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Effects of Acute Exposure to a 1439 MHz Electromagnetic Field on the Microcirculatory Parameters in Rat Brain

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Abstract. *The aim of this study was to determine the potential of radio-frequency electromagnetic fields (RF-EMF) to affect cerebral microcirculation, including blood-brain barrier function, in rat brain. Materials and Methods: The head of the rat was exposed for 10 min to 1439 MHz RF-EMF having three intensity doses: 0.6, 2.4, 4.8 W/kg of brain averaged specific absorption rate (BASAR). Four microcirculatory parameters: blood-brain barrier permeability, leukocyte behavior, plasma velocity, and vessel diameter were measured before and after RF-EMF exposure using a closed cranial window method. Results: No extravasation of intravenously injected dyes from pial venules was found at any BASAR level. No significant changes in the number of endothelial-adhering leukocytes after exposure were found. The hemodynamics indicated that the plasma velocities and vessel diameters remained constant within the physiological range throughout each exposure. Conclusion: These findings suggest that there were no effects on the cerebral microcirculation under the given RF-EMF exposure conditions.*

The possibility of radio-frequency electromagnetic fields (RF-EMF) of mobile phones to cause possible adverse health effects is the subject of numerous studies and publications. Permeability changes in the blood-brain barrier (BBB) have been a matter of concern as this could pose a health hazard to the brain. The early studies reported that BBB permeability could be affected by RF-EMF only when the exposure was high enough to cause a temperature increase (1-3). In contrast to these observations, one of the most remarkable findings in recent years was the BBB disruption reported by Salford *et al.* who demonstrated that albumin leakage sites were found in the rat brain after 915 MHz-EMF exposure even under non-thermal intensity levels (4, 5). The low level RF-EMF effect on BBB permeability was subsequently found in several *in vivo* and *in vitro* studies (6, 7). However, several other studies failed to replicate the findings of Salford *et al.*(8-11).

The BBB function is not the only important parameter of cerebral microcirculation. Leukocyte behavior, blood flow velocity and vessel diameter are also valuable parameters for evaluation of the cerebral microcirculation (12, 13). For example, an increase in leukocyte adhesion to the endothelium in pial venules reflects inflammatory responses in the brain (14). Many inflammations are also accompanied by changes in BBB permeability (15). These phenomena suggest a strong relationship between leukocyte behavior and BBB function. Therefore, simultaneous investigation of several parameters is important.

Many studies on BBB permeability in relation to RF-EMF exposure have been investigated with a histological approach in postmortem animals (8, 9, 11). Other parameters such as hemodynamic changes need direct observation of the cerebral microcirculation in living animals. Therefore, an *in vivo* observation method of the cerebral microcirculation under the RF-EMF exposure conditions is required.

The aim of present study was to investigate the possible effects of acute exposure to RF-EMF on the cerebral microcirculation, including several of the parameters mentioned above. The parameters were evaluated using a closed cranial window (CCW) method which allows the direct observation of the cerebral microcirculation (16). We modified the CCW so as it would be usable even during RF-EMF irradiation of the rat brain. Four microcirculatory

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Key Words: Closed cranial window, radio-frequency, electromagnetic field, blood-brain barrier, leukocyte behavior, cerebral microcirculation, acute exposure.

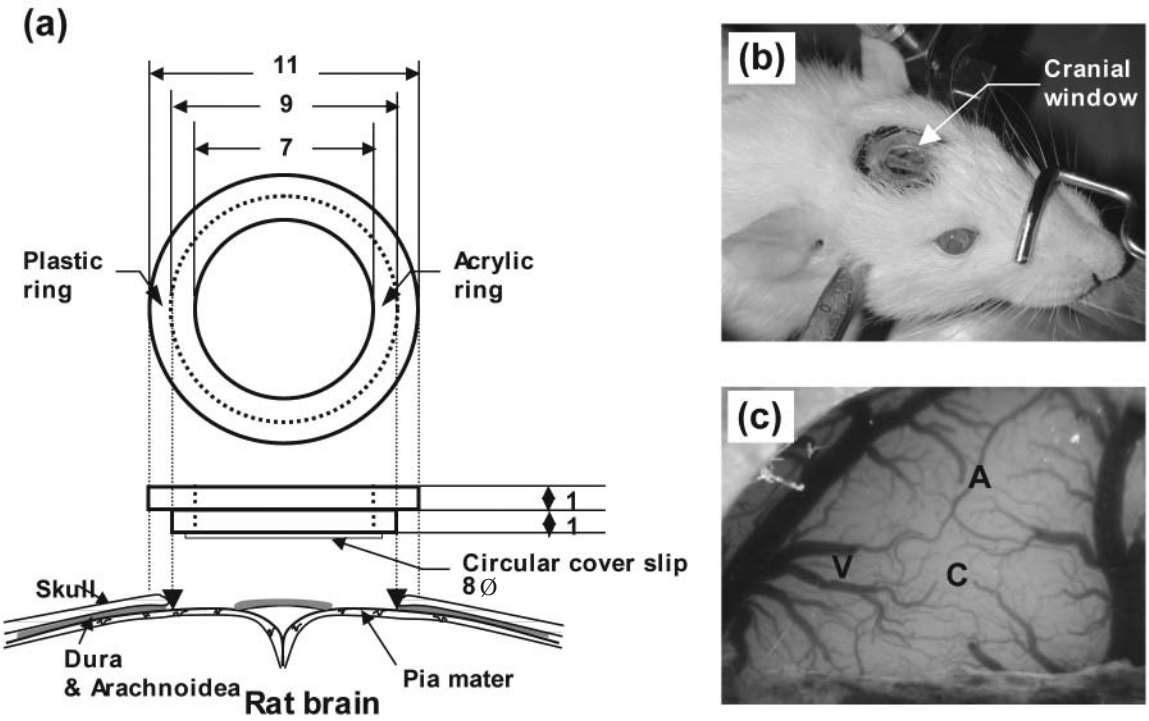

Figure 1. *Design and overview of cranial window for long-term observation. The window, made of acrylic, plastic and glass, with a convex shape (a) was implanted into the parietal region on the rat brain (b). (c) The pial microvessels, A: arteriole, V: venule and C: capillary, were clearly observable throughout the experimental period.*

parameters, BBB permeability, leukocyte behavior, plasma velocity and vessel diameter were measured before and after the exposure of rat brain to 1439 MHz RF-EMF at 0.6, 2.4, and 4.8 W/kg of brain averaged specific absorption rate (BASAR). These exposure levels range from a low level, comparable to the study by Salford *et al.* to a moderate level of 2.4-fold, that of the current safety guideline for exposure to localized RF-EMF (17).

Materials and Methods

Animals. Male Sprague-Dawley rats (10-11 weeks old, 386±22 g, Tokyo Laboratory Animals Science Co. Ltd, Japan) were used in this experiment. They were fed a standard pellet diet and water *ad libitum* and were maintained with a 12-h light/dark cycle, at a temperature of 23.0˚C±1˚C and a relative humidity of 50%±10%. All experimental procedures were conducted in accordance with the ethical guidelines for animal experiments at the National Institute of Public Health, Japan.

Preparation of closed cranial window. The closed cranial window (CCW) setup used in the present study was developed for long-term observation of pial microcirculation under RF-EMF exposure conditions. Therefore, no metal materials were used. The CCW was made of acrylic and plastic and had a convex shape which allows it to be inserted into the skull hole. The bottom of the window had a circular cover-glass 8.0 mm in diameter (Figure 1a).

The CCW was implanted into the parietal region of the rats. The animals were anesthetized with an intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). The head of each rat was fixed in a stereotaxic apparatus. After removal of hair, skin and connective tissues from the parietal region, a 10-mm circular skull hole was made using a dental drill with cold saline drip to prevent heat generation during the drilling. Subsequently, the dura mater and arachnoid were carefully removed from the cerebral surface to expose the pia mater. The window was inserted into the hole of the skull and fixed with cyanoacrylate glue (Figure 1b). All animals were exposed to RF-EMF at least one week after the window implantation to allow for recovery (16).

RF-EMF exposure. The exposure system consisted of a small anechoic chamber, a carousel type rat holder and a monopole antenna (Figure 2) (18). Eight rats (one anesthetized rat and seven dummy rats) were exposed at once to the same brain averaged specific absorption rate (BASAR). The dummy rats formed in the shape of the anesthetized rat were made with tissue-equivalent material which has similar electrical properties of the rat tissues. We used these dummy rats for homogeneous exposure of rat brain to RF-EMF. Their heads were positioned toward the antenna placed at the center of the rat holder. The exposure was performed for 10 min using a 1439 MHz near-field TDMA (time division multiple access) signal for PDC (Personal Digital Cellular, Japanese cellular telephone standard) system to simulate the exposure from mobile phones. The PDC signal has pulsed 6.67 ms waveforms at repetition intervals of 20 ms, *i.e.* the peak power is

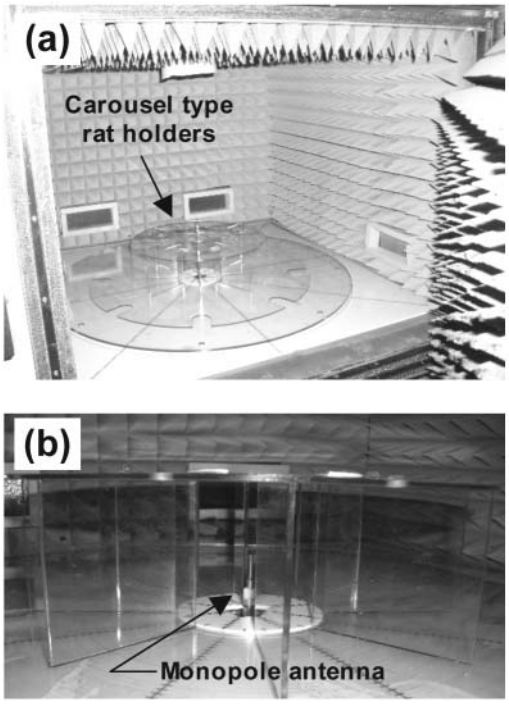

Figure 2. *Exposure system. The RF-EMF exposure was performed in a small anechoic chamber (a) equipped with a monopole antenna (b). The anesthetized rat was held in an acrylic holder and its head was positioned toward the antenna placed at the center of the chamber.*

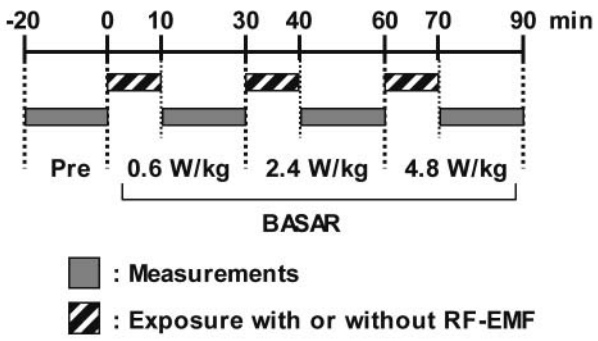

Figure 3. *Experimental protocol. The exposure of 1439 MHz RF-EMF for 10 min and subsequent measurements for 20 min were sequentially performed from pre-measurement up to 4.8 W/kg of brain averaged SAR (BASAR). Thus, a region of cerebral microcirculation in the same rat could be observed repeatedly.*

3-fold higher than the temporal average. The field intensity was adjusted to deliver 0.6, 2.4, or 4.8 W/kg in BASAR. Whole-body exposures occured concomitantly; the relative value of whole body averaged SAR was 1/3.7 of the BASAR.

In the case of acute response to an inflammatory or pathophysiological event in the cerebral microcirculatory parameter, which we selected here, the changes in those parameters are evoked over several tens of minutes. For example, the blood-brain barrier disruption, accompanied by extravasation of FITC-dextran from blood vessels, in combination with increases in leukocyte adhesion and constriction of pial arterioles is observed within 20 min after a topical application of compound 48/80, the inflammatory mediator (14). Therefore, we chose a 10 min exposure followed by 20 min observation, sequentially performed from BASAR 0.6 W/kg to 4.8 W/kg in the present experiment (Figure 3). After the exposure, the rat was immediately placed in a stereotaxic apparatus for microscopic observations.

Intravital-microscopic observation. The rat pial microcirculation within the CCW was monitored using an intravital-microscopic system while the animal was under anesthesia. The rats were anesthetized with an intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and with a subcutaneous injection of pentobarbital (25 mg/kg). The head of each animal was fixed in the stereotaxic apparatus.

Two types of intravital-microscopic monitoring systems were used. One system was an intravital-fluorescence microscopic system. This system consisted of a fluorescent microscope (BX50WI, Olympus Optical Co. Ltd., Tokyo, Japan) and an image-intensified camera (C2400-80, Hamamatsu Photonics K.K., Hamamatsu, Japan). The light source was a mercury lamp (U-ULH, Olympus Optical Co. Ltd.). The filter cube has two types of excitation filter, of 524 nm and 490 nm. The second system was a confocal laser-scanning microscopic system (CLSM). The CLSM consisted of a microscope (BX50WI, Olympus Optical Co. Ltd.), an argon-ion laser (643-YOKO-AO2, Omnichrome, Chino, CA, USA), a confocal scanning unit (CSU-10, Yokogawa Electric Co., Tokyo, Japan), and an image-intensified CCD camera (C2400-80, Hamamatsu Photonics K.K.). The light source was an argon laser with a 488 nm wavelength. The confocal scanning unit obtained real time images through a fluorescence filter (490 nm). The images of the pial microvascular bed through each system were recorded at 30 frames per second to a video cassette recorder (WV-DR7, Sony, Tokyo, Japan) with a video timer (VTG-33, FOR.A Co. Ltd., Tokyo, Japan). All the images were digitized and later analyzed off-line.

BBB permeability. We evaluated BBB permeability using two methods which were widely used in previous studies (14, 19). One aimed to examine the appearance of BBB disruption, while another observed the time-dependent changes in BBB permeability.

The transient extravasation of sodium fluorescein (MW: 376, Sigma-Aldrich, Inc, St. Louis, MO, USA), as an indicator of low molecule leakage, was monitored using the intravital-fluorescence microscope in four rats. Sodium fluorescein (2%, 100 μl/kg) was intravenously injected just before each experimental period and the image of the pia mater, including pial venules and the extravascular region, was recorded under the microscope with a fluorescent excitation wavelength at 490 nm. To evaluate the extent of leakage of the fluorescent dye in detail, the representative fluorescent images of pial venules under each condition were observed using a CLSM. The fluorescence intensity profile of the image including pial venules and the surrounding extravascular region was measured at 5 sec after the dye injection by the Scion Image software (Scion Corp., Frederick, MD, USA).

The extravascular accumulation of FITC-dextran (Mean MW: 250 kDa, Sigma-Aldrich, Inc.), as an indicator of large molecule

leakage, was monitored through the CCW under the intravital-fluorescence microscope in six rats (two out of six rats were also used for evaluation with sodium fluorescein mentioned above). FITC-dextran (25 mg/kg) was intravenously injected 5 min before the pre-measurement period. An image of the pia mater was recorded through the fluorescence microscope with a fluorescent excitation wavelength at 490 nm during each experimental period. The averaged fluorescence intensity in the arbitrary area of the pia mater was measured off line. Only this evaluation was performed twice with or without the exposure to RF-EMF using the same six rats to detect the differences in fluorescent intensity between two time-courses without metabolic clearance of the dye.

Leukocyte behavior. The changes in the behavior of leukocytes were evaluated using the number of leukocytes having interactions with the endothelium of pial venules in six rats. We measured the numbers of sticking leukocytes, which were defined as cells attached to the same endothelium area for more than 30 s. The leukocytes were labeled with rhodamine 6G (0.1 mg/kg, Wako Pure Chemical Industries Ltd, Osaka, Japan), injected intravenously and were examined under the intravital-fluorescence microscope with a fluorescent excitation wavelength at 524 nm. The numbers of sticking leukocytes were counted in a 100 μm length of four pial venules in each rat. In addition, the number was compared between postcapillary venules (8-30 μm) and collecting venules (31-50 μm) to investigate the detailed changes in each pial venule.

Hemodynamics. The changes in the plasma velocity were evaluated using the velocity of microspheres flowing in the pial venules. This was performed simultaneously with the evaluation of leukocyte behavior using the same six rats. Fluorescence microspheres (2.5% solids-latex, 1.0 μm YG, Polysciences Inc., Warrington, PA, USA) were intravenously injected at each experimental period and their motion was observed under the intravital-fluorescence microscope with a fluorescent excitation wavelength at 490 nm. The drifting distance of microsphere flowing along the central axis of pial venules was measured from the video image frame by frame. The velocity was calculated as the distance traveled for 1/30 sec of the video frame. The plasma velocity was expressed as the average of three measurements and values compared between postcapillary venules and collecting venules.

The diameter of pial venules was measured using the sodium fluorescein CLSM image in four rats. Four venules were selected in each rat and the diameter changes of the same region were measured repeatedly.

Window temperature. Changes in the temperature in the CCW were measured using infrared thermography (TVS-5301, Nippon Avionics Co. Ltd., Tokyo, Japan) with or without RF-EMF exposure for three rats in each group. Before and immediately after each exposure to RF-EMF, the parietal region of the rat brain equipped with the CCW was placed under the thermograph and the temperature of the CCW was recorded.

Statistical analysis. All results are presented as means ± standard errors. The statistical analysis was carried out using a Mann-Whitney *U*-test or Kruskal-Wallis test followed by Scheffe test; $p<0.05$ values were considered statistically significant.

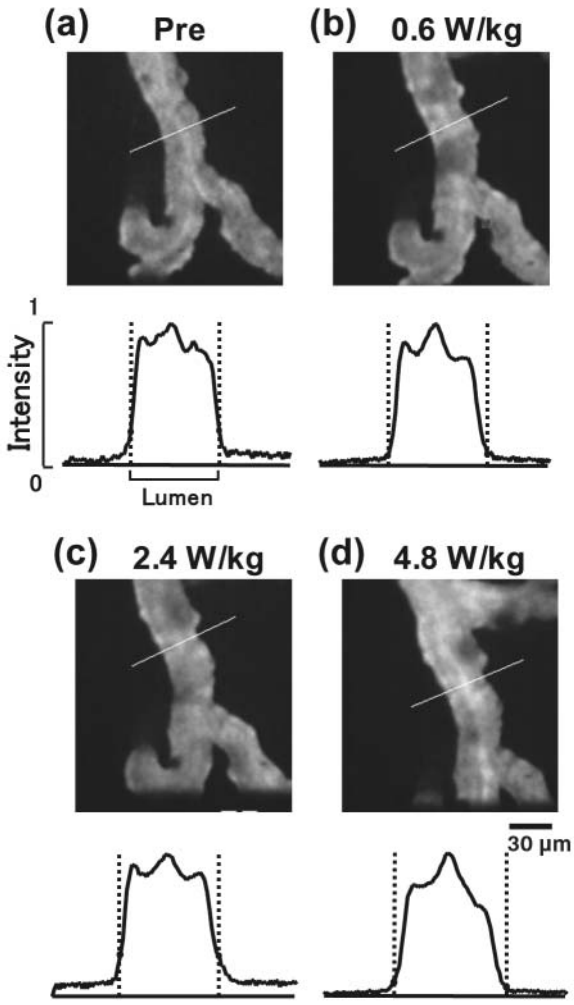

Figure 4. *Image analysis of sodium fluorescein leakage from pial venules. Upper photographs show the images of the pial venules as observed under CLSM. The corresponding graphs below ead. show the fluorescence profiles along the white line indicated in each image. The fluorescence was normalized to the maximum intensity on the pial venule. Although the fluorescence outside the pial venules increased on BBB disruption (data not shown), no increases were seen in pre-measurement (a), 0.6 W/kg (b), 2.4 W/kg (c), or 4.8 W/kg (d) BASAR.*

Results

BBB permeability. The BBB permeability in the rat brain after the 1439 MHz RF-EMF exposure was examined using two types of fluorescent dyes. No leakage of sodium fluorescein in the pia mater was observed at any intensity of RF-EMF exposure. Detailed quantification of the extravasation of sodium fluorescein around pial venules is shown in Figure 4. The increase in fluorescence outside the pial venule, which was seen in the positive control of BBB disruption (data not shown), were not found in any animal after RF-EMF exposure.

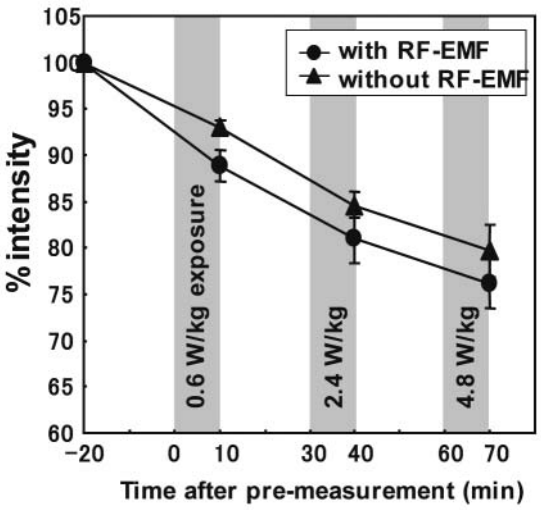

Figure 5. *Time-course of fluorescence intensity in the pia mater region. After intravenous injection of FITC-dextran (Mean MW: 250 kDa), the intensity, indicating accumulation of extravasated FITC-dextran, was monitored until the end of the experiment in six rats. The measurement in each rat was performed twice with or without 1439 MHz RF-EMF exposure. No significant differences were found between the two conditions, with or without the exposure, in each experimental period.*

The intensity of FITC-dextran in the pia mater region decreased time-dependently (Figure 5). However, neither the time-course pattern or the intensity at each experimental period showed any significant differences between the conditions with or without RF-EMF exposure (Figure 5).

Leukocyte behavior. To evaluate the leukocyte behavior in the pial microcirculation after exposure, we measured the number of leukocytes having interactions with the endothelium in pial venules. In postcapillary and collecting venules, no statistical changes in the number of sticking leukocytes compared to the pre-measurement were observed even at maximal BASAR exposure (Figure 6).

Hemodynamics. Plasma velocities and vessel diameters were measured for evaluation of the changes in hemodynamics after the exposure. Although the plasma velocity in postcapillary venules tended to increase at BASAR 2.4 and 4.8 W/kg, there were no significant differences in the velocities compared to pre-measurement or 0.6 W/kg of BASAR values (Figure 7a). In collecting venules, no significant differences in the velocities were found among different exposures (Figure 7b). The changes in diameter of pial venules (n=16) of four rats are shown as a percentage of the pre-exposure diameter. There were no significant differences in the diameter: BASAR 0.6 W/kg=97.8%±2.8%, 2.4 W/kg=97.8%±4.8%, 4.8 W/kg=101.7%±4.3%.

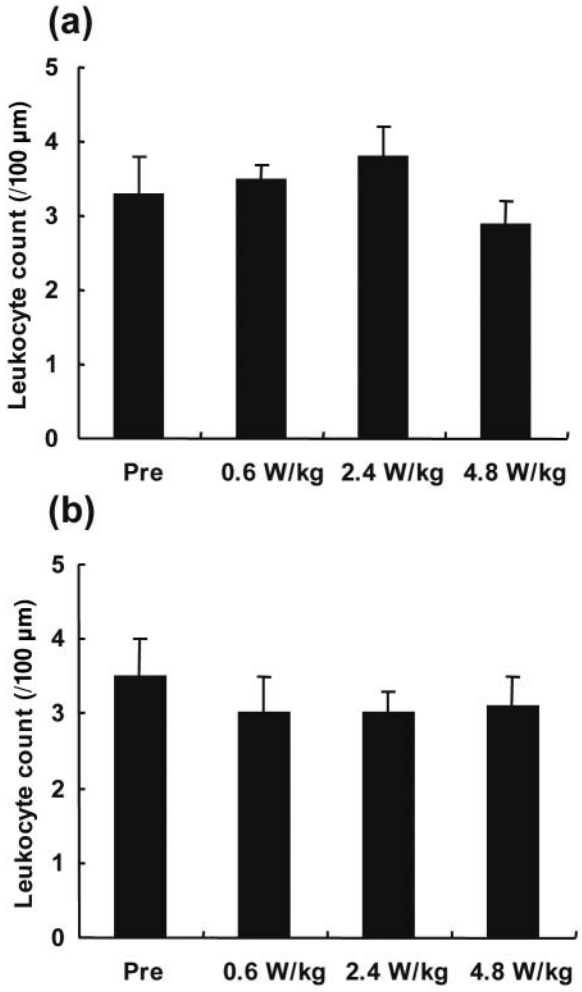

Figure 6. *The numbers of sticking leukocytes having interactions with endothelial cells in pial venules. The change in numbers was monitored in postcapillary venules (a: 8-30 μm, n=12) and collecting venules (b: 31-50 μm, n=12) of six rats. There were no statistically significant changes in the number in postcapillary or collecting venules at any BASAR compared with pre-measurement values.*

Window temperature. The temperature difference between pre- and post-exposure at BASAR 0.6 W/kg, 2.4 W/kg and 4.8 W/kg were –0.45˚C±0.14˚C, 0.20˚C±0.12˚C, and 0.70˚C±0.18˚C, respectively. The corresponding values without exposure were –0.33˚C±0.24˚C, –0.09˚C±0.08˚C, and 0.08˚C±0.17˚C, respectively. A slight temperature increase was found at BASAR 2.4 W/kg and at 4.8 W/kg, but a statistically significant increase was only indicated at 4.8 W/kg ($p<0.05$).

Discussion

This study demonstrates the advantages of our newly developed closed cranial window (CCW) method for evaluating the acute effects of RF-EMF exposure on the

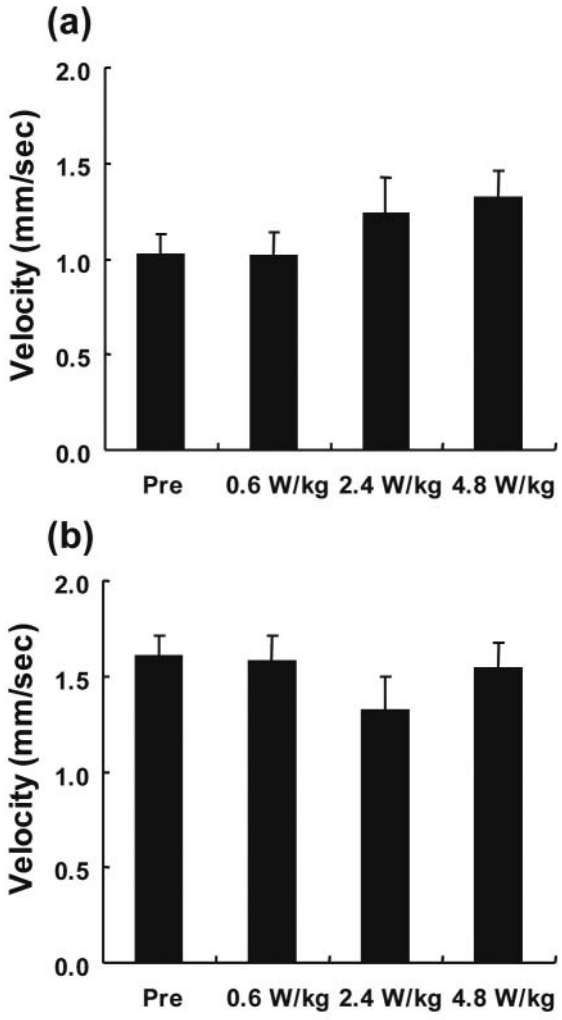

Figure 7. *The plasma velocity in the pial venules. The velocity was measured in postcapillary venules (a: 8-30 μm, n=12) and collecting venules (b: 31-50 μm, n=12) of six rats. There were no statistically significant changes in the velocity in postcapillary or collecting venules at any BASAR compared with pre-measurement values.*

cerebral microcirculation. The four cerebral microcirculatory parameters, BBB permeability, leukocyte behavior, plasma velocity and vessel diameter, evaluated before and after the 10 min exposure to 1439 MHz RF-EMF at 0.6, 2.4, and 4.8 W/kg of BASAR provides a plausible method for the evaluation of effects of EMF from mobile phones on brain tissue. However, no noticeable changes were observed in the four parameters under the given conditions.

Our CCW method allows for direct observation of the cerebral microcirculatory parameters in rat brain exposed to RF-EMF. One of the advantages of this method was the ability to directly observe dynamic changes in the parameters *in vivo*, which cannot be seen in postmortem animals. We succeeded in making the CCW of non-metallic materials (acrylic, plastic and glass) to avoid EMF interaction, and implanting it into the rat parietal region. This enables evaluation of the effects of RF-EMF exposure on the cerebral microcirculation in the same rat repeatedly in the long-term.

No changes in the BBB permeability were found under the present conditions. BBB permeability was evaluated using two fluorescent dyes, sodium fluorescein and FITC-dextran. Sodium fluorescein, as a small molecule, was applied to confirm the BBB disruption (19, 20). On the other hand, FITC-dextran, as a large molecule, is useful for measurement of the time-dependent changes in BBB permeability (21, 22). Under inflammatory conditions, both dyes extravasate from cerebral microvessels, especially venules causing an increase in fluorescent intensity around the microvessels (23). We confirmed these phenomena in our previous study in which a BBB leakage was intentionally induced by cortical perfusion with 1 M mannitol solution (16). Therefore, if the RF-EMF exposure were to induce an inflammatory response in the cerebral microvessels, a transient increase in fluorescent intensity of sodium fluorescein or time-dependent changes in the intensity of FITC-dextran would be found around the pial venules. However, there were no changes even at 4.8 W/kg of the maximal BASAR level. Tsurita *et al.* (8) used the same type of exposure system equipped with a monopole antenna and showed no albumin leakage in the rat brain after 1439 MHz RF-EMF exposure at 2 W/kg of BASAR using histological evaluation. Our results support theirs.

Another microcirculatory parameter, leukocyte behavior, is also important, because leukocytes play a part in immune function in the brain. Under inflammatory conditions, leukocyte behavior indicates augmented adhesion to the endothelial cells in microvessels. Many investigators found the enhancement of leukocyte adhesion in the cerebral microcirculation after brain disorders (24-26) or after experimental application of inflammatory mediators (14, 27, 28). For example, Yuan *et al.* (26) found an increase in the number of sticking leukocytes in rat pial venules after radiation treatment. We assume that if the RF-EMF exposure induces a pathophysiological condition around or inside cerebral microvessels, changes in the leukocyte behavior would be manifested as an increase in the numbers of sticking leukocytes. However, we found no significant difference in the numbers between pre and post exposures at any BASAR value. An increase in endothelial-adhering leukocytes was found in mice after 15 days successive exposure to 50 Hz extremely low frequency EMF in our laboratory (29). However, our present findings suggest that leukocyte behavior is not affected by 1439 MHz RF-EMF, at least under acute exposure.

Inflammatory or pathophysiological responses in the brain are also detected as changes in hemodynamics in

cerebral microcirculation. We focused on plasma velocities and vessel diameters in rat pial venules, because these changes have been recognized under inflammatory conditions (28). In particular, a recent study found that the blood flow in the human brain increased after a pulse-modulated 900 MHz RF-EMF exposure for 30 min (30). Thus, some effects on the plasma velocity and vessel diameters were expected in our animal study. However, in the present study, neither parameter showed any response to the 1439 MHz RF-EMF exposure even at 4.8 W/kg of the maximal BASAR level, which is about 18-fold that used in the human volunteer study (30). The discrepancy of the effect between the human study and ours may derive from a difference of species or exposure conditions.

We used multi-parameter evaluation in our microcirculatory study. Although many investigators have reported the effects of RF-EMF on the cerebral microcirculation, these studies mainly focused on the BBB permeability of cerebral vessels. Moreover, many of them used histological evaluation (4, 8, 11, 31). On the contrary, we not only examined BBB permeability but also other microcirculatory parameters, leukocyte behavior and hemodynamic changes using intravital microscopic observation, and demonstrated no effects of 1439 MHz RF-EMF on any of these parameters. Several reports have shown that the BBB disruption is accompanied with changes in leukocyte behavior (14, 32) or hemodynamics (33) under inflammatory conditions in the rat brain. Therefore, our findings of the multi-parameter evaluation supported the lack of increase in the BBB permeability under RF-EMF exposure as previously reported (8-11).

The basic restriction of local exposure to RF-EMF for general environment in the ICNIRP guidelines is 2 W/kg (17). Several studies evaluating changes in BBB permeability after RF-EMF exposure found that the exposure under a lower SAR level than 2 W/kg did not modify the BBB permeability (8, 9, 31). In the present study, our result on BBB permeability supports their findings. Moreover, the four microcirculatory parameters, including BBB permeability, showed no significant changes after the exposure to 1439 MHz RF-EMF even at 4.8 W/kg of BASAR. Therefore, these findings suggest that the exposure of animals to RF-EMF lower than the basic restriction of the local exposure (2 W/kg) does not induce any changes in cerebral microcirculation, if a presumption of the existence of a dose-response relationship between the intensity of the RF-EMF exposure and biological responses is accepted.

Conclusion

We introduced the CCW method for evaluating the effects of acute exposure for 10 min to 1439 MHz RF-EMF at 0.6, 2.4, 4.8 W/kg of BASAR on the cerebral microcirculation. The four microcirculatory parameters examined, *i.e.* BBB permeability, leukocyte behavior, plasma velocity and vessel diameter, revealed no significant differences in values between pre and post 1439 MHz RF-EMF exposure of the rat brain. These findings suggest that, with acute exposure, there were no changes in the cerebral microcirculation under the RF-EMF exposure conditions, even at a level 2.4-fold higher than the local exposure limit of the ICNIRP guidelines. However, further studies are required under other exposure conditions (*e.g.*, subchronic exposure, other frequencies) to validate our conclusion.

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References

1 Lin JC and Lin MF: Microwave hyperthermia-induced blood-brain barrier alterations. Radiat Res *89*: 77-87, 1982.
2 Goldman H, Lin JC, Murphy S and Lin MF: Cerebrovascular permeability to 86Rb in the rat after exposure to pulsed microwaves. Bioelectromagnetics *5*: 323-330, 1984.
3 Moriyama E, Salcman M and Broadwell RD: Blood-brain barrier alteration after microwave-induced hyperthermia is purely a thermal effect: I. Temperature and power measurements. Surg Neurol *35*: 177-182, 1991.
4 Salford LG, Brun A, Sturesson K, Eberhardt JL and Persson BR: Permeability of the blood-brain barrier induced by 915 MHz electromagnetic radiation, continuous wave and modulated at 8, 16, 50, and 200 Hz. Microsc Res Tech *27*: 535-542, 1994.
5 Salford LG, Brun AE, Eberhardt JL, Malmgren L and Persson BR: Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environ Health Perspect *111*: 881-883, 2003.
6 Aubineau P and Töre F: 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. Friendrich G (ed.). Forschungsgemeinschaft Funk e. V. Germany, pp. 82-83, 2005.
7 Schirmacher A, Winters S, Fischer S, Goeke J, Galla HJ, Kullnick U, Ringelstein EB and Stogbauer F: Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the blood-brain barrier *in vitro*. Bioelectromagnetics *21*: 338-345, 2000.
8 Tsurita G, Nagawa H, Ueno S, Watanabe S and Taki M: Biological and morphological effects on the brain after exposure of rats to a 1439 MHz TDMA field. Bioelectromagnetics *21*: 364-371, 2000.
9 Kuribayashi M, Wang J, Fujiwara O, Doi Y, Nabae K, Tamano S, Ogiso T, Asamoto M and Shirai T: Lack of effects of 1439 MHz electromagnetic near field exposure on the blood-brain barrier in immature and young rats. Bioelectromagnetics *26*: 578-588, 2005.

10 Franke H, Streckert J, Bitz A, Goeke J, Hansen V, Ringelstein EB, Nattkamper H, Galla HJ and Stogbauer F: Effects of Universal Mobile Telecommunications System (UMTS) electromagnetic fields on the blood-brain barrier *in vitro*. Radiat Res *164*: 258-269, 2005.
11 Finnie JW, Blumbergs PC, Cai Z, Manavis J and Kuchel TR: Effect of mobile telephony on blood-brain barrier permeability in the fetal mouse brain. Pathology *38*: 63-65, 2006.
12 Dirnagl U: Cerebral ischemia: the microcirculation as trigger and target. Prog Brain Res *96*: 49-65, 1993.
13 Uhl E, Pickelmann S, Baethmann A and Schurer L: Influence of platelet-activating factor on cerebral microcirculation in rats: part 1. Systemic application. Stroke *30*: 873-879, 1999.
14 Mayhan WG: Leukocyte adherence contributes to disruption of the blood-brain barrier during activation of mast cells. Brain Res *869*: 112-120, 2000.
15 Schilling L and Wahl M: Opening of the blood-brain barrier during cortical superfusion with histamine. Brain Res *653*: 289-296, 1994.
16 Masuda H, Ushiyama A, Okano H and Ohkubo C: Chronological observation of the pial microcirculation using a chronically implanted cranial window method in the rat. *In*: Microcirculation Annual 2000. Tsuchiya M, Asano M, Tokita T and Takahashi K (Eds). Nihon-Igakukan. Japan, pp. 151-152, 2000.
17 International Commission on Non-Ionizing Radiation Protection: Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz) Health Phys *74*: 494-522, 1998.
18 Watanabe S, Taki M and Yamanaka Y: A microwave exposure setup for the head of Sprague-Dawley rats. Proc. XXVIth General Assembly of the International Union of Radio Science (URSI), pp. 863, 1999.
19 Kawamura S, Schurer L, Goetz A, Kempski O, Schmucker B and Baethmann A: An improved closed cranial window technique for investigation of blood-brain barrier function and cerebral vasomotor control in the rat. Int J Microcirc Clin Exp *9*: 369-383, 1990.
20 Yong T and Linthicum DS: Microvascular leakage in mouse pial venules induced by bradykinin Brain Inj *10*: 385-393, 1996.
21 Mayhan WG: VEGF increases permeability of the blood-brain barrier via a nitric oxide synthase/cGMP-dependent pathway. Am J Physiol *276*: C1148-1153, 1999.
22 Mayhan WG: Nitric oxide donor-induced increase in permeability of the blood-brain barrier. Brain Res *866*: 101-108, 2000.
23 Olesen SP: Leakiness of rat brain microvessels to fluorescent probes following craniotomy. Acta Physiol Scand *130*: 63-68, 1987.
24 Kubes P, Kurose I and Granger DN: NO donors prevent integrin-induced leukocyte adhesion but not P-selectin-dependent rolling in postischemic venules. Am J Physiol *267*: H931-937, 1994.
25 Ritter LS, Orozco JA, Coull BM, McDonagh PF and Rosenblum WI: Leukocyte accumulation and hemodynamic changes in the cerebral microcirculation during early reperfusion after stroke. Stroke *31*: 1153-1161, 2000.
26 Yuan H, Gaber MW, McColgan T, Naimark MD, Kiani MF and Merchant TE: Radiation-induced permeability and leukocyte adhesion in the rat blood-brain barrier: modulation with anti-ICAM-1 antibodies. Brain Res *969*: 59-69, 2003.
27 Lindauer U, Dreier J, Angstwurm K, Rubin I, Villringer A, Einhaupl KM and Dirnagl U: Role of nitric oxide synthase inhibition in leukocyte-endothelium interaction in the rat pial microvasculature. J Cereb Blood Flow Metab *16*: 1143-1152, 1996.
28 Uhl E, Pickelmann S, Rohrich F, Baethmann A and Schurer L: Influence of platelet-activating factor on cerebral microcirculation in rats: part 2. Local application. Stroke *30*: 880-886, 1999.
29 Ushiyama A, Masuda H, Hirota S and Ohkubo C: Subchronic effects on leukocyte-endothelial interactions in mice by whole body exposure to extremely low frequency electromagnetic fields. In Vivo *18*: 425-432, 2004.
30 Huber R, Treyer V, Schuderer J, Berthold T, Buck A, Kuster N, Landolt HP and Achermann P: Exposure to pulse-modulated radio frequency electromagnetic fields affects regional cerebral blood flow. Eur J Neurosci *21*: 1000-1006, 2005.
31 Fritze K, Sommer C, Schmitz B, Mies G, Hossmann KA, Kiessling M and Wiessner C: Effect of global system for mobile communication (GSM) microwave exposure on blood-brain barrier permeability in rat. Acta Neuropathol (Berl) *94*: 465-470, 1997.
32 Gaber MW, Yuan H, Killmar JT, Naimark MD, Kiani MF and Merchant TE: 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 Res Brain Res Protoc *13*: 1-10, 2004.
33 Mayhan WG: Effect of lipopolysaccharide on the permeability and reactivity of the cerebral microcirculation: role of inducible nitric oxide synthase. Brain Res *792*: 353-357, 1998.

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