8 Magnetic Field Influences on the Microcirculation

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CONTENTS

8.1 INTRODUCTION

Microcirculation consists of structurally and functionally differentiated small blood vessels: small muscular arteries, arterioles, metarterioles, capillaries, postcapillary venules, venules, and lymphatic capillaries (reviewed by Ohkubo and Okano, 2004). 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 Brånemark, 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).

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 (reviewed by WHO, 2006). 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. Furthermore, when taken with the presence of water channels, aquaporins in the continuous endothelium of microvascular beds, it is most likely that these molecules are responsible for the exclusive water pathways through the walls of all microvessels with continuous endothelium (Michel and Curry, 1999).

For evaluating the effects of magnetic fields including static magnetic fields (SMFs) and electromagnetic fields (EMFs) on microcirculatory system, various microcirculatory preparations, for example, rabbit ear chamber (REC) (Asano et al., 1965; Okano et al., 1999; Gmitrov and Ohkubo, 2002a,b; Gmitrov et al., 2002); dorsal skinfold chamber (DSC) in mice (Ushiyama and Ohkubo, 2004; Ushiyama et al., 2004a,b; Traikov et al., 2005, 2006; Morris and Skalak, 2007), rats (Morris and Skalak, 2005), and hamsters (Brix et al., 2008; Strieth et al., 2008; Strelczyk et al., 2009; Gellrich et al., 2014); and cranial window (CW) in rats (Masuda et al., 2007a–c, 2009, 2011), have been used to observe and analyze microcirculation. These preparations allow noninvasive, continuous measurement of hemodynamics, blood velocity, angiogenesis, and metabolites, for example, pH and pO_2, transport of molecules and particles, and cell-to-cell interactions in vivo (Jain, 1997).

Effects of magnetic fields on the circulatory system have been reviewed in experimental animals (Ohkubo and Okano, 2004, 2011; Saunders, 2005; Tenforde, 2005; WHO, 2006; McKay et al., 2007; Ohkubo et al., 2007; Robertson et al., 2007; Nittby et al., 2008; Ruggiero, 2008; Funk et al., 2009; McNamee et al., 2009; Ohkubo and Okano, 2011; Yu and Shang, 2014) and in humans (Jauchem, 1997; Chakeres and de Vocht, 2005; Crozier and Liu, 2005; van Rongen, 2005; McKay et al., 2007; Robertson et al., 2007; Funk et al., 2009; McNamee et al., 2009; Jokela and Saunders, 2011; Ohkubo and Okano, 2011), and possible interpretations of the effects have been discussed using theoretical models (Tenforde et al., 1983; Kinouchi et al., 1996; ICNIRP, 1998, 2009, 2010, 2014; Luo et al., 2005; Tenforde, 2005; Kainz et al., 2010). There are many therapeutic applications of locally increased blood flow. It has been suggested that magnetic fields could have the potential to modify microcirculatory perfusion. Regarding the effects of magnetic fields on microcirculation and microvasculature, McKay et al. (2007) reviewed that nearly half of the cited experiments (10/27) are related to either a vasodilatory effect, increased blood flow, or increased blood pressure (BP). Conversely, 3 of the 27 studies reported a decrease in blood perfusion/pressure. Four studies reported no effect. The remaining 10 studies found that magnetic fields could trigger either vasodilation or vasoconstriction depending on the initial tone of the vessel. In terms of cellular effects of magnetic fields related to perfusion, 4 of a total of 19 studies reported an increase in nitric oxide (NO) activity from magnetic field exposures (one of these studies used a model with an altered vessel state prior to exposure), 1 found a bidirectional effect, and 5 found no effect. Nine studies reported vascular development effects (seven reported increased angiogenesis; two reported decreased angiogenesis). Other cellular effects were reported in three studies.

8.2 STATIC MAGNETIC FIELDS

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 BP (reviewed by Ohkubo and Okano, 2004, 2011). There are many therapeutic applications for locally increased blood flow. It is suggested that magnetic fields have the

potential to modify microcirculatory perfusion. However, there might be safety concerns on magnetic resonance imaging (MRI) and magnetic levitation for transportation using strong-intensity SMF with Tesla (T) levels (reviewed by Silva et al., 2006). 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 and Ohkubo, 2002a,b; Gmitrov et al., 2002). BP in a central artery contralateral to that of an ear lobe having the REC, fixed on the microscope stage, was monitored by a BP monitoring system.

Using the REC methods, Ohkubo and Xu (1997) firstly demonstrated that cutaneous microcirculation was modulated by moderate-intensity SMF with millitesla (mT) levels: the bidirectional effects of a 1, 5, and 10 mT SMF on cutaneous microcirculation were found in conscious rabbits. 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 bidirectional effects (activation/inhibition) of 10 min exposure to 1.0 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 (ACh) to cause vasodilation, and 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 earlier were performed at Department of Environmental Health (the 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: an 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., 1990, 1994). Prato et al. (1990) reported that exposure to low-field (0.15 T, for 23.2 min) MRI increases BBB permeability. In addition, Prato et al. (1994) found that exposures to SMF alone for 22.5 min, without radio frequency (RF)-specific absorption rate (SAR) and temporal gradient, increased BBB permeability at 1.5 and 1.89 T. In contrast, Yamaguchi-Sekino et al. (2012) obtained the following results: acute exposure (up to 3 h) to ultrahigh magnetic field (17.2 T generated by an MRI system) does not alter BBB permeability in rats. However, so far, there has been no explanation available for these inconsistent results of BBB alteration between different intensities of SMF.

In another aspect, previously, researches have shown that acute exposure (30–40 min) to SMF (30–223 mT) increased skin vasomotion amplitude (Li et al., 2007; Yan et al., 2011). In resting skin blood flow in healthy young men, Yan et al. (2011) found that SMF exposure at the maximum

magnetic flux density B_{max} of 223 mT for 30 min induced a significant increase in vasomotion amplitude, mainly reflecting the intrinsic myogenic and endothelial-related metabolic activities, by placing a magnet to the center of the middle finger prominence, and, after removal of the SMF, the vasomotion amplitude vanished gradually. In an animal (rat) model, Li et al. (2007) reported significant enhancement of vasomotion amplitude, mainly reflecting the endothelial-related metabolic activity (0.01–0.05 Hz) in the skin stressed by pressure loading over the trochanter area upon exposure to an SMF at B_{max} of 30 mT for 40 min. In their study, prolonged surface loading caused significant reduction of the endothelial-related metabolic activity and increased the myogenic activity; that is, it induced a higher vascular tone in tissues that had been stressed as compared with the unstressed ones (Li et al., 2007). In contrast, SMF significantly increased the endothelial-dependent vasodilation and subsequently increased blood flow in the stressed skin (Li et al., 2007). The modulating effect of SMF on the vasomotion amplitude might be related to the vascular tone modified by prolonged compressive loading (Li et al., 2007).

In contrast to the aforementioned studies (Li et al., 2007; Yan et al., 2011), Xu et al. (2013) focused on examining the subchronic effects of moderate-intensity inhomogeneous SMF on peripheral hemodynamics. Their study indicated that SMF exposure of B_{max} 160 mT for 3 weeks seems to have tendency to modulate the vasomotion amplitude at 0.05 Hz in the range of endothelial-related metabolic activity for rats and contract the increased vasomotion amplitude in the ischemic area, but did not induce significant change in any one of these parameters during the SMF exposure period of 3–7 weeks investigated. These results suggest that SMF may have a regulatory effect on rhythmic vasomotion in the ischemic area by smoothing the vasomotion amplitude, mainly reflecting the endothelial-related metabolic activity, in the early stage of the wound healing process. The physiological implication is that smoothing or buffering the vasomotion amplitude may play a key role on inherent hemodynamic control mechanisms for rhythmic vasomotion and endothelial-dependent vasodilation.

In the context of angiogenesis, Ruggiero et al. (2004) reported that 3 h exposure to 0.2 T SMF did not apparently affect the basal pattern of vascularization or chick embryo viability using the chick embryo chorioallantoic membrane (CAM) assay. Prostaglandin E_1 and fetal calf serum elicited a strong angiogenic response in sham-exposed eggs. This angiogenic response was significantly inhibited by 3 h exposure to 0.2 T SMF. These findings point to possible use of SMF in inhibiting angiogenesis. Preclinical studies examining the inhibition of angiogenesis by SMF exposure have turned to the use of SMF in the treatment of cancer in terms of tumor angiogenesis. Brix et al. (2008) evaluated SMF effects on capillary flow of red blood cells (RBCs) in unanesthetized Syrian golden hamsters, using a DSC technique for intravital fluorescence microscopy. Capillary RBC velocities (v_{RBC}), capillary diameters (D), arteriolar diameters (D_{art}), and functional vessel densities (FVDs) were measured in striated skin muscle at different magnetic flux densities. Exposure above 500 mT for 1 h resulted in a significant reduction of v_{RBC} in capillaries compared with the baseline value. At B_{max} of 587 mT, v_{RBC} was reduced (40%). Flow reduction was reversible when the field strength was decreased below the threshold level. In contrast, mean values determined at different exposure levels for the parameters D, D_{art}, and FVD did not vary (5%). Blood flow through capillary networks is affected by SMF directed perpendicular to the vessels.

The same research group further analyzed the effects of SMF (≤587 mT) on tumor microcirculation (Strieth et al., 2008). In vivo fluorescence microscopy was performed in A-Mel-3 tumors growing in DSC preparations of hamsters. Short time exposure (≥150 mT) resulted in a significant reduction of v_{RBC} and segmental blood flow in tumor microvessels. At B_{max} of 587 mT, a reversible reduction of v_{RBC} (40%) and of FVD (15%) was observed. Prolongation of the exposure time (1 min to 3 h) resulted in reductions. Microvessel diameters and leukocyte–endothelial cell interactions remained unaffected by SMF exposures. However, in contrast to tumor-free striated muscle controls, exposure at B_{max} of 587 mT induced a significant increase in platelet–endothelial cell adherence in a time-dependent manner that was reversible after reducing the strength of the SMF.

The authors assumed that these reversible changes may have implications for functional measurements of tumor microcirculation by MRI and new therapeutic strategies using strong SMF.

The same research group further evaluated the effects of an SMF (586 mT, for 3 h) on tumor angiogenesis and growth (Strelczyk et al., 2009). The analysis of microcirculatory parameters revealed a significant reduction of parameters, FVD, D, and v_{RBC}, in tumors after SMF exposure compared with the control tumors. These changes reflect retarded vessel maturation by antiangiogenesis. The increased edema after SMF exposure indicated an increased tumor microvessel leakiness possibly enhancing drug uptake.

In addition, combining SMF exposure (587 mT, for 2–3 h) with paclitaxel chemotherapy, tumor growth was analyzed (Gellrich et al., 2014). SMF inhibited tumor angiogenesis and increased tumor microvessel permeability significantly. This was not mediated by inflammatory leukocyte–endothelial cell interactions. Further, SMF increased the effectiveness of paclitaxel chemotherapy significantly. These findings support that SMF possibly open the blood–tumor barrier to small molecular therapeutics (Brix et al., 2008; Strieth et al., 2008; Strelczyk et al., 2009; Gellrich et al., 2014).

Atef et al. (1995) showed that the kinetics of oxyhemoglobin auto-oxidation decreased in the auto-oxidation reaction rate of 2%–5.9% and 10%–17%, under the SMF exposure of 100–250 and 350–400 mT, respectively. Djordjevich et al. (2012) indicated that subchronic continuous exposure to 16 mT SMF for 28 days caused lymphocyte and granulocyte redistribution between the spleen and blood in mice. These results suggested that observed changes were not due to an unspecific stress response, but that they were rather caused by specific adaptation to subchronic SMF exposure.

In another aspect of SMF effects on erythrocytes, Lin et al. (2013a,b) found that 0.8 T SMF coupled with the slow cooling procedure increased survival rates of frozen–thawed human erythrocytes without any negative effects on cell morphology or function. They suggest that the SMF cryoprotective effect is due to enhanced biophysical stability of the cell membrane, which reduces dehydration damage to the erythrocyte membrane during the slow cooling procedure.

As for clinical trials, Kim et al. (2010) performed the following experiment using single photon emission computed tomography (SPECT): the permanent magnet (0.3 T, unipolar, disk shaped, 4 cm diameter, and 1 cm thick) was placed on the right frontotemporal region of the brain for 20 min for each of 14 healthy subjects. Technetium (Tc)-99m ethylcysteinate dimer (ECD) perfusion SPECT was taken to compare the cerebral blood flow (CBF) patterns in the subjects exposed to the SMF with those of the resting and sham conditions. They found that the regional CBF (rCBF) was significantly increased in the right frontal and parietal regions and also the right insula. In contrast, rCBF was rather decreased in the left frontal and left parietal regions. These results suggested that 0.3 T SMF induces an increase in rCBF in the targeted brain areas noninvasively, which may result from a decrease in rCBF in contralateral regions.

Wang et al. (2009a) investigated the effects of gradient SMF (0.2–0.4 T, 2.09 T/m, 1–11 days) on angiogenesis both in vitro and in vivo. An 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used as an in vitro method to detect the proliferation ability of human umbilical vein endothelial cells (HUVECs). Two kinds of in vivo models, a chick CAM and a matrigel plug, were used to detect the effects of gradient SMF on angiogenesis. The results showed that the proliferation ability of HUVEC was significantly inhibited 24 h after the onset of exposure. With regard to the CAM model, vascular numbers in the CAM that was continuously exposed to the gradient SMF were all less than those in normal condition. In accordance with the gross appearance, the contents of hemoglobin (Hb) in the models exposed to gradient SMF for 7–9 days were also less. In addition, similar to the CAM model, the results of vascular density and Hb contents in the mouse matrigel plug also demonstrated that the gradient SMF exposure for 7–11 days inhibited vascularization. These findings indicate that gradient SMF might inhibit or prevent new blood vessel formation and could be helpful for the treatment of some diseases relevant to pathological angiogenesis.

Other reports have shown that an SMF of strong intensity higher than a few Tesla has bioeffects (Tenforde et al., 1983; Higashi et al., 1993, 1995; Shiga et al., 1993; Denegre et al., 1998;

Ichioka et al., 2000, 2003; Snyder et al., 2000; Haik et al., 2001; Kotani et al., 2002; Zborowski et al., 2003; Yamamoto et al., 2004; Valiron et al., 2005; Hsieh et al., 2008; ICNIRP, 2009, 2014; Tao and Huang, 2011; Mian et al., 2013; Glover et al., 2014; Theysohn et al., 2014). For instance, an SMF of strong intensity with an extremely high magnetic gradient (B_{max} of 8 T, the maximum gradient G_{max} of 400 T^2/m) could induce some bioeffects on paramagnetic Hb by magnetic attraction in a high gradient or diamagnetic Hb by magnetic repulsion in a high gradient, retarding the mean blood velocity in peripheral circulation, partly due to the asymmetric distribution of RBCs with different magnetic susceptibilities, and magnetically induced movement of diamagnetic water vapor at the skin surface, which may lead to a skin temperature decrease (Ichioka et al., 2003). Moreover, it has been shown that RBCs rotate and orient so that the concave surface is aligned parallel to strong uniform SMF due to magnetic torque (Higashi et al., 1993). From a rheological point of view, concerning the SMF effects on blood viscosity, different results were obtained, depending on the exposure conditions (Haik et al., 2001; Yamamoto et al., 2004; Tao and Huang, 2011). Haik et al. (2001) reported an increase in viscosity of blood flowing parallel to inhomogeneous strong SMF at B_{max} of 3, 5, and 10 T. Yamamoto et al. (2004) also indicated an increased viscosity of both fully oxygenated and fully deoxygenated blood and greater increase in blood viscosity of deoxygenated blood relative to that of oxygenated blood in 1.5 T homogeneous SMF exposure for 36 min. In contrast, Tao and Huang (2011) demonstrated that acute exposure to 1.33 T homogeneous SMF reduced blood viscosity when it was applied parallel to the flow direction (for an exposure duration of 1 min, short chains of RBC were formed; for an exposure duration of 12 min, long cluster chains of RBC were formed) (Tao and Huang, 2011). This process could enable the RBC to pass through the blood vessels in a more streamlined fashion, thereby reducing the blood viscosity (Tao and Huang, 2011).

These observations suggested that both inhomogeneous and homogeneous high-intensity SMFs (Tesla level) may modulate in vivo hemodynamics. However, the underlying mechanisms and physiological consequences have not yet been fully understood. Because our applied SMF at B_{max} of 160 mT was much lower in the magnetic force compared with the SMF of several Tesla, different plausible mechanisms might exist between them, such as through a Ca^{2+}–calmodulin-regulated NO–cGMP signaling pathway. Takeshige and Sato (1996) suggested a mechanism of SMF action for the promotion of hemodynamic responses in which an SMF at B_{max} of 130 mT might inhibit acetylcholinesterase (AChE). The recovery of circulation is assumed to be partly due to the enhanced release of ACh by the SMF exposure, activating the cholinergic vasodilator nerve endings innervated to the muscle artery (Takeshige and Sato, 1996). The inhibitory effect of SMF on AChE was also observed in the magnetic flux density of 0.8 mT or more (Ravera et al., 2010). In addition, it is also suggested that an SMF at B_{max} of 5.5 mT should have a potential to counteract the action of a nitric oxide synthase (NOS) inhibitor L-NAME, presumably via increased endogenous ACh release (Okano and Ohkubo, 2005b, 2006). The increased (upregulated) effect of a 120 μT SMF on endothelial nitric oxide synthase (eNOS) expression was also confirmed in HUVEC (Martino et al., 2010).

Muehsam et al. (2013) found that exposure for 10–30 min to SMF of B_{max} 186 mT resulted in more rapid Hb deoxygenation, using a reducing agent dithiothreitol in an in vitro cell-free preparation. Thus, SMF significantly increased the rate of Hb deoxygenation occurring several minutes to several hours after the end of SMF exposure (Muehsam et al., 2013). The observation that SMF pretreatment of Hb alone, or of the deoxygenation solution itself, failed to yield a significant effect suggests that SMF exposure acted upon the interaction of Hb with the deoxygenation solution (Muehsam et al., 2013). With regard to the mechanism, they speculated that these SMF modalities modified protein/solvation structure in a manner that altered the energy required for deoxygenation (Muehsam et al., 2013). They further speculated that the mT-range SMF could induce the action of the Lorentz force on charges bound at the protein/water interface based on the Lorentz–Langevin model for weak magnetic field bioeffects (Muehsam and Pilla, 2009a,b). This model suggested that weak exogenous ac/dc magnetic fields can act on an ion/ligand bound in a molecular cleft, based upon the assumption that the receptor molecule is able to detect the Larmor trajectory of an ion or ligand within the binding site. To date, however, there is insufficient direct experimental evidence

pertaining to this model. Further studies are required to better understand the mechanisms of SMF bioeffects on hemodynamic function. Spectral analysis would be useful to examine these effects in more detail with a view of hemodynamics.

8.2.1 Discussion and Conclusion

In summary, significant circulatory system responses to SMF have been recently reviewed in experimental animals and/or humans: SMF exposures between 1.0 and 8 T for anywhere between 10 min and 12 weeks can influence cutaneous microcirculation, hemodynamics, arterial BP, and/or angiogenesis in vivo (Ohkubo and Okano, 2004, 2011; Chakeres and de Vocht, 2005; Crozier and Liu, 2005; Saunders, 2005; Tenforde, 2005; van Rongen, 2005; WHO, 2006; McKay et al., 2007; Ohkubo et al., 2007; Robertson et al., 2007; Ruggiero, 2008; Funk et al., 2009; McNamee et al., 2009; Ohkubo and Okano, 2011; Yu and Shang, 2014).

Ten of a total of 38 studies in the last two decades report either an increase in blood flow, an elevation in BP, or increased angiogenesis. In contrast, 14 of the 38 studies indicate either a decrease in blood flow, a reduction in BP, or reduced angiogenesis. Eight studies report no effect. The remaining six studies found that SMF exposures could trigger either vasodilation or vasoconstriction depending on the initial tone of the vessel, which induce bidirectional effect. For a summary, please refer to Table 8.1.

In particular, a series of studies, as shown in Table 8.1, have demonstrated that cutaneous microcirculation, hemodynamics, and/or arterial BP 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, it was concluded that significant bioresponses to therapeutic signals occur when the state of the target is far from the homeostasis or the equilibrium of hemodynamics (Ohkubo and Okano, 2004).

In the context of neoplasia, Strelczyk group reported that SMF reduces RBC velocity and increases platelet adhesion without affecting the leukocytes or smooth muscle cells of the vessel walls, as demonstrated by the lack of changes in mean arterial BP and vessel diameters (Brix et al., 2008; Strieth et al., 2008; Strelczyk et al., 2009; Gellrich et al., 2014). These findings support that SMF possibly open the blood–tumor barrier to small molecular therapeutics (Brix et al., 2008; Strieth et al., 2008; Strelczyk et al., 2009; Gellrich et al., 2014).

The mechanisms of moderate-intensity SMF effects could be mediated by suppressing or enhancing the action of biochemical effectors, thereby inducing homeostatic effects bidirectionally. The potent mechanisms of SMF effects have often been linked to 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). In addition, moderate-intensity SMF exposures have been reported to perturb distribution of membrane proteins and glycoproteins, receptors, cytoskeleton, and transmembrane fluxes of different ions, especially calcium $[Ca^{2+}]_i$, that, in turn, interfere with many different physiological activities, including phagocytosis (Dini, 2010). Wang et al. (2009b) reported that over time periods of many hours to several days, moderate-intensity SMF-initiated changes to Ca^{2+} can modulate signaling pathways, leading to significant changes in gene expression, cell behavior, and phenotype. Furthermore, Wang et al. (2010) revealed that moderate-intensity SMF reproduced several responses elicited by ZM241385, a selective $A_{2A}R$ antagonist and also a Parkinson's disease (PD) drug candidate, in PC12 cells including altered Ca^{2+} flux, increased ATP levels, reduced cAMP levels, reduced NO production, reduced p44/42 mitogen-activated protein kinase (MAPK) phosphorylation, inhibited proliferation, and reduced iron uptake. SMF also counteracted several PD-relevant endpoints exacerbated by $A_{2A}R$ agonist CGS21680 in a manner similar to ZM241385; these include reduction of increased expression of $A_{2A}R$, reversal of altered Ca^{2+} efflux, dampening of increased adenosine production, reduction of enhanced proliferation and associated p44/42 MAPK phosphorylation, and inhibition of neurite outgrowth (Wang et al., 2010).

TABLE 8.1
Summary of SMF Effects on Hemodynamics, BP, and/or Angiogenesis In Vivo

Increased Effect (*n* = 10)	**Exposure Conditions: Intensity; Duration; Subjects**
Prato et al. (1990)	0.15 T; 23.2 min; rats (BBB)
Prato et al. (1994)	1.5 and 1.89 T; 22.5 min; rats (BBB)
Xu et al. (1998)	180 mT; 1–3 weeks; rabbits (REC)*
Xu et al. (2001)	1.0 and 10.0 mT; 10 min; mice
Gmitrov (2002)	250 mT; 40 min; rabbits (REC)
Gmitrov and Ohkubo (2002a)	250 mT; 40 min; rabbits (REC)
Gmitrov and Ohkubo (2002b)	250 mT; 40 min; rabbits (REC)
Okano et al. (2005a)[a]	25 mT; 2–12 weeks; rats*
Li et al. (2007)	30 mT; 40 min; rats*
Yan et al. (2011)	223 mT; 30 min; humans*
Decreased Effect (*n* = 14)	**Exposure Conditions: Intensity; Duration; Subjects**
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)[c]	10 and 25 mT; 2–8 weeks; SHR*
Okano and Ohkubo (2005b)[c]	180 mT; 5–8 weeks; SHR*
Okano and Ohkubo (2006)[c]	180 mT; 6 weeks; SHR*
Okano et al. (2005b)[c]	5 mT; 2–8 weeks; SHR*
Okano and Ohkubo (2007)[b]	12 mT; 2–10 weeks; rats*
Brix et al. (2008)[d]	538 and 587 mT; 1 h; hamsters (DSC)*
Strieth et al. (2008)[d]	149–587 mT; 3 h; hamsters (DSC)*
Strelczyk et al. (2009)[d]	587 mT; 3 h; hamsters (DSC)*
Ruggiero et al. (2004)	200 mT; 3 h; chick embryo*
Wang et al. (2009a,b)	400 mT; 7–11 days; mouse* and chick embryo*
Mayrovitz and Groseclose (2005)	400 mT; 45 min; humans*
Bidirectional Effect (*n* = 6)	**Exposure Conditions: Intensity; Duration; Subjects**
Ohkubo and Xu (1997)	1, 5, and 10 mT; 10 min; rabbits (REC)*
Okano et al. (1999)	1 mT; 10 min; rabbits (REC)*
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 (DSC)
Kim et al. (2010)	300 mT; 20 min; humans*
No Effect (*n* = 8)	**Exposure Conditions: Intensity; Duration; Subjects**
Yamaguchi-Sekino et al. (2012)	17.2 T; 3 h; rats (BBB)
Kangarlu et al. (1999)	8 T; 1 h for humans*; 3 h for pigs*
Steyn et al. (2000)	27 mT; 46 h; horses*
Kuipers et al. (2007)	60 mT; 1 h; humans*
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*

BBB, blood–brain barrier; REC, rabbit ear chamber; DSC, dorsal skinfold chamber.

[a] Initial state of subject: pharmacologically induced hypotension.

[b] Initial state of subject: pharmacologically induced hypertension.

[c] Initial state of subject: spontaneous hypertensive rats (SHR).

[d] Tumor microvessels.

* Conscious conditions (without anesthesia).

Csillag et al. (2014) investigated that effects of SMF exposure on a murine model of allergic inflammation and also on human provoked skin allergy. Inhomogeneous SMF was generated with an apparatus validated previously, providing a peak-to-peak magnetic induction of the dominant SMF component 389 mT by 39 T/m lateral gradient in the in vivo and in vitro experiments and 192 mT by 19 T/m in the human study at the 3 mm target distance. They found that even a single 30 min exposure of mice to SMF immediately following intranasal ragweed pollen extract (RWPE) challenge significantly lowered the increase in the total antioxidant capacity of the airways and decreased allergic inflammation. Repeated (on 3 consecutive days) or prolonged (60 min) exposure to SMF after RWPE challenge decreased the severity of allergic responses more efficiently than a single 30 min treatment. SMF exposure did not alter reactive oxygen species (ROS) production by RWPE under cell-free conditions, while diminished RWPE induced increase in the ROS levels in A549 epithelial cells. Results of the human skin prick tests indicated that SMF exposure had no significant direct effect on provoked mast cell degranulation. The observed beneficial effects of SMF are likely owing to the mobilization of cellular ROS-eliminating mechanisms rather than direct modulation of ROS production by pollen NAD(P)H oxidases.

Ruggiero (2008) proposed that some proteins might not be the sole candidates for this role of sensory molecules: large and complex polymers like glycosaminoglycans, which show a precise array of electric charges on their surface, as well as small second messengers with stereospecific positioning of charges such as inositol 1,4,5-trisphosphate, might play a direct or indirect role in the cell response to SMF. Ruggiero (2008) further speculated that some genes are sensitive to SMF and others to variable EMF. Ruggiero (2008) termed these genes as *magnetic-sensitivity-conferring genes* and proposed that an approach should first identify which genes are expressed in magnetic field–sensitive cells, but not in nonsensitive cells.

Further study concerning the magnetic-sensitivity-conferring genes is an intriguing areas of research and also shed light on the signal transduction and biophysical mechanisms of the magnetic sense in animals (Ritz et al., 2004; Rodgers et al., 2009; Yoshii et al., 2009; Gegear et al., 2010; Nishimura et al., 2010; Eder et al., 2012; Maeda et al., 2012; Stoneham et al., 2012; Wiltschko and Wiltschko, 2012; Dodson et al., 2013; Lee et al., 2014; Neil et al., 2014; Solov'yov et al., 2014) and humans (Foley et al., 2011). In particular, recent studies on the effects of magnetic field on the biological clock through the radical pair mechanism have been in progress in the relatively weak magnetic fields less than 1 mT (Yoshii et al., 2009). Furthermore, studies on the subtle effects of RF–EMFs have been recently focused on the mechanism of spin biochemistry (Usselman et al., 2014). Technological advances in spin biochemistry will enable to understand the influence of various kinds of magnetic fields on biological systems.

One of the other established physical mechanisms is *magnetic induction* (van Rongen et al., 2007) in relatively high intensity of SMF over 1 T generating from MRI devices. This mechanism originates from the following two types of interaction:

1. *Electrodynamic interactions with moving electrolytes*: An SMF exerts Lorentz forces on moving ionic charge carriers and thereby gives rise to induced electric fields and currents. This interaction is the basis of magnetically induced potentials associated with flowing blood. More recently, using a uniform SMF of 0.2 T, Kainz et al. (2010) successfully demonstrated the experimental and theoretical validation of a magnetohydrodynamic (MHD) solver for blood flow analysis. The measured voltage value probably induced by MHD signal was 245 μV. The computational MHD results can then be correlated with the actual measurements. The authors hope to develop an MHD-based biomarker to noninvasively estimate the blood flow for the evaluation of heart failure. This MHD theory is also supported by other studies (Oster et al., 2014; Srivastava, 2014).
2. *Induced electric fields and currents*: Time-varying magnetic fields induce electric currents in living tissues in accordance with Faraday's law of induction. Electric currents may also be induced by SMF (Gupta et al., 2008). Gupta et al. (2008) suggested that, in

the bore of 1.5 T MRI magnet, this induced voltage distorts the electrocardiogram (ECG) signal of the patient and appears as an elevation of the T-wave of the ECG signal. The flow of blood through the aortic arch is perpendicular to the SMF and coincides with the occurrence of the T-wave of the ECG. The induced electric fields could be enhanced by movement in an SMF (Glover and Bowtell, 2008). Glover and Bowtell (2008) measured in situ surface electric fields induced by typical human body movements such as walking or turning in the *fringe* magnetic fields of a whole-body 3 T MRI scanner. These values were 0.15, 0.077, and 0.015 V/m for the upper abdomen, head, and across the tongue, respectively. A peak electric field of 0.3 V/m was measured for the chest. The speed of movements was not specified in this study. In a body moving at a constant speed of 0.5 m/s into a 4 T magnet, Crozier and Liu (2005) estimated the maximum induced electric field strength to be 2 V/m, which is equal to the apparent threshold for peripheral nerve stimulation in the frequency range from 10 Hz to 1 kHz (ICNIRP, 1998). It should be noted, however, that frequencies associated with body movements are likely to be ≤10 Hz, the frequency below which more negative resting membrane potential decreases the electrical excitability of neurons due to the slow inactivation of voltage-gated sodium ion channels (Bezanilla, 2002). Head translational and rotational frequencies during walking, for example, vary from 0.4 to 4.0 Hz (Grossman et al., 1988; Pozzo et al., 1990; MacDougall and Moore, 2005).

Moreover, when considering a MHD Lorentz force acting on ions, Roberts et al. (2011) recently discovered that SMF from high-strength MRI machines (3 and 7 T, for 25 min) induces nystagmus in all normal humans and that a Lorentz force, derived from ionic currents in the endolymph and pushing on the cupula, best explains this effect. Furthermore, Ward et al. (2014) recorded eye movements in the SMF of a 7 T MRI machine for 25 min in nine individuals with unilateral labyrinthine hypofunction, as determined by head impulse testing and vestibular-evoked myogenic potentials (VEMPs). Eye movements were recorded using infrared video-oculography. Static head positions were varied in pitch with the body supine, and slow-phase eye velocity (SPV) was assessed. All subjects exhibited predominantly horizontal nystagmus after entering the magnet head first, lying supine. The SPV direction reversed when entering feet first. Pitching chin to chest caused subjects to reach a null point for horizontal SPV. Right unilateral vestibular hypofunction (UVH) subjects developed slow-phase-up nystagmus and left UVH subjects, slow-phase-down nystagmus. Vertical and torsional components were consistent with superior semicircular canal excitation or inhibition, respectively, of the intact ear. These findings provide compelling support for the hypothesis that magnetic vestibular stimulation (MVS) is a result of a Lorentz force and suggest that the function of individual structures within the labyrinth can be assessed with MVS. We speculate that a Lorentz force acting on ions in the endolymph could also be induced in the microvessels.

In another aspect of other medical applications, magnetic drug delivery system (MDDS), which is a technology to control the drug kinetics from outside the body by external magnetic force using spatially inhomogeneous SMF or dc/ac magnetic fields, has been investigated (Mishima et al., 2007; Nishijima et al., 2008, 2009; Hirota et al., 2009; Chorny et al., 2010; Morais, 2010; Nakagawa et al., 2012; Wang et al., 2014). In particular, under SMF exposure conditions with regard to MDDS using suspension of the ferromagnetic particles, the trajectory of the ferromagnetic particles in the blood vessel was calculated, and the possibility of the navigation of the drug has been discussed (Mishima et al., 2007; Nishijima et al., 2008, 2009; Hirota et al., 2009; Nakagawa et al., 2012). The drug navigation probability to the desired direction was confirmed to be higher than 80% using a high-temperature superconducting magnet of B_{max} 4.5 T (Nishijima et al., 2008). A rat experiment was also performed successfully using a permanent magnet of B_{max} 0.3 T (Nishijima et al., 2008). Moreover, the suspension of the magnetite was injected into the blood vessel of the pig, and the magnetite was successfully navigated and/or accumulated by the high-temperature superconducting magnet (Nishijima et al., 2009).

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 (Cavopol et al., 1995; McLean et al., 1995) and myosin phosphorylation (Engström et al., 2002) have been found mostly in the absolute field gradient range of more than 1.0 mT/mm in the target tissues or cells. However, these hypotheses have not been tested well in vivo compared with in vitro, and the effects and the underlying mechanisms remain elusive. In particular, to reveal and clarify the effects and mechanisms of spatial magnetic gradient in vitro as well as in vivo, it is necessary to carry out the experiments comparing the spatially homogeneous and inhomogeneous SMF.

8.3 EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELDS

Many researches for exploring biological and health hazardous effects on extremely low-frequency EMFs (ELF–EMF) have been done. It has been reported that the suppression of natural killer (NK) cell activity in mice after subchronic and chronic exposures to 60 Hz EMF 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.

In the fields of ELF–EMF exposure effects on the microcirculatory system in vivo, Ohkubo research group (Xu et al., 2001; Ushiyama and Ohkubo, 2004; Ushiyama et al., 2004a,b) has investigated the effects. Xu et al. (2001) investigated acute hemodynamic effects of not only SMF but also ELF–EMF at a threshold level on modulating the muscle capillary microcirculation in pentobarbital-anesthetized mice. The skin in a tibialis anterior was circularly removed with 1.5 mm diameter for intravital-microscopic recording of the capillary blood velocity in the tibialis anterior muscle. Fluorescein isothiocyanate-labeled dextran (FITC-Dx) was used for an in vivo fluorescent plasma marker of the muscle capillaries. Following a bolus injection of FITC–Dx solution into the caudal vein, the peak blood velocity in the muscle capillaries was measured prior to, during, and following exposure to SMF or 50 Hz EMF using a fluorescence epi-illumination system. The whole body of experimental animals, placed on the observing stage of a fluorescence microscope, was exposed to SMF of 0.3, 1.0, and 10 mT or 50 Hz EMF of 0.3 and 1.0 mT for 10 min using a specially devised electromagnet. For sham exposure, the electromagnet was not energized. During exposure and postexposure to SMF of 10.0 mT, the peak blood velocity significantly increased as compared to sham exposure. After the withdrawal of SMF and 50 Hz EMF of 1.0 mT, significant similar effects on the blood velocity were present or enhanced. These findings suggest that field intensity of 1.0 mT might be considered as a threshold level for enhancing muscle microcirculation under pentobarbital-induced hypnosis.

Furthermore, Ohkubo research group employed an in vivo microscopic approach using DSC method and quantified the effect on immune responses at microcircular level in subcutaneous tissue (Ushiyama and Ohkubo, 2004; Ushiyama et al., 2004a,b). They explored the effect of ELF–EMF (0.3, 1.0, 3.0, 10, or 30 mT) of 50 Hz on microcirculatory system, acute and subchronic effects on leukocyte–endothelium interactions in subcutaneous tissue of mice. They developed a nonmetallic DSC, which is made of polyacetal resin (Duracon™). This nonmetallic frame chamber can be applied to studies for ELF–EMF exposure without any resultant thermal effects. The adherent leukocyte count to the endothelium is 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, they 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).

Ushiyama and Ohkubo (2004) reported that significant increase in endothelium-adhering and rolling leukocytes was detected in venules following acute EMF exposure (50 Hz, 30 mT, for 30 min). 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 EMFs 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.

Ushiyama et al. (2004a) reported similar result from the experiments of subchronic exposure. In this experiment, mice with DSC were maintained under the various intensity (0.3, 1.0, and 3.0 mT) of 50 Hz EMF for 17 consecutive days. They demonstrated that a significant increased number of rolling leukocytes compared to preexposure status was observed only in the highest exposure level group (3.0 mT). In other groups, no significant change in rolling count was observed in any measuring time point. They 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.

In a recent in vivo study, Gutiérrez-Mercado et al. (2013) investigated the effects of ELF–EMF on the capillaries of some circumventricular organs (CVOs). They showed that an ELF–EMF (120 Hz, 0.66 mT, for 2 h/day for a 7-day period) induces a vasodilation as well as an increase in the permeability to nonliposoluble substances in rats. All animals were administered colloidal carbon (CC) intravenously to study, through optical and transmission electron microscopy, the capillary permeability in CVO and the BBB in brain areas. An increase in capillary permeability to CC was detected in the ELF–EMF-exposed group as well as a significant increase in vascular area (capillary vasodilation); none of these effects were observed in individuals of the control (no treatment) and sham ELF–EMF groups. They suggested that the ELF–EMF has over structural and permeability characteristics of CVO capillaries and to a lesser extent on brain regions with BBB.

Using a combination of in vivo and in vitro approaches, Delle Monache et al. (2013) investigated the effect and underlying mechanism of ELF–EMF (50 Hz, 2 mT) on endothelial cell models HUVEC and MS1 (mouse pancreatic endothelial cells) measuring cell status and proliferation, motility, and tubule formation ability. MS1 cells when injected in mice determined a rapid tumorlike growth that was significantly reduced in mice inoculated with ELF–EMF-exposed cells. In particular, histological analysis of tumors derived from mice inoculated with ELF–EMF-exposed MS1 cells indicated a reduction of hemangioma size and of blood-filled spaces and hemorrhage. In parallel, in vitro proliferation of MS1 treated with ELF–EMF was significantly inhibited. They also found that the ELF–EMF exposure downregulated the process of proliferation, migration, and formation of tubule-like structures in HUVEC. In particular, ELF–EMF exposure significantly reduced the expression and activation levels of vascular endothelial growth factor receptor 2 (VEGFR2), suggesting a direct or indirect influence of ELF–EMF on VEGF receptors placed on cellular membrane. In conclusion, ELF–EMF reduced, in vivo and in vitro, the ability of endothelial cells to form new vessels, most probably affecting VEGF signal transduction pathway that was less responsive to activation. They suggested that these findings could not only explain the mechanism of antiangiogenic action exerted by ELF–EMF but also promote the possible development of new therapeutic applications for treatment of those diseases where excessive angiogenesis is involved.

Robertson et al. (2007) reviewed several mechanisms of protection by various types of ELF–EMF, such as heat shock proteins (HSPs), opioids, collateral blood flow, and NO induction, and the evidence supporting the use of ELF–EMF as a means of providing protection in each of these mechanisms. The comments were that, although there are few studies demonstrating direct protection

with ELF–EMF therapies, there are many published reports suggesting that ELF–EMF may be able to influence some of the biochemical systems with protective applications (Robertson et al., 2007).

As for clinical studies, McNamee et al. (2010, 2011) indicated that ELF–EMF (60 Hz, 0.2 and 1.8 mT) did not significantly affect perfusion, heart rate, or mean arterial pressure. The decrease in perfusion and heart rate trends over time appears to be associated with a combination of inactivity (resulting in decreased body temperatures) and reduced physiological arousal. In contrast, Nishimura et al. (2011) suggested that repeated exposure to an ELF–EMF (6 and 8 Hz, 1 μT, 10 V/m, for at least two 10–15 min sessions per week, over a period of 4 weeks) has a BP-lowering effect on humans with mild-to-moderate hypertension.

8.3.1 Discussion and Conclusion

An early in vivo study reported that during the postexposure period of 50 Hz EMF at 1.0 mT, the peak blood velocity significantly increased as compared to sham exposure (Xu et al., 2001). In contrast, the microcirculatory parameters of pia mater were not affected by similar condition (Ushiyama and Ohkubo, 2004; Ushiyama et al., 2004a). Therefore, it was concluded that no evidence about health hazardous effect by low intensity of ELF–EMF exposure is observed (Ushiyama and Ohkubo, 2004; Ushiyama et al., 2004a). However, Gutiérrez-Mercado et al. (2013) suggested that ELF–EMF (120 Hz, 0.66 mT, for 2 h/day for a 7-day period) has over structural and permeability characteristics of some CVO capillaries and to a lesser extent on brain regions with BBB. In vivo and in vitro studies, ELF–EMF (50 Hz, 2 mT) reduced the ability of endothelial cells to form new vessels, most probably affecting VEGF signal transduction pathway that was less responsive to activation (Delle Monache et al., 2013). Several mechanisms of protection by ELF–EMF have been reported, such as HSP, opioids, collateral blood flow, and NO induction (Robertson et al., 2007). The comments were that, although there are few studies demonstrating direct protection with ELF–EMF therapies, there are many published reports suggesting that ELF–EMF may be able to influence some of the biochemical systems with protective applications (Robertson et al., 2007). Few clinical reports have addressed the effects of ELF–EMF. Therefore, further exploration is required to comprehensively evaluate and understand the effects of ELF–EMF exposure on microcirculation along with the resulting physiological consequences.

8.4 PULSED ELECTROMAGNETIC FIELD

In the past few decades since Bassett's reports (Bassett et al., 1982a,b), therapy with pulsed electromagnetic fields (PEMFs) has led to clinical trials, commercial production, and availability of devices for promoting the healing of bone nonunions in the clinic (Chalidia et al., 2011; Griffin et al., 2011). Accordingly, it has been clarified that PEMF stimulation could promote neovascularization and improve the perfusion (Lin et al., 1993; Smith et al., 2004; Kavak et al., 2009; McKay et al., 2010; Nikolaeva et al., 2010; Pan et al., 2013).

Lin et al. (1993) reported that PEMF exposure (10 Hz, 5 mT, for 6 h/day) in rabbits induced the increase of blood flow and fibroblasts at the defects from 2 to 4 weeks after operation. These results suggested that PEMF enhanced the blood flow and increased the fibroblasts at the defect. At the same time, PEMFs directly stimulated the collagen production from the fibroblasts, thus accelerating the healing process of the ligament.

Smith et al. (2004) reported that local PEMF stimulation (18.8 T/s [positive amplitude] and 8 T/s [negative amplitude] for 2 min) produced significant vasodilation, compared to prestimulation values, in cremasteric arterioles in anesthetized rats. This dilation occurred after 2 min of stimulation (9% diameter increase) and after 1 h of stimulation (8.7% diameter increase). Rats receiving sham stimulation demonstrated no statistically significant change in arteriolar diameter following either sham stimulation period. PEMF stimulation of the cremaster did not affect systemic arterial pressure or heart rate, nor was it associated with a change in tissue environmental temperature.

These results suggested that local application of a specific PEMF waveform can elicit significant arteriolar vasodilation.

Kavak et al. (2009) examined the PEMF (50 Hz, 5 mT) effects of thoracic aorta rings obtained from streptozotocin-induced diabetic and healthy control rats to determine if PEMF could ameliorate problems associated with diabetes. Streptozotocin was given via tail vein to produce diabetes mellitus (DM). The PEMF stimulation occurred four times daily for 30 min at 15 min intervals repeated daily for 30 days. Thoracic aorta rings from both DM and non-DM rats exposed to PEMF were evaluated for contraction and relaxation responses and membrane potential changes in the presence or absence of chemical agents that were selected to test various modes of action. Relaxation response of thoracic aorta rings was significantly reduced in DM than non-DM group. PEMF significantly increased the relaxation response of the diabetic rings to ACh and reduced the concentration response to phenylephrine. Resting membrane potential was significantly higher in DM than in non-DM group. Inhibitors of NO, both nitro-l-arginine (l-NO-ARG) and l-NO-ARG + indometacin combination, produced a significant transient hyperpolarization in all groups. Inhibitors of potassium channel activity, charybdotoxin or apamin, produced a membrane depolarization. However, PEMF did not induce any significant effect on the membrane potential in DM group. It was concluded that treatment with PEMF ameliorated the diabetes-induced impairments in the relaxation response of these rings.

Pan et al. (2013) indicated that PEMF stimulation (15 Hz, 1.2 mT, 8 h/day for 28 days) enhanced acute hind limb ischemia-related perfusion and angiogenesis, associated with upregulating fibroblast growth factor (FGF)-2 expression and activating the extracellular signal-regulated kinase (ERK)1/2 pathway in diabetic rats. They suggested that PEMF stimulation may be valuable for the treatment of diabetic patients with ischemic injury.

McKay et al. (2010) investigated the acute effect of a PEMF (72 Hz, 225 μT, 6.7 mV/m, for 30 and 60 min) on blood flow in the skeletal microvasculature of a male Sprague–Dawley rat model. ACh (0.1, 1.0, and 10.0 mM) was used to perturb normal blood flow and to delineate the differential effects of PEMF, based on the degree of vessel dilation. The authors found that there were no significant effects of PEMF on peak blood flow, heart rate, and myogenic activity, but a small attenuation effect on anesthetic-induced respiratory depression was noted.

Borsody et al. (2013) investigated an effective means of PEMF stimulation of the facial nerve for the purpose of increasing CBF. In normal anesthetized dog and sheep, a focal PEMF was directed toward the facial nerve within the temporal bone by placing a 6.5 cm figure 8 stimulation coil over the ear. In an initial set of experiments, CBF was measured by laser Doppler flowmetry and the cerebral vasculature was visualized by angiography. The effect of facial nerve stimulation was found to be dependent on stimulation power, frequency, and the precise positioning of the stimulation coil. Furthermore, an increase in CBF was not observed after direct electrical stimulation in the middle ear space, indicating that nonspecific stimulation of the tympanic plexus, an intervening neural structure with vasoactive effects, was not responsible for the increase in CBF after PEMF stimulation. Subsequent experiments using perfusion MRI demonstrated reproducible increases in CBF throughout the forebrain that manifested bilaterally, albeit with an ipsilateral predominance. Moreover, Borsody et al. (2014) performed an additional experiment using an ischemic stroke dog model involving injection of autologous blood clot into the internal carotid artery that reliably embolizes to the middle cerebral artery. Facial nerve stimulation caused a significant improvement in perfusion in the hemisphere affected by ischemic stroke and a reduction in ischemic core volume in comparison to sham stimulation control. The ATP/total phosphate ratio showed a large decrease poststroke in the control group versus a normal level in the stimulation group. The same stimulation administered to dogs with brain hemorrhage did not cause hematoma enlargement. They concluded that these results support the development and evaluation of a noninvasive facial nerve stimulator device as a treatment of ischemic stroke (Borsody et al., 2013, 2014).

For clinical evaluation, Nikolaeva et al. (2010) reported that for children with diabetic polyneuropathy, after local PEMF (16 Hz, 45 mT, for 15 min), along with improvements in clinical

measures, the microcirculatory bed showed a virtually twofold increase in blood influx with predominant changes in efflux with maintained high shunting parameter. Myogenic tone reacted most strongly to PEMF, with weak reactions from the endothelium and neurogenic tone. This state of the microcirculatory bed can be evaluated as an adaptive mechanism, ensuring effective circulation, its shunting, and activation of the myogenic component of regulation.

Mesquita et al. (2013) investigated the effects of ELF (1 Hz)–PEMF called repetitive transcranial magnetic stimulation (rTMS) on motor cortex CBF and tissue oxygenation in seven healthy adults, during/after 20 min stimulation. Noninvasive optical methods are employed: diffuse correlation spectroscopy (DCS) for blood flow and diffuse optical spectroscopy (DOS) for Hb concentrations. A significant increase in median CBF (33%) on the side ipsilateral to stimulation was observed during rTMS and persisted after discontinuation. The measured hemodynamic parameter variations enabled computation of relative changes in cerebral metabolic rate of oxygen consumption during rTMS, which increased significantly (28%) in the stimulated hemisphere. By contrast, hemodynamic changes from baseline were not observed contralateral to rTMS administration. They suggested that these findings provide new information about hemodynamic/metabolic responses to rTMS and demonstrated the feasibility of DCS/DOS for noninvasive monitoring of TMS-induced physiological effects.

8.4.1 Discussion and Conclusion

Though the available evidence suggests that specific PEMF stimulation may offer some benefit in the treatment of microcirculatory disorders, it is inconclusive and insufficient to inform current practice. More definitive conclusions on treatment effect await further well-conducted randomized controlled trials. Further studies are required to explore the effects and underlying mechanisms.

8.5 RADIO FREQUENCY–ELECTROMAGNETIC FIELDS

There is some concern that the exposure to RF–EMFs emitted by cellular phones could cause adverse health effects (Mann et al., 2000). The 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 (Ohkubo et al., 2007). The BBB function is important for maintenance of brain homeostasis (Mayhan, 2001). Many researchers have reported that RF–EMF exposures increase the blood flow in the microcirculation of musculature over the past two decades (Shrivastav et al., 1983; Sharma and Hoopes, 2003). Most of the results were, however, obtained under thermal conditions induced by high intensity of RF–EMF exposure. Thus, those responses were attributed to the increase in body or local region temperature.

There had been little information about the effects of RF–EMF exposures on microcirculation under nonthermal conditions until 1994. In this year, Salford et al. (1994) reported that albumin leakage sites were found in the rat brain after 915 MHz–EMF exposure for 2 h even under nonthermal intensity levels, which is less than 0.08 W/kg of whole-body averaged SAR. Because permeability change of BBB has been a matter of concern as it could result in health hazard on the brain, many research groups attempted to confirm their results. However, a few studies (Schirmacher et al., 2000; Aubineau and Tore, 2005) found in the low-level RF–EMF affect BBB permeability in vivo and in vitro, whereas others (Tsurita et al., 2000; Franke et al., 2005a,b; Kuribayashi et al., 2005; Finnie et al., 2006) failed to replicate Salford's findings (Salford et al., 1994, 2003).

The cerebral microcirculatory dynamics including BBB function have been a target to evaluate the biological effects of RF–EMF (Masuda et al., 2007a–c, 2009, 2011). 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 the 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 exposures 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 (Yuan et al., 2003; Gaber et al., 2004; Masuda et al., 2007a–c, 2009, 2011). 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. To investigate the possible effects of the exposure to RF–EMF on cerebral microcirculation including several parameters mentioned earlier, Masuda et al. (2007a–c) introduced the CCW method into rats and observed the changes in the parameters in the brain after acute or subchronic exposure to RF–EMF using the exposure system consisted of a small anechoic chamber and a monopole antenna. The head of each rat was positioned toward the central antenna and was locally exposed to 1439 MHz electromagnetic near-field of the time division multiple access (TDMA) signal 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 min 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 postexposures 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 postexposures. No extravasation of two kinds of fluorescence dyes, FITC–Dx (MW: 250 kDa) and sodium fluorescein (MW: 376 kDa), 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 postexposure in plasma velocities or adherent leukocyte counts. No extravasation of the two kinds of fluorescence dyes from the pial venule was noticed.

McQuade et al. (2009) carried out a study designed to confirm whether exposure to 915 MHz radiation, using a similar transverse electromagnetic (TEM) transmission line exposure cell and similar exposure parameters to those used by Salford and colleagues, caused the extravasation of albumin in rat brain tissue. These authors exposed or sham-exposed the rats (28–46 per group) for 30 min to CW 915 MHz or 915 MHz radiation pulse modulated at 16 or 270 Hz at whole-body SARs ranging between 1.8 mW/kg and 20 W/kg and examined the brain tissue shortly after exposure. The authors examined coronal sections from three or more regions along the rostro-caudal axis, assigning scores for extracellular extravasation across the whole section. Separate brain regions in each section were distinguished but these results were not presented. Overall, McQuade et al. (2009) reported little or no extracellular extravasation of albumin in the brain tissue of any exposure group compared to sham-exposed animals, in contrast to the effects seen in the positive control groups.

de Gannes et al. (2009) also used improved staining techniques, as well as those originally used by the Salford research group, in order to identify albumin extravasation and the presence of dark neurons in rat brains 14 or 50 days after the head-only exposure or sham exposure of rats (8 rats per group) for 2 h to a Global System for Mobile Communications (GSM)-900 signal at brain-averaged SARs of 140 mW/kg and 2.0 W/kg. In addition, de Gannes et al. (2009) used a more specific marker for neuronal degeneration than the one used by the Salford research group and also looked for the presence of apoptotic neurons. Like McQuade et al. (2009) and Masuda et al. (2009), de Gannes et al. (2009) also used a cage-control group and a positive control group. The authors reported that they were unable to find any evidence of the increase in albumin extravasation or number of dark neurons in 12 different regions of the brain tissue of exposed animals, although clear increases in both were seen in the positive control group.

Huber et al. (2002, 2003, 2005) examined the effects of RF–EMF exposures on rCBF, electroencephalogram (EEG), and heart rate variability (HRV). They reported that (1) pulse-modulated

RF–EMF alters waking rCBF and (2) pulse modulation of RF–EMF is necessary to induce waking and sleep EEG changes and (3) affects HRV. They speculated that pulse-modulated RF–EMF exposure may provide a new, noninvasive method for modifying brain function for experimental, diagnostic, and therapeutic purposes.

The effects of RF–EMF exposures on skin temperature were measured under normal blood flow and without blood flow in the rabbit ear (Jia et al., 2007). The results showed the following: (1) physiological blood flow clearly modified RF–EMF-induced thermal elevation in the pinna as blood flow significantly suppressed temperature increases even at 34.3 W/kg and (2) under normal blood flow conditions, exposures at 2.3 and 10.0 W/kg, approximating existing safety limits for the general public (2 W/kg) and occupational exposure (10 W/kg), did not induce significant temperature rises in the rabbit ear. However, 2.3 W/kg induced local skin temperature elevation under no blood flow conditions. The results demonstrate that the physiological effects of blood flow should be considered when extrapolating modeling data to living animals and particular caution is needed when interpreting the results of modeling studies that do not include blood flow.

Other in vivo effects of RF–EMF (GSM signals) have been investigated in the skin of hairless rat (Masuda et al., 2006; Sanchez et al., 2006, 2008). The results of 2 h acute exposure (Masuda et al., 2006) and 12-week chronic exposure (Sanchez et al., 2006) did not demonstrate major histological variations, and there was no evidence that GSM signals alter HSP expression in rat skin (Sanchez et al., 2008).

8.5.1 Discussion and Conclusion

Masuda et al. (2007a–c, 2009, 2011) focused on the cerebral microcirculatory parameters and investigated the acute and subchronic effects on the exposure to RF–EMF on those parameters. According to the results of these 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 exposures lower than the local permissible level (2.0 W/kg) in the ICNIRP guidelines (ICNIRP, 1998) do not induce any changes in cerebral microcirculation, if a presumption for dose–response relationship between intensities of the RF–EMF exposures and biological responses is accepted. The values of brain-averaged SAR in the present exposures were 0.6, 2.4, and 4.8 W/kg. These exposure levels range from a low level comparable to the study by Salford et al. (1994) to a moderate level of 2.4 times higher than that of the safety guideline. Several studies that evaluated changes in BBB permeability after RF–EMF exposure found that the exposure under lower SAR level than the 2.0 W/kg did not modify the BBB permeability (Tsurita et al., 2000; Kuribayashi et al., 2005; Franke et al., 2005a,b; Finnie et al., 2006). These results not only support the previous findings but also provide new information for considering the effects of RF–EMF exposures on the microcirculation.

The second is that the multiparameter evaluation supports the lack of increase in the BBB permeability under RF–EMF exposures. Although many investigators have reported the effects of RF–EMF on BBB permeability, these studies mainly used histological evaluation (Salford et al., 1994, 2003; Fritze et al., 1997; Tsurita et al., 2000; Kuribayashi et al., 2005; Franke et al., 2005a,b; Finnie et al., 2006; Nittby et al., 2008). On the contrary, Masuda et al. (2007a–c, 2009, 2011), McQuade et al. (2009), and de Gannes et al. (2009) 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, the findings by these investigators 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 these phenomena.

Thus, many confirmation studies in vivo and in vitro have been performed to confirm since Salford's findings (Salford et al., 1994). Most of them suggest no effects of RF–EMF exposure

on the BBB. However, these investigators recognize that no one can replicate the Salford's studies (1994, 2003) with the same methodology they used, because their methodology involves much uncertainty (SSM, 2009; Stam, 2010). Therefore, there is still considerable disagreement about the Salford's reports (SSM, 2009; Stam, 2010).

8.6 OVERALL CONCLUSION

There is an importance of understanding the effects of various kinds of magnetic fields with different intensities and frequencies on microcirculatory system. Methodologically speaking, pharmacological treatments as well as pathological animal models are useful for experimental evaluation of the effects and mechanisms of magnetic fields. It may have direct and indirect role in the interaction of magnetic fields with different tissues and organs. The results could be useful in applying specific SMF and PEMF for microcirculatory disorders. In contrast, the results obtained from exposure to most of ELF–EMF and RF–EMF failed to show any changes in microcirculatory system except for leukocyte and endothelial cell interaction. The range of EMF investigated is very higher than that of the international exposure guidelines. These microcirculatory studies in the animal models, combined with in vivo real-time molecular imaging techniques and in vitro pathway analysis, can contribute to evaluate therapeutic application and possible health risks of magnetic fields.

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