RF: Factors influencing leukocyte adherence in microvessels. *Thromb Haemost* 38: 823-830, 1977.

Received November 10, 2003

Accepted January 30, 2004