Environmentalist (2007) 27:395–402
DOI 10.1007/s10669-007-9082-z

EMF effects on microcirculatory system

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Published online: 6 July 2007
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Abstract Authors review the importance of studying the effects of electromagnetic fields (EMF) on microcirculatory system, especially in respect of possibility that vasculature may have direct and indirect role in interaction of static magnetic fields (SMF). We outline the physiological importance of microcirculation and relatively new methods of evaluation technique in vivo and explain in details the local and/or whole body exposure effects of SMF with range of 0.3–180 mT, power frequency EMF with range of 0.1–30 mT and microwaves at 1.5 GHz with range 0.08–8 W/kg brain average specific absorption rate (SAR) on microcirculatory systems in different tissues in experimental animals.

Keywords EMF · SMF · SAR · Microcirculation · In vivo · Animals

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1 Introduction

Relatively few papers review the effects of EMF on microcirculatory system (McKay et al. 2007). The circulatory system is the transport system of the organism that supplies O_2, ions, hormones, and substances absorbed from the gastrointestinal tract to the tissues, and returns CO_2 to the lungs and other products of metabolism to the liver and kidneys. It also has a central role in the regulation of body temperature, and the distribution of the hormones and other agents that regulate tissue and cell function. The principal function of microcirculation is exchanging physiological substances between blood and tissues, and the compensatory adjustments should contribute to the efficacy of the exchange process: lumen dimensions, length, tortuosity, diameter of branch ratios, vascular density, wall thickness, vessel diameter (vasomotion), blood flow velocity, blood viscosity, intramicrovascular hematocrit, leukocyte-endothelial cell interaction and others.

Microcirculation consists of structurally and functionally differentiated small blood vessels: small muscular arteries, arterioles, metarterioles, capillaries, postcapillary venules, venules, and lymphatic capillaries. In the cutaneous microvascular beds, the connection between the arterioles and the venules is made by some thoroughfare channels including capillaries or arteriolar-venular shunts. In most vascular beds, the precapillary resistance vessels are responsible for the largest function of the resistance in a vascular bed, and hence are the major components that influence regional hemodynamics and total peripheral resistance. Smooth muscle cells are found in all of these except the blood capillaries and lymphatic capillaries. The blood capillary wall is composed of a single layer of endothelial cells. The lymphatic capillaries are composed of endothelium-lined vessels similar to blood capillaries.

Fluid and protein that have extravasated from the blood capillaries partially enter the lymphatic capillaries and are transported via the lymphatic system back to the blood vascular system. Postcapillary venules play an important role in fluid and cellular exchange and are the major site of leukocyte migration into tissue spaces.

Rhythmical and spontaneous changes in both the diameter of arterioles and the volume and velocity of blood flow due to constriction and dilation of the vascular smooth muscle are known as vasomotion (Asano and Branemark 1972). The quantitative description of spontaneous arteriolar vasomotion requires data on frequency, amplitude, diameter, and branching order of the vessels observed. Fluid absorbed into lymphatic capillaries is passively transported through dynamic changes due to arteriolar vasomotion in cutaneous tissue. Frequency and amplitude of spontaneous vasomotion could play an important role in disease. In microangiopathies, lymphedema, and essential hypertension, altered patterns of arteriolar vasomotion could constitute an additional pathogenetic factor (Funk and Intaglietta 1983). Extracellular control of the smooth muscle cells is exerted through neurogenic, hormonal, local and myogenic mechanisms (Mulvany 1983).

For evaluating EMF exposure effects on microcirculatory system, various microcirculatory preparations, e.g., rabbit ear chamber (REC) (Asano et al. 1965), dorsal skin fold chamber (DSC) in mice (Ushiyama and Ohkubo 2004) and cranial window (CW) in mice and rats (Ushiyama et al. 2003; Masuda et al. 2003), have been used to observe and analyze microcirculation in our group. These preparations allow non-invasive, continuous measurement of hemodynamics, blood velocity, angiogenesis, metabolites, e.g., pH and pO_2, transport of molecules and particles, and cell to cell interactions in vivo (Jain 1997).

2 Static magnetic fields (SMF)

This is an area of research that would benefit from increased investigation because SMF therapy could be useful for circulatory diseases including ischemic pain and hypertension, primarily due to the modulation of blood flow or blood pressure. However, there might be safety concerns on magnetic resonance imaging (MRI) and magnetic levitation for transportation using strong intensity SMF with Tesla (T) levels. Therefore, the knowledge of the SMF effects on microcirculation is extremely important in consideration of the human health, and the relation of capillary formation and tumor growth.

Several attempts have been made to explore the parameters of microcirculation and microvasculature when tissue and/or blood vessels have been exposed to SMF. In particular, the REC offers the advantages of superior optical quality. Due to the longer duration of an individual measurement, we have exclusively utilized REC to investigate the effects of SMF on microcirculation using microphotoelectric plethysmography (MPPG) monitoring system. REC is a round-table chamber made of acryl resin for disk with an observing table and three holding pillars, a sustaining ring, and a glass window. The methods for installation of REC and its availability to the bioelectromagnetic research have been published in detail (Ohkubo and Xu 1997; Xu et al. 1998; Okano et al. 1999; Gmitrov et al. 2002). Blood pressure in a central artery contralateral to that of an ear lobe having the REC, fixed on the microscope stage, was monitored by a blood pressure monitoring system.

Using these methods, we firstly demonstrated that cutaneous microcirculation was modulated by moderate-intensity SMF with milli Tesla (mT) levels: the biphasic effects of a 1, 5, and 10 mT SMF on cutaneous microcirculation were found in conscious rabbits (Ohkubo and Xu 1997). A 10 min exposure to SMF induced changes in vasomotion in a non-dose dependent manner. When the initial vessel diameter was less than a certain value, SMF exposure caused an increase in vessel diameter (vasodilation). In contrast, when the initial diameter was greater than a certain value, SMF exposure caused a decrease in vessel diameter (vasoconstriction). Based on these results, it would appear that the initial state of the vessel is of importance when considering SMF effects on microcirculation and microvasculature.

Likewise, this observation using REC is reflected in the following studies: the biphasic effects (activation/inhibition) of 10 min exposure to 1 mT SMF on cutaneous microcirculation were found in conscious rabbits treated with vasoactive agents (Okano et al. 1999). When high vascular tone was induced by norepinephrine to cause vasoconstriction, the SMF exposure led to increased vasomotion and caused vasodilation. In contrast, low vascular tone was induced by acetylcholine to cause vasodilation, the SMF exposure led to decreased vasomotion and caused vasoconstriction. Other studies without using REC have also been well reviewed by McKay et al. (2007). Our studies described above were performed at Department of Environmental Health (former Department of Physiological Hygiene), National Institute of Public Health, Japan.

Similar findings were reported by an independent laboratory using different techniques without using REC for the microvessels of rat skeletal muscle: a SMF exposure (70 mT for 15 min) had a restorative effect on microvascular tone (Morris and Skalak 2005). When vessels had high tone (constricted), the SMF acted to reduce tone, and when vessels had low tone (dilated), the SMF acted to increase tone. This response was amplified when the vessels had an initial diameter of less than 30 μm.

The effects of higher SMF applying for MRI on blood brain barrier (BBB) permeability were investigated in rats using a radioactive tracer, ^{153}Gd-DTPA (Prato et al. 1994). Exposures to SMF alone for 45 min, without radiofrequency (RF)-SAR and temporal gradient, increased BBB permeability at 1.5 T and 1.89 T.

2.1 Discussion and conclusion

In summary, significant circulatory system responses to SMF have been recently reviewed in experimental animals and/or humans: SMF exposures between 1 mT and 8 T for anywhere between 10 min and 12 weeks can influence cutaneous microcirculation, hemodynamics, and/or arterial blood pressure (Saunders 2005; Tenforde 2005; McKay et al. 2007). Five of a total of 26 studies in the last two decades report either an increase in blood flow, or an elevation in blood pressure. In contrast, nine of the 26 studies indicate either a decrease in blood flow or a reduction in blood pressure. Seven studies report no effect. The remaining five studies found that SMF exposures could trigger either vasodilation or vasoconstriction depending on the initial tone of the vessel. For a summary, please refer to Table 1.

In particular, our series of studies, as shown in Table 1, have demonstrated that cutaneous microcirculation, hemodynamics, and/or arterial blood pressure were modulated by moderate-intensity SMF with mT levels in pharmacologically treated animals and genetically hypertensive animals, while no changes were observed in normal animals when the initial state of the vessel was not identified. Therefore, we concluded that significant bioresponses to therapeutic signals occur when the state of the target is far from the homeostasis (Ohkubo and Okano 2004).

The mechanisms of SMF effects in the mT range could be mediated by suppressing or enhancing the action of biochemical effectors, thereby inducing homeostatic effects biphasically. The potent mechanisms of SMF effects have often been linked to nitric oxide (NO) pathway, Ca^{2+} dependent pathway, sympathetic nervous system (e.g., baroreflex sensitivity and the action of sympathetic agonists or antagonists), and neurohumoral regulatory system (e.g., production and secretion of angiotensin II and aldosterone), as reviewed by McKay et al. (2007).

There are a number of important dosimetry issues that could exhibit significant effects on microcirculation. It has been shown that it is more appropriate to consider biological responses to SMF through the hypothesis of intensity windows, instead of intensity-response dependence (Markov et al. 2004). Furthermore, it has been reported that the gradient component of SMF might be responsible for the physiological responses in vivo (Okano and Ohkubo 2005a, b), because the in vitro effects of gradient fields on action potential generation (McLean et al. 1995; Cavopol et al. 1995) and myosin phosphorylation (Engström et al. 2002) have been found mostly in the absolute field gradient range of more than 1 mT/mm in the target tissues or cells. However, these hypotheses have not been tested, and the effects and underlying mechanisms remain elusive. In particular, to reveal and clarify the effects and mechanisms of spatial magnetic gradient, it is necessary to carry out the experiments comparing the spatially homogeneous and inhomogeneous SMF.

Table 1 Summary of SMF effects on hemodynamics and blood pressure (BP)

Increased hymodynamics or BP (n = 5)	*Exposure*
Prato et al. (1994)	1.5, and 1.89 T; 45 min; rats
Xu et al. (1998)	180 mT; 4 weeks; rabbits
Xu et al. (2000)	0.3, 1, and 10 mT; 10 min; mice
Gmitrov et al. (2002)	250 mT; 40 min; rabbits
Okano et al. (2005a)[c]	10, and 25 mT; 12 weeks; rats
Decreased hymodynamics or BP (n = 9)	*Exposure*
Ichioka et al. (2000)	8 T; 20 min; rats
Ichioka et al. (2003)	8 T; 20 min; rats
Okano and Ohkubo (2003a)[b]	5.5 mT; 30 min; rabbits
Okano and Ohkubo (2003b)[a]	10, and 25 mT; 12 weeks; SHR
Okano and Ohkubo (2005b)[a]	180 mT; 14 weeks; SHR
Okano and Ohkubo (2006)[a]	180 mT; 6 weeks; SHR
Okano and Ohkubo (2007)[b]	12 mT; 10 weeks; rats
Okano et al. (2005b)[a]	1, and 5 mT; 12 weeks; SHR
Mayrovitz and Groseclose (2005)	400 mT; 45 min; humans
Biphasic effect (n = 5)	*Exposure*
Ohkubo and Xu (1997)	1, 5, and 10 mT; 10 min; rabbits
Okano et al. (1999)	1 mT; 10 min; rabbits
Okano and Ohkubo (2001)	5.5 mT; 30 min; rabbits
Okano and Ohkubo (2005a)	5.5 mT; 30 min; rabbits
Morris and Skalak (2005)	70 mT; 15 min; rats
No effect (n = 7)	*Exposure*
Kangarlu et al. (1999)	8 T; 1 h for humans; 3 h for pigs
Steyn et al. (2000)	27 mT; 48 h; horses
Mayrovitz et al. (2001)	100 mT; 36 min; humans
Mayrovitz et al. (2005)	85 mT; 20 min; humans
Hinman (2002)	50 mT; 15 min; humans
Martel et al. (2002)	80 mT; 30 min; humans
Kuipers et al. (2006)	60 mT; 1 h ; humans

[a] Initial state of subjects: spontaneously hypertensive rats (SHR)

[b] Initial state of subjects: pharmacologically induced hypertension

[c] Initial state of subjects: pharmacologically induced hypotension

3 Extremely low frequency-electromagnetic fields (ELF-EMF)

Many researches for exploring biological and health hazardous effects on ELF-EMF have been done, however, there are few reports evaluating the exposure effects on the microcirculatory system in vivo. Therefore, we aimed to clarify the effects on the microcirculation by ELF-EMF exposure. To pursue the effect quantitatively, we employed two in vivo microscopic approaches; dorsal skinfold chamber (DSC) method and CW method. By using DSC method, we quantified the effect on immune responses at microcircular level in subcutaneous tissue. CW method was applied to clarify the effect on the pial microcirculation and the growth of implanted brain tumor.

3.1 DSC model and the effect on the leukocyte-endothelial interaction

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. demonstrate the suppression of natural killer (NK) cell activity in mice after subchronic and chronic exposure to 60 Hz magnetic fields at 1.0 mT (House et al. 1996, House and McCormick 2000), although the specific mechanisms related to these phenomena are unclear. Moreover, it is uncertain whether the same effects occur under normal physiological conditions. To better understand EMF interactions with in vivo systems, we explore the effect of ELF-EMF on microcirculatory system, acute and subchronic effects on leukocyte-endothelium interactions in subcutaneous tissue of mice. To attain this purpose we developed a non-metallic DSC, which made of polyacetal resin (Duracon^{TM}) (Ushiyama et al. 2004a). This non-metallic frame chamber can be applied to studies for ELF-EMF exposure without any resultant thermal effects. The adherent leukocyte counts to the endothelium are one of the good indicators for estimating pathophysiological conditions, particularly rolling counts always increased when the immune system is activated. In a series of experiments, 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 cytokines, which induce expression of the cell adhesion molecules on endothelial cell surfaces. Under inflammatory conditions this process permits leukocytes to adhere to tissues (Waldman and Knight 1996).

According to the result of short term exposure, significant increase in endothelium-adhering and rolling leukocytes was detected in venules following EMF exposure under the condition of extremely high intensity magnetic field (30 mT) (Ushiyama and Ohkubo 2004), although these vessels showed no abnormal pathophysiology or inflammation under the intravital microscopy. 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 effect will not be directly related to the health effect because increased interaction was found only in the 30 mT exposure group.

Similar result was obtained from the experiments of subchronic exposure (Ushiyama et al. 2004b). In this experiment, mice with DSC were maintained under the various intensity of 50 Hz electromagnetic field for 15 consecutive days. We demonstrated that a significant increased number of rolling leukocytes compared to pre-exposure status was observed only in highest exposure level (3 mT) group. In other groups, no significant change in rolling count was observed in any measuring time point. We also measured TNF-α and IL-1β concentration in serum, however, there was no significant change among three groups, suggesting the sensitivity against these molecules is not good enough or other unknown mechanism is underlied. We are continuing further study to figure out the molecular mechanism of this phenomenon.

3.2 CW model and the effect on the brain tumor and pial microcirculation

Although a couple of epidemiological reports have suggested the weak relationship between brain tumor and ELF-EMF exposure (Ahlbom et al. 2001), there were few reports of in vivo study focused on brain tumor. Therefore we explored the subchronic effects of whole body exposure to ELF-EMF on the growing implanted brain tumor and cerebral microcirculation in a mice CW. This CW model makes us possible to observe the cerebral microcirculation with high resolution and to clarify the pathophysiological effect of ELF-EMF using brain tumor bearing model (Fukumura 2006). In this experiment, small pieces of human glioma U87 tissue were implanted to the CW of SCID (Severe combined immuno-deficiency) mice prior to ELF-EMF exposure. Mice were divided into 3 exposure groups with 50 Hz EMF at 0.3 mT and 3 mT (exposure groups) and without EMF (sham exposure group) and exposed for 18 consecutive days (Ushiyama et al. 2003). During/after the exposure, we measured tumor growth (size of tumor tissue), the microcirculatory parameters, plasma leakage of rhodamine-labeled albumin and leukocytes-adhesion to the tumor blood vessels with a real time confocal microscopy.

Results indicated that, ELF-EMF exposure did not affect the growth of tumor. This suggested that ELF-EMF has no effect to accelerate or decelerate the growth of tumor under relatively high magnetic density environment. As the intensive angiogenesis was observed around the tumor tissue accompanied with the tumor growth, we evaluated the microcirculatory parameters and permeability of angiogenic vessels at 15th day of exposure. Microcirculatory parameters including vascular density, number of segments per unit area, mean diameter and volume density did not show any statistical difference among three groups. In U87 mice, tumor vessels showed high permeability compared with the vessels of mice without tumor did. However, no difference in permeability coefficient was found the three exposure groups. Adhesive leukocytes tended to increase their numbers after exposure compared with those of sham exposure group, however, there was no statistical significant difference. As a conclusion, we could not find any evidence that the subchronic exposure of 50 Hz EMF may affect the growth of brain tumor and microcirculatory parameters including BBB function in the pial tissue. Further investigation will be required for resolving this phenomenon and elucidating the physiological significance.

3.3 Discussion and conclusion

Our animal study suggests that some immunological responses in subcutaneous tissue were induced by high intensity of ELF-EMF exposure. However, the estimated threshold intensity will be more than 10 mT for acute exposure and 1 mT for subchronic exposure. On the other, the microcirculatory parameters of pia mater were not affected by similar condition. Therefore, we can conclude no evidence about health hazardous effect by low intensity of ELF-EMF exposure is observed.

4 Radio-frequency electromagnetic fields (RF-EMF)

BBB function has been focused on as one of the important research topic related to the adverse health effects of RF-EMF on tissue or organ microcirculation. Many researchers have reported that the exposure to RF-EMF increases the blood flow in the microcirculation of musculature over the past two decades (Shrivastav et al. 1983; Sharma and Hoopes 2003). Most of them were, however, obtained under the thermal conditions which induced by high intensity of RF-EMF exposure. Thus, those responses were attributed to the increase in body or local region temperature.

On the other hand, there had been little information about the effects of RF-EMF exposure on microcirculation under non-thermal conditions until 1994. In this year, Salford et al. reported that albumin leakage sites were found in the rat brain after 915 MHz-EMF exposure even under non thermal intensity levels (Salford et al. 1994, 2003). Because permeability change of BBB has been a matter of concern as it could result in health hazard on brain, many research groups attempted to confirm their results. However, a few studies (Aubineau and Tore 2005; Schirmacher 2000) found the low level RF-EMF affect BBB permeability in vivo and in vitro, whereas others (Tsurita et al. 2000; Kuribayashi et al. 2005; Franke et al. 2005; Finnie et al. 2006) failed to replicate Salford's findings.

The cerebral microcirculatory dynamics including BBB function has been a target in our studies to evaluate the biological effects of RF-EMF. As indicators of dynamic changes in microcirculation, there are several parameters, such as blood flow velocity, vessel diameter changes and leukocyte behavior. Those changes are often observed in tissue or organs under pathophysiological conditions. For example, the BBB disruption and the increase in leukocyte adhesiveness to endothelium in pial venules were found in inflammatory brain (Mayhan 2000; Gaber et al. 2004). Therefore, simultaneous investigation of several parameters is helpful to assess the effects of RF-EMF exposure on the microcirculation.

The closed cranial window (CCW) method is one of the useful techniques to evaluate the cerebral microcirculatory parameters in experimental animals in vivo (Masuda et al. 2000; Yuan et al. 2003). This method allows for direct observation of pial microvasculature and several blood cells via a transparent glass window implanted on the parietal region of the brain (Fig. 1). To investigate the possible effects of the exposure to RF-EMF on cerebral microcirculation including several parameters mentioned above, we introduced the CCW method into rats and observed the changes in the parameters in the brain after acute or subchronic exposure to RF-EMF (Masuda et al. 2001a, b).

The exposure system we used consisted of a small anechoic chamber and a monopole antenna. The head of

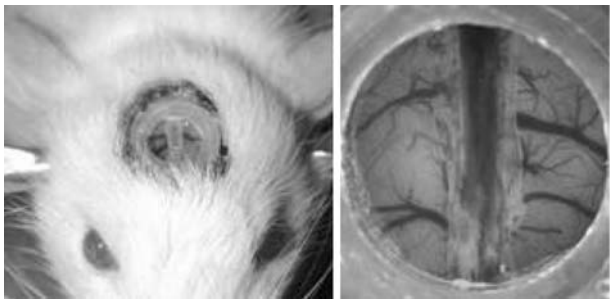

Fig. 1 Overview of rat pial microcirculation via the closed cranial window

each rat was positioned toward the central antenna and was locally exposed to 1,439 MHz electromagnetic near-field TDMA (time division multiple access) signal for exposure was controlled by mean SAR of the brain. The values of brain averaged SAR were 0.6, 2.4, and 4.8 W/kg for acute exposure experiment, and 2.4 W/kg for subchronic exposure experiment, respectively. The exposure duration was 10 minutes for the acute exposure, and was 60 min everyday, 5 days a week for 4 weeks for subchronic exposure. The pial microcirculation including vascular diameters, plasma velocities, leukocyte behavior and BBB function within the CW was observed using intravital fluorescence microscopy.

As results in acute exposure experiment, the values in the diameters and maximal plasma velocity of the pial venule of pre- and post-exposures did not significantly differ from each other for any tested SAR. Corresponding to the increase in SAR, the number of rolling leukocytes on the venular endothelia tended to decrease, however, no significant differences were recognized between the values for pre- and post-exposures. No extravasation of two kinds of fluorescence dyes, FITC-Dx (MW: 250 kDa) and sodium fluorescein (MW: 376), from the pial venule was noticed due to any SAR. Furthermore, in subchronic exposure experiment, no significant differences were recognized between the values for pre- and post-exposure in plasma velocities or adherent leukocyte counts. No extravasation of the two kinds of fluorescence dyes from the pial venule was noticed.

4.1 Discussion and conclusion

We focused on the cerebral microcirculatory parameters and investigated the acute and subchrocnic effects on the exposure to RF-EMF on those parameters. As results of our studies, no significant changes were found at least in vascular diameters, plasma velocities, leukocyte behavior or BBB function either after acute or subchronic exposure experiment. These findings lead to the following two suggestions.

The first is that the RF-EMF exposure lower than the local permissible level (2.0 W/kg) in the ICNIRP guidelines (1998) does not induce any changes in cerebral microcirculation, if a presumption for dose-response relationship between intensities of the RF-EMF exposure and biological responses is accepted. The values of brain averaged SAR in the present exposure were 0.6, 2.4, and 4.8 W/kg. These exposure levels range from a low level comparable to the study by Salford to a moderate level of 2.4 times the safety guideline. Several studies which evaluated changes in BBB permeability after RF-EMF exposure found that the exposure under lower SAR levels than the 2.0 W/kg did not modify the BBB permeability (Tsurita et al. 2000; Kuribayashi et al. 2005; Franke et al. 2005; Finnie et al. 2006). Our present results not only support their findings, but also provide new information for considering the effects of RF-EMF exposure on the microcirculation.

The second is that the multi-parameter evaluation supports the lack of increase in the BBB permeability under RF-EMF exposure. Although many investigators have reported the effects of RF-EMF on BBB permeability, these studies mainly used histological evaluation (Salford et al. 1994; Tsurita et al. 2000; Finnie et al. 2006; Fritze et al. 1997). On the contrary, we examined not only BBB permeability but also other microcirculatory parameters in vivo. Several reports have shown that the BBB disruption is accompanied with changes in leukocyte behaviors (Mayhan 2000; Gaber et al. 2004) or hemodynamics (Mayhan 1998) under inflammatory condition in the rat brain. Therefore, our findings strengthen the negative results that the RF-EMF exposure does not induce BBB disruption.

However, further studies are required under other exposure conditions to confirm this phenomena.

5 Overall conclusion

There is an importance of understanding the effects of magnetic fields on microcirculatory system. It may have direct and indirect role in interaction of magnetic fields with different tissues. The results from our studies could be useful in applying them for microcirculatory disorders. In addition, results obtained from exposure to ELF and RF-EMF failed to show any changes in microcirculatory system except for leukocyte and endothelial cell interaction. Levels of EMF used in our studies are very higher than the international exposure guidelines. These animal studies can contribute to evaluate possible health risks of EMF.

References

Ahlbom, I. C., Cardis, E., Green, A., Linet, M., Savitz, D., & Swerdlow, A. (2001). Review of the epidemiologic literature on EMF and health. *Environmental Health Perspectives, 109*(Suppl 6), 911–933.

Asano, M., & Brånemark, P. I. (1972). Microphotoelectric plethysmography using a titanium chamber in man. In H. Harders (Ed.), *Advances in microcirculation*, (Vol. 4, pp. 131–160). Basel: S. Karger.

Asano, M., Yoshida, K., & Tatai, K. (1965). Microphotoelectric plethysmography using a rabbit ear chamber. *Journal of Applied Physiology, 20*, 1056–1062.

Aubineau, P., & Tore, F. (2005). Head exposure to 900 MHz microwaves induces plasma protein extravasation in the rat brain and dura mater at non-thermal SAR levels. In *Monografie Workshops: Influences of RF- and electromagnetic field interaction*. Berufsgenossenschaft der Feinmechanik und Elektrotechnik, pp. 82–83.

Cavopol, A. V., Wamil, A. W., Holcomb, R. R., & McLean, M. J. (1995). Measurement and analysis of static magnetic fields that block action potentials in cultured neurons. *Bioelectromagnetics, 16*, 197–206.

Engström, S., Markov, M. S., McLean, M. J., Holcomb, R. R., & Markov, J. M. (2002). Effects of non-uniform static magnetic fields on the rate of myosin phosphorylation. *Bioelectromagnetics, 23*, 475–479.

Finnie, J. W., Blumbergs, P. C., Cai, Z., Manavis, J., & Kuchel, T. R. (2006). Effect of mobile telephony on blood-brain barrier permeability in the fetal mouse brain. *Pathology, 38*, 63–65.

Franke, H., Streckert, J., Bitz, A., Goeke, J., Hansen, V., Ringelstein, E. B., Nattkamper, H., Galla, H. J., & Stogbauer, F. (2005). Effects of universal mobile telecommunications system (UMTS) electromagnetic fields on the blood-brain barrier in vitro. *Radiation Research, 164*, 258–269.

Fritze, K., Sommer, C., Schmitz, B., Mies, G., Hossmann, K. A., Kiessling, M., & Wiessner, C. (1997). Effect of global system for mobile communication (GSM) microwave exposure on blood-brain barrier permeability in rat. *Acta Neuropathologica (Berl), 94*, 465–470.

Fukumura, D. (2006). Window models. In D. Shepro (Ed.), *Microvascular research: Biology and pathology* (pp. 151–157). San Diego, CA, USA: Elsevier Science.

Funk, W., & Intaglietta, M. (1983). Spontaneous arteriolar vasomotion. In K. Messemer, & F. Hammersen (Eds.), *Progress in applied microcirculation* (Vol. 3, pp. 66–82). Basel: Karger.

Gaber, M. W., Yuan, H., Killmar, J. T., Naimark, M. D., Kiani, M. F., & Merchant, T. E. (2004). An intravital microscopy study of radiation-induced changes in permeability and leukocyte-endothelial cell interactions in the microvessels of the rat pia mater and cremaster muscle. *Brain research. Brain Research Protocols, 13*, 1–10.

Gmitrov, J., Ohkubo, C., & Okano, H. (2002). Effect of 0.25 T static magnetic field on microcirculation in rabbits. *Bioelectromagnetics, 23*, 224–229.

Hinman, M. R. (2002). Comparative effect of positive and negative static magnetic fields on heart rate and blood pressure in healthy adults. *Clinical Rehabilitation, 16*, 669–674.

House, R. V., & McCormick, D. L. (2000). Modulation of natural killer cell function after exposure to 60 Hz magnetic fields: Confirmation of the effect in mature B6C3F1 mice. *Radiation Research, 153*, 722–724.

House, R. V., Ratajczak, H. V., Gauger, J. R., Johnson, T. R., Thomas, P. T., & McCormick, D. L. (1996). Immune function and host defence in rodents exposed to 60-Hz magnetic fields. *Fundamental and Applied Toxicology, 34*, 228–239.

Ichioka, S., Minegishi, M., Iwasaka, M., Shibata, M., Nakatsuka, T., Ando, J., & Ueno, S. (2003). Skin temperature changes induced by strong static magnetic field exposure. *Bioelectromagnetics, 24*, 380–386.

Ichioka, S., Minegishi, M., Iwasaka, M., Shibata, M., Nakatsuka, T., Harii, K., Kamiya, A., & Ueno, S. (2000). High-intensity static magnetic fields modulate skin microcirculation and temperature in vivo. *Bioelectromagnetics, 21*, 183–188.

ICNIRP (International Commission on Non-Ionizing Radiation Protection). (1998). Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz), *Health Physics, 74*, 494–522.

Jain, R. K. (1997). The Eugene M. Landis award lecture 1996 delivery of molecular and cellular medicine to solid tumors. *Microcirculation, 4*, 1–23.

Kangarlu, A., Burgess, R. E., Zhu, H., Nakayama, T., Hamlin, R. L., Abduljalil, A. M., & Robitaille, P. M. (1999). Cognitive, cardiac, and physiological safety studies in ultra high field magnetic resonance imaging. *Magnetic Resonance Imaging, 17*, 1407–1416.

Kuipers, N. T., Sauder, C. L., & Ray, C. A. (2006). Influence of static magnetic fields on pain perception and sympathetic nerve activity in humans. *Journal of Applied Physiology*, *102*, 1410–1415.

Kuribayashi, M., Wang, J., Fujiwara, O., Doi, Y., Nabae, K., Tamano, S., Ogiso, T., Asamoto, M., & Shirai, T. (2005). Lack of effects of 1439 MHz electromagnetic near field exposure on the blood-brain barrier in immature and young rats. *Bioelectromagnetics, 26*, 578–588.

Markov, M. S., Williams, C. D., Cameron, I. L., Hardman, W. E., & Salvatore, J. R. (2004) Can magnetic fields inhibit angiogenesis and tumor growth? In P. J. Rosch, & M. S. Markov (Eds.), *Bioelectromagnetic medicine* (pp. 625–636). NY: Marcel Dekker Inc.

Martel, G. F., Andrews, S. C., & Roseboom, C. G. (2002). Comparison of static and placebo magnets on resting forearm blood flow in young, healthy men. *The Journal of Orthopaedic and Sports Physical Therapy, 32*, 518–524.

Masuda, H., Ushiyama, A., Okano, H., & Ohkubo, C. (2000). Chronological observation of the pial microcirculation using a chronically implanted cranial window method in the rat. In M. Tsuchiya, M. Asano, T. Tokioka, & K. Takahashi (Eds.), *Microcirculation annual 2000* (pp.151–152). Tokyo: Nihon-Igakukan.

Masuda H., Hirota S., Ushiyama A., Wake K., Watanabe S., Taki M., & Ohkubo, C. (2003). In vivo evaluation of exposure effects to radio-frequency electromagnetic fields on the cerebral circulation in rats. *Abstract book of the Blood-Brain Barrier (BBB) Workshop of COST281-Potential Health Implications From Mobile Communication Systems*. Reisensburg, Germany, pp. 28–29.

Masuda H., Ushiyama A., Wake K., Watanabe S., Taki M., & Ohkubo C. (2001a). Acute effects of local exposure to radio-frequency electromagnetic fields on the cerebral microcirculation in rats. *The 23rd Annual Meeting of Bioelectromagnetic Society Abstract Book*, pp.139–140.

Masuda H., Ushiyama A., Wake K., Watanabe S., Taki M., & Ohkubo C. (2001b). Chronic effects of local exposure to radiofrequency electromagnetic fields on the cerebral microcirculation in rats. *2001 Asia-Pacific Radio Science Conference Digest*, p.410.

Mayhan, W. G. (1998). Effect of lipopolysaccharide on the permeability and reactivity of the cerebral microcirculation: Role of inducible nitric oxide synthase. *Brain Research, 792*, 353–357.

Mayhan, W. G. (2000). Leukocyte adherence contributes to disruption of the blood-brain barrier during activation of mast cells. *Brain Research, 869*, 112–120.

Mayrovitz, H. N., Groseclose, E. E., & King, D. (2005). No effect of 85 mT permanent magnets on Laser-Doppler measured blood flow response to inspiratory gasps. *Bioelectromagnetics, 26*, 331–335.

Mayrovitz, H. N., Groseclose, E. E., Markov, M., & Pilla, A. A. (2001). Effects of permanent magnets on resting skin blood perfusion in healthy persons assessed by Laser Doppler Flowmetry and Imaging. *Bioelectromagnetics, 22*, 494–502.

McKay, J. C., Prato, F. S., & Thomas, A. W. (2007). A literature review: The effects of magnetic field exposure on blood flow and blood vessels in the microvasculature. *Bioelectromagnetics, 28*, 81–98.

McLean, M. J., Holcomb, R. R., Wamil, A. W., Pickett, J. D., & Cavopol, A. V. (1995). Blockade of sensory neuron action potentials by a static magnetic field in the 10 mT range. *Bioelectromagnetics, 16*, 20–32.

Morris, C., & Skalak, T. (2005) Static magnetic fields alter arteriolar tone in vivo. *Bioelectromagnetics, 26*, 1–9.

Mulvany, M. J. (1983). Functional characteristics of vascular smooth muscle. In K. Messemer, & F. Hammersen (Eds.) *Progress in applied microcirculation* (Vol. 3, pp. 4–18). Basel: Karger.

Ohkubo, C., & Okano, H. (2004). Static magnetic field and microcirculation. In P. J. Rosch, & M. S. Markov (Eds.), *Bioelectromagnetic medicine*, (pp. 563–591). NY: Marcel Dekker Inc.

Ohkubo, C., & Xu, S. (1997). Acute effects of static magnetic fields on cutaneous microcirculation in rabbits. *In Vivo, 11*, 221–225.

Okano, H., Gmitrov, J., & Ohkubo, C. (1999). Biphasic effects of static magnetic fields on cutaneous microcirculation in rabbits. *Bioelectromagnetics, 20*, 161–171.

Okano, H., Masuda, H., & Ohkubo, C. (2005a). Effects of 25 mT static magnetic field on blood pressure in reserpine-induced hypotensive wistar-kyoto rats. *Bioelectromagnetics, 26*, 36–48.

Okano, H., Masuda, H., & Ohkubo, C. (2005b). Decreased plasma levels of nitric oxide metabolites, angiotensin II and aldosterone in spontaneously hypertensive rats exposed to 5 mT static magnetic field. *Bioelectromagnetics, 26*, 161–172.

Okano, H., & Ohkubo, C. (2001). Modulatory effects of static magnetic fields on blood pressure in rabbits. *Bioelectromagnetics, 22*, 408–418.

Okano, H., & Ohkubo, C. (2003a). Anti-pressor effects of whole-body exposure to static magnetic field on pharmacologically induced hypertension in conscious rabbits. *Bioelectromagnetics, 24*, 139–147.

Okano, H., & Ohkubo, C. (2003b). Effects of static magnetic fields on plasma levels of angiotensin II and aldosterone associated with arterial blood pressure in genetically hypertensive rats. *Bioelectromagnetics, 24*, 403–412.

Okano, H., & Ohkubo, C. (2005a). Effects of neck exposure to 5.5 mT static magnetic field on pharmacologically modulated blood pressure in conscious rabbits. *Bioelectromagnetics, 26*, 469–480.

Okano, H., & Ohkubo, C. (2005b). Exposure to a moderate-intensity static magnetic field enhances the hypotensive effect of a calcium channel blocker in spontaneously hypertensive rats. *Bioelectromagnetics, 26*, 611–623.

Okano, H., & Ohkubo, C. (2006). Elevated plasma nitric oxide metabolites in hypertension: Synergistic vasodepressor effects of a static magnetic field and nicardipine in spontaneously hypertensive rats. *Clinical Hemorheology and Microcirculation, 34*, 303–308. .

Okano, H., & Ohkubo, C. (2007). Effects of 12 mT static magnetic field on sympathetic agonist-induced hypertension and hemodynamic changes in wistar rats. *Bioelectromagnetics*, *28*, 369–378.

Prato, F. S., Wills, J. M., Roger, J., Frappier, H., Drost, D. J., Lee, T. Y., Shivers, R. R., & Zabel, P. (1994). Blood-brain barrier permeability in rats is altered by exposure to magnetic fields associated with magnetic resonance imaging at 1.5 T. *Microscopy Research and Technique, 27*, 528–534.

Salford, L. G., Brun, A. E., Eberhardt, J. L., Malmgren, L., & Persson, B. R. (2003) Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. *Environ Health Perspect, 111*, 881–883, discussion A408.

Salford, L. G., Brun, A., Sturesson, K., Eberhardt, J. L., & Persson, B. R. (1994). Permeability of the blood-brain barrier induced by 915 MHz electromagnetic radiation, continuous wave and modulated at 8, 16, 50, and 200 Hz. *Microscopy Research and Technique, 27*, 535–542.

Saunders, R. (2005). Static magnetic fields: Animal studies. *Progress in Biophysics and Molecular Biology, 87*, 225–239.

Schirmacher, A., Winters, S., Fischer, S., Goeke, J., Galla, H. J., Kullnick, U., Ringelstein, E. B., & Stogbauer F. (2000). Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the blood-brain barrier in vitro. *Bioelectromagnetics, 21*, 338–345.

Sharma, H.S., & Hoopes, P. J. (2003). Hyperthermia induced pathophysiology of the central nervous system. *International Journal of Hyperthermia, 19*, 325–354.

Shrivastav, S., Kaelin, W. G. Jr., Joines, W. T., & Jirtle, R. L. (1983). Microwave hyperthermia and its effect on tumor blood flow in rats. *Cancer Research, 43*, 4665–4669.

Steyn, P. F., Ramey, D. W., Kirschvink, J., & Uhrig, J. (2000). Effect of a static magnetic field on blood flow to the metacarpus in horses. *Journal of the American Veterinary Medical Association, 217*, 874–877.

Tenforde, T. S. (2005). Magnetically induced electric fields and currents in the circulatory system. *Progress in Biophysics and Molecular Biology, 87*, 279–288.

Tsurita, G., Nagawa, H., Ueno, S., Watanabe, S., & Taki, M. (2000). Biological and morphological effects on the brain after exposure of rats to a 1439 MHz TDMA field. *Bioelectromagnetics, 21*, 364–371.

Ushiyama, A., Masuda, H., & Ohkubo, C. (2003) Effects of subchronic exposure to extremely low frequency electromagnetic fields on tumor growth and angiogenesis in the mouse cranial window. In M. Asano, & S. Miura (Eds.), *Microcirculation annual 2003* (pp. 101–102). Tokyo: Nihon-Igakukan.

Ushiyama, A., Yamada, S., & Ohkubo, C. (2004a). Microcirculatory parameters measured in subcutaneous tissue of the mouse using a novel dorsal skinfold chamber. *Microvascular Research, 68*, 147–152.

Ushiyama, A., Masuda, H., Hirota, S., & Ohkubo, C. (2004b). Subchronic effects on leukocyte-endothelial interactions in mice by whole body exposure to extremely low frequency electromagnetic fields. *In Vivo 18*, 425–432.

Ushiyama, A., & Ohkubo, C. (2004) Acute effects of low-frequency electromagnetic fields on leukocyte-endothelial interactions in vivo. *In Vivo, 18*, 125–132.

Waldman, W. J., & Knight, D. A. (1996). Cytokine-mediated induction of endothelial adhesion molecule and histocompatibility leukocyte antigen expression by cytomegalovirus-activated T cells. *The American Journal of Pathology, 148*, 105–119.

Xu, S, Okano, H., & Ohkubo, C. (1998) Subchronic effects of static magnetic fields on cutaneous microcirculation in rabbits. *In Vivo, 12*, 383–389.

Xu, S., Okano, H., & Ohkubo, C. (2000). 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.

Yuan, H., Gaber, M. W., McColgan, T., Naimark, M. D., Kiani, M. F., & Merchant, T. E. (2003). Radiation-induced permeability and leukocyte adhesion in the rat blood-brain barrier: Modulation with anti-ICAM-1 antibodies. *Brain Research, 969*, 59–69.