Journal of Applied Biomedicine 15 (2017) 112–118

Contents lists available at ScienceDirect

Journal of Applied Biomedicine

journal homepage: www.elsevier.com/locate/jab

Original Research Article

Electromagnetic field (10 Hz, 1 mT) protects mesenchymal stem cells from oxygen-glucose deprivation-induced cell death by reducing intracellular Ca^{2+} and reactive oxygen species

Jong Hyeok Jung*, Jae Young Kim*

Department of Life Science, Gachon University, Seongnam-Si, Republic of Korea

ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form 25 October 2016
Accepted 3 November 2016
Available online 24 November 2016

Keywords:
Ca^{2+}
Electromagnetic fields
Mesenchymal stem cell
Oxygen and glucose deprivation
Reactive oxygen species

ABSTRACT

Protective effects of electromagnetic fields (EMFs) against oxygen and glucose deprivation (OGD)-induced human mesenchymal stem cell (MSC) death were studied. Cell survival, intracellular calcium and ROS/RNS levels were measured after culturing MSCs for 3 h under OGD with or without EMF exposure. The survival rate of cells cultured under OGD condition was significantly reduced compared to control cells, while cells cultured in OGD with 10 Hz/1 mT EMF exposure had higher survival ratio than that in equivalent non-exposed cells. This protective effect of EMF was not observed at different frequency/intensity combinations such as 10 Hz/0.01 mT, 10 Hz/0.1 mT, 50 Hz/1 mT and 100 Hz/1 mT. ROS/RNS levels of cells cultured under OGD conditions significantly increased compared to the control level while 10 Hz/1 mT EMF alleviated this effect. Intracellular calcium levels in OGD group were higher than control while those in OGD plus 10 Hz/1 mT EMF group were significantly lower than OGD group. Addition of Ca^{2+} chelator promoted protective effects of EMF against OGD-induced MSC death. Our results suggest that 10 Hz/1 mT EMF exposure protects MSCs from OGD-induced cell death and the underlying mechanisms of the protection are reduction of intracellular levels of Ca^{2+} and ROS/RNS.
© 2016 Faculty of Health and Social Sciences, University of South Bohemia in Ceske Budejovice. Published by Elsevier Sp. z o.o. All rights reserved.

Introduction

Ischemic brain injury can be caused by cardiac arrest, profound hypotension or hypovolaemia due to blood loss (Howard et al., 2011). Two major mechanisms of neural damage during ischemia are reduction of oxygen and glucose supply (Guo et al., 2011). Since patients with ischemic brain injury suffer from persistent neurological deficits and severe irreversible neuronal cell loss, cell-based therapeutic approaches have attracted a great deal of attention (Scheibe et al., 2012). Mesenchymal stem cells (MSC) are attractive cytotherapy candidates for ischemic neuronal injury owing to their neuroprotective (Crigler et al., 2006; Scuteri et al., 2006; Wilkins et al., 2009) and immunosuppressive properties (Aggarwal and Pittenger, 2005; Chen et al., 2006). Indeed, functional recovery has been achieved in a rodent ischemic neuronal injury model when MSC were injected directly into the infarct site (Chen et al., 2001, 2003; Kurozumi et al., 2005; Nomura et al., 2005; Zhao et al., 2006) or administered intravenously (Chen et al., 2003). Such protective effects of MSC are thought to be due to the secretion of various growth factors including glial-derived neurotrophic factor, nerve growth factor, and vascular endothelial growth factor (Caplan and Dennis, 2006; Chen et al., 2002; Parekkadan et al., 2007; Wagner et al., 2007). However, the regenerative potential of MSC has been considered to be transient (Lee et al., 2009). One of the reasons for this temporal effect may be unfavorable conditions for MSC such as hypoxia or inflammation. Hypoxia suppresses the growth (Holzwarth et al., 2010) and differentiation (Holzwarth et al., 2010; Yang et al., 2011) of MSC, and hypoxia plus serum deprivation significantly promote cell death (Zhu et al., 2006).

Electromagnetic fields (EMFs) are capable of altering cell proliferation and differentiation, cell cycles, apoptosis, DNA replication, and gene expression (Pesce et al., 2013). In recent studies with human cells, extremely low frequency EMF has been shown to induce production of reactive oxygen species (ROS),

Abbreviations: DCFH-DA, 2′,7′-dichlorofluorescin-diacetate; EMFs, electromagnetic fields; I/R, ischemia-reperfusion; MSC, mesenchymal stem cell; OGD, oxygen and glucose deprivation; ROS, reactive oxygen species; EDTA, ethylenediaminetetraacetic acid; NAC, *N*-acetylcysteine.

* Corresponding authors at: Department of Life Science, Gachon University, Seongnam-daero 1342, Seongnam-Si, Gyeonggi-do, 461-701, Republic of Korea.
E-mail addresses: bright5887@naver.com (J.H. Jung), jykim85@gachon.ac.kr (J.Y. Kim).

http://dx.doi.org/10.1016/j.jab.2016.11.003
1214-021X/© 2016 Faculty of Health and Social Sciences, University of South Bohemia in Ceske Budejovice. Published by Elsevier Sp. z o.o. All rights reserved.

which may be harmful to the cells (Henrykowska et al., 2009; Park et al., 2013; Reale et al., 2014). However, in other studies, extremely low frequency EMF has been shown to exert protective effects against rat cardiac ischemia-reperfusion (I/R) injury (George et al., 2008) and to lead an increase in ROS production of phorbol-12-myristate-13-acetate stimulated neutrophils, which may have beneficial effect on inflammatory processes at the site of infection (Poniedzialek et al., 2013).

In this study, we examined a protective effect of EMF against oxygen and glucose deprivation (OGD)-induced cell death of human MSC using an *in vitro* OGD model previously established to mimic pathological conditions of central nervous system ischemia (Beck et al., 2003). Additionally, we identified possible mechanisms for protective effects of EMF.

Materials and methods

Cell culture

Human MSC were obtained from umbilical cord matrix (PromoCell GmbH, Heidelberg, Germany). Cells were grown in MSC growth medium (PromoCell) supplemented with 1x antibiotic-antimycotic (Invitrogen, GIBCO BRL, MD, USA) and 5 μg/ml Plasmocin (Invivogen, San Diego, CA, USA) in 100 mm-cell culture dishes. Cells were grown at 37 °C in a 5% CO_2 atmosphere under humid conditions and passaged by 0.25% trypsin-EDTA (Invitrogen) when they reached 80–90% confluence.

Oxygen and glucose deprivation (OGD) and electromagnetic field exposure

Cell injury during ischemia occurs due to reduced oxygen and glucose supply. Thus, culturing cells under OGD conditions is thought to mimic the pathological conditions of ischemia. In the present study, MSC were subjected to OGD as previously described (Beck et al., 2003). The same batch of human MSCs in exponential growth phase was randomly divided into three groups: 1) a control group cultured under normal condition (20% O_2 in the presence of glucose), 2) an OGD group incubated in OGD condition (1% O_2 in the absence of glucose), and 3) an OGD + EMF group cultured under OGD condition with EMF exposure. OGD group and OGD + EMF group were placed outside and inside the Helmholtz coil system in the same incubator, respectively, while control group was placed in a separate incubator without an EMF exposure system, because of different O_2 conditions. MSC were washed twice with PBS, then incubated in OGD medium composed of Dulbecco's modified Eagle's medium (DMEM, Welgene, Gyongsan, Korea) without glucose supplemented with 1× antibiotic-antimycotic (Invitrogen) and 5 μg/ml Plasmocin (Invivogen) in a Heracell gas addition incubator (Heraeus Instruments GmbH, Hanau, Germany) containing 94% N_2, 1% O_2 and 5% CO_2 for 3 h. To investigate the effects of Ethylenediaminetetraacetic acid (EDTA) and N-acetylcysteine (NAC), 0.5 M EDTA solution (pH 8.0, Amresco, Solon, USA) and 0.5 M NAC(Sigma-Aldrich, St. Louis, MO, USA) solution was added to the OGD medium to obtain a final concentration of 2 mM EDTA or 1 mM NAC.

The EMF was generated by two coils arranged in a Helmholtz configuration oriented to produce a vertical magnetic field with parameters as follows: axial symmetry—2D; diameter of two coils—inner 15 cm, outer 26 cm; distance between coils—18 cm; coil diameter—18AWG; number of loops—1000. The system was placed in the center of a gas addition incubator (Fig. 1), and cells were exposed to various conditions with continuous sinusoidal extremely low frequency EMF (10, 50, or 100 Hz/1 mT and 10 Hz/0.01, 0.1, or 1 mT) using an AC power source (PCR-1000L, Kikusui, Japan). Cell culture dishes were maintained at the center of a uniform field area. A relatively uniform magnetic field was detected inside the coil but it was not detected outside the coil by a tesla meter TM-701 (Kanetech-Bensenville, IL, USA). A thermometric probe placed inside and outside the EMF generator revealed no significant temperature difference between culture media of exposed or unexposed cells.

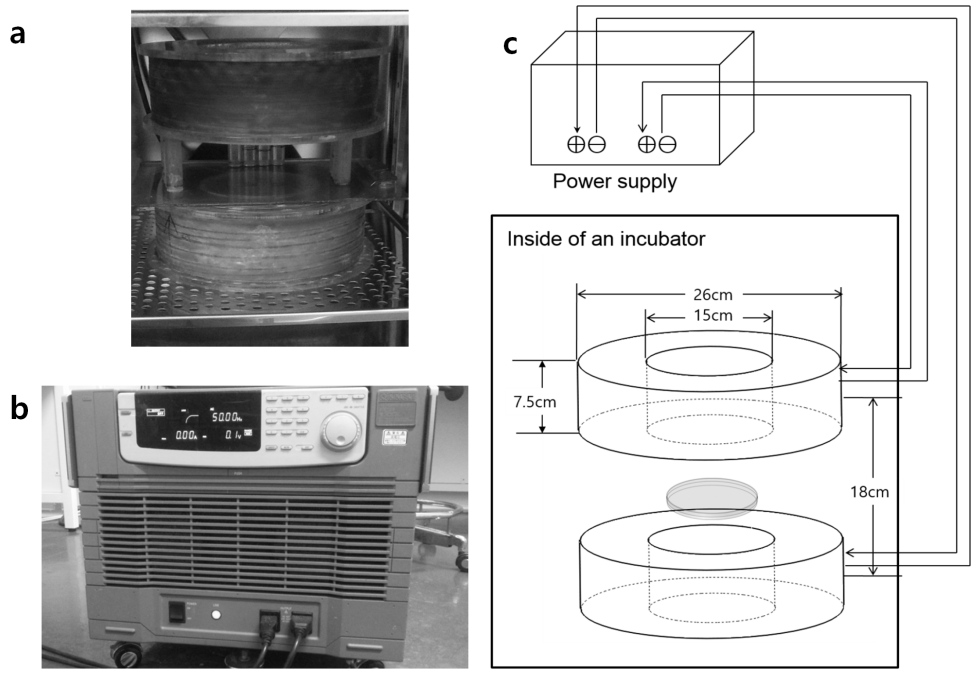

Fig. 1. Overview of EMF exposure system. A photograph of a helmholtz coil installed in the incubator (a), power supply (b) and a schematic diagram of coils with specification (c).

Cell viability measurement

Cells were plated at a density of 1.25×10^5 in 500 μl of culture medium in 4-well plates. Cells were trypsinized and diluted in high-glucose containing DMEM medium (Welgene, Gyongsan, Korea), after which 10 μl aliquots were stained with 0.4% trypan blue (Acros Organics, New Jersey, USA) in PBS to determine the cell viability. The number of viable MSC was counted under a light microscope using a hemocytometer (Paul Marienfield GmbH & Co. KG, Germany) at 100× magnification.

EZ-Cytox Enhanced Cell Viability Assay Kit (Daeil lab service, Seoul, Korea) was also used to confirm the results. Briefly, plated cells were treated with Ez-cytox solution and incubated for 30 min at 37 °C. Cell culture supernatants were then placed into 96-well plates and the absorbance was measured using a μQuant Scanning Microplate Spectrophotometer (Bio-Tek, Winooski, VT, USA) at 450 nm.

Fluorometric determination of reactive oxygen and nitrogen species (ROS/RNS)

2′,7′–dichlorofluorescin-diacetate (DCFH-DA) is a general dye to detect changes in redox balance as that changes of its signal can be a result of intracellular ROS/RNS generation (Gomes et al., 2005). Cells were plated at a density of 2.5×10^4–3.0×10^4 in 200 μl of culture medium in 96-well black Plates 1 day before treatment. Following exposure to 20% or 1% O_2 for 3 h, cells were loaded with 10 μM DCFH-DA (Sigma-Aldrich) for 30 min at 37 °C. The fluorescence was then measured at an excitation wavelength of 485 nm and emission wavelength of 535 nm using a Victor X4 Multilabel Plate Reader (Perkin Elmer, MA, USA).

Fluorometric determination of calcium ions

Cells were plated at a density of 2.5×10^4 in 200 μl of culture medium in 96-well black Plates 1 day before treatment. Following exposure to 20% or 1% O_2 for 0.5, 1, 2 or 3 h, cells were washed with calcium-free HBSS containing 20 mM HEPES (assay buffer, Molecular Probes, Eugene, OR, USA). Next, 100 μl Fluo-4-NW-dye-mix (Molecular Probes) was added, after which the samples were incubated for 30 min at 37 °C, followed by 30 min incubation in the dark at room temperature. The fluorescence was measured with an excitation wavelength of 485 nm and emission wavelength of 535 nm using a Victor X4 Multilabel Plate Reader (PerkinElmer).

Statistical analysis

Significant differences among groups were identified by one-way analysis of variance (ANOVA) with Tukey's HSD post hoc test using SPSS software (version 12.0; SPSS, Inc., Chicago, IL, USA). All experiments were performed in duplicate or triplicate. Data from at least two independent experiments were pooled for statistical analysis. Values are expressed as the means ± standard deviation (SD). Differences were considered significant at p-value < 0.05.

Results

EMF protects MSC from OGD-induced cytotoxicity

We investigated the protective effects of extremely low frequency of EMF on survival of human MSC using an *in vitro* oxygen and glucose deprivation (OGD) model. To determine whether the protective effect depends on the frequencies of the

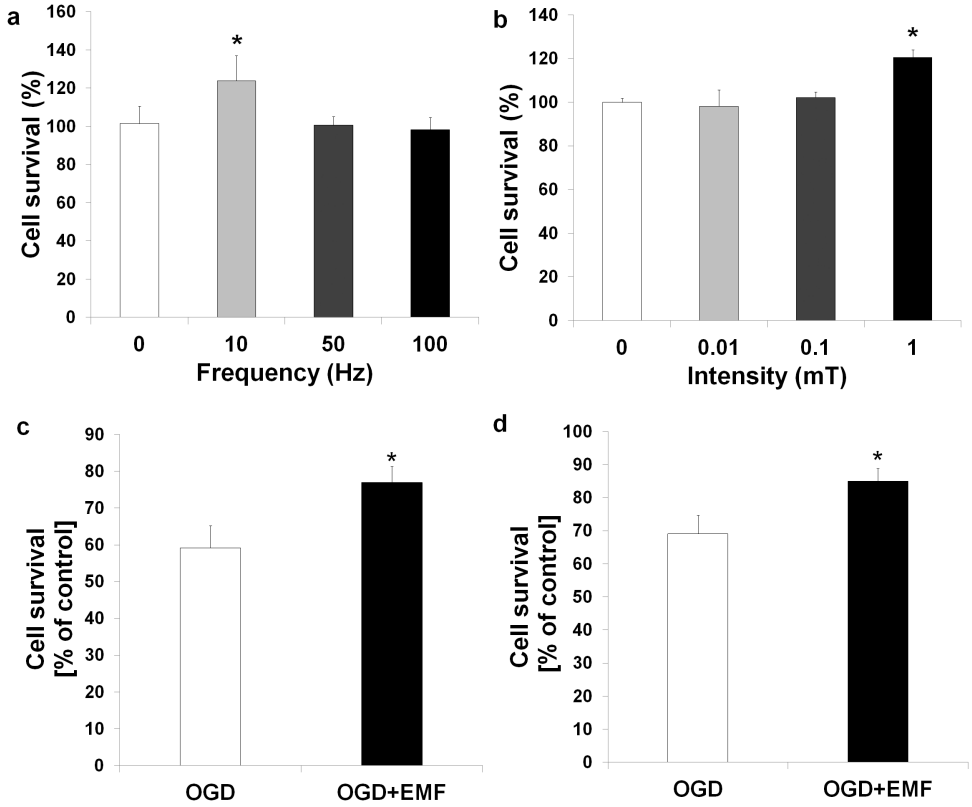

Fig. 2. EMF protects MSC from OGD-induced cytotoxicity and the protective effects are dependent on frequency and intensity. (a) Cells were exposed to 1 mT EMF at various frequencies (10, 50 or 100 Hz). (b) Cells were exposed to 10 Hz EMF at different intensities (0.01, 0.1 or 1 mT). Cell survival was determined by Ez-Cytox cell viability assay. (c and d) Cells were exposed to 10 Hz/1 mT EMF and cell survival was determined by trypan blue staining (c) or Ez-Cytox assay (d). Bar graphs represented as mean ± S.D. of two (a, b and d) or three (c) independent experiments ($n = 9$ or 12). *$p < 0.05$ compared with the control.

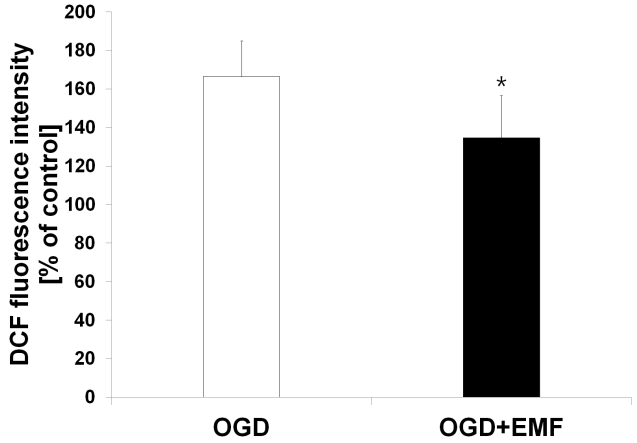

Fig. 3. EMF reduces OGD-induced intracellular ROS/RNS production of MSC. Bar graphs represented as mean ± S.D. of two independent experiments ($n = 6$). $^*p < 0.05$ compared with the control.

field, we investigated cell survival after exposure to fields at three different frequencies (10, 50 or 100 Hz). 10 Hz/1 mT EMF exerted a protective effect, while 50 or 100 Hz/1 mT EMF exposure did not, indicating a frequency-specific protective effect (Fig. 2a). Next, we tested the intensity specificity using three different field intensities (0.01, 0.1 or 1 mT). 0.01 or 0.1 mT/10 Hz EMF exposure did not exert a protective effect, while 1 mT/10 Hz EMF protected the cells (Fig. 2b). The survival rate of the cells cultured under OGD condition was reduced by approximately 40% relative to the control cells. However, the survival rate of the cells exposed to 10 Hz/1 mT EMF during cell culture under the same OGD condition was about 20% higher than that in equivalent non-exposed cells (Fig. 2c). The results were confirmed by Ez-Cytox cell viability assay kits and found to be similar to those obtained by trypan blue staining (Fig. 2d). These results demonstrated that the protection of MSC from OGD-induced cell death requires EMF at a specific frequency (10 Hz) and intensity (1 mT).

EMF reduces OGD-induced intracellular ROS/RNS and calcium levels of MSC

We examined intracellular ROS/RNS levels of MSCs since it is known that OGD-induced cell death is associated with increased cellular ROS levels (Howard et al., 2011). ROS/RNS levels of cells cultured under OGD increased to approximately 165% of the control levels, while those of cells exposed to 10 Hz/1 mT EMF were about 135% of the control (Fig. 3). To determine whether

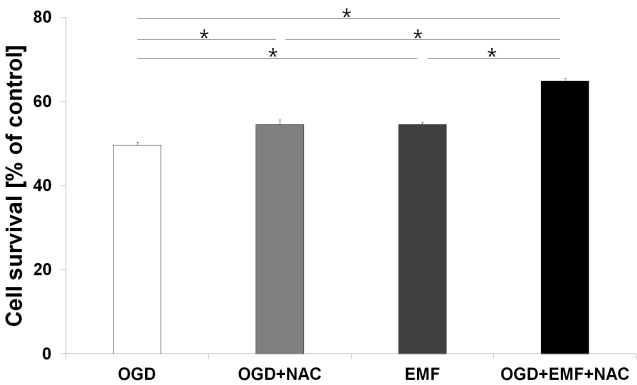

Fig. 4. NAC promotes the suppressive effects of EMF against OGD-induced cell death. Bar graphs represented as mean ± S.D. of two independent experiments ($n = 9$). $^*p < 0.05$ compared with the control.

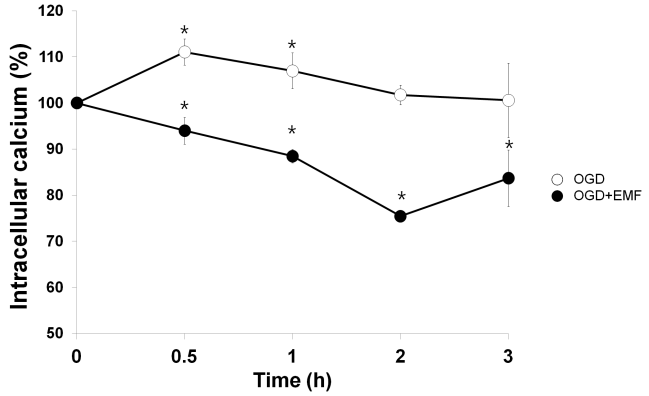

Fig. 5. EMF reduces intracellular calcium levels of MSC cultured under OGD conditions. Graphs represented as mean ± S.D. of two independent experiments ($n = 9$). $^*p < 0.05$.

simultaneous scavenging of ROS by EMF exposure and antioxidant treatment exerts more protective effects, we investigated cell survival after exposure to 10 Hz/1 mT EMF and 1 mM NAC. As shown in Fig. 4, co-exposure to 1 mM NAC and 10 Hz/1 mT EMF exerted more profound effects than exposure to EMF alone.

It is well known that OGD results in an early increase in intracellular calcium level in neurons (Nikonenko et al., 2005; Pisani et al., 1998); therefore, we examined the calcium levels. Intracellular calcium levels in the OGD group increased at 30 min and 1 h and then returned to the control levels, while those in the OGD plus 10 Hz/1 mT EMF group were significantly lower than in the OGD group at all time points tested (Fig. 5).

We next tested the additive effects of EDTA, which is a chelating agent for metal ions such as Ca^{2+} and Fe^{3+}. 10 Hz/1 mT EMF exposure decreased intracellular calcium levels in a time-dependent manner, and this reduction became more profound when cells were subjected to EMF together with EDTA (Fig. 6). After culturing cells for 3 h under OGD, EMF exposure alone and EMF plus EDTA treatment resulted in approximately 25% and 40% of reduction in intracellular calcium levels, respectively. Addition of EDTA without EMF exposure also decreased the intracellular calcium levels. In addition, EDTA was found to exert an additive effect on 10 Hz/1 mT EMF-induced reduction of intracellular ROS/RNS production. OGD-induced intracellular ROS/RNS production was significantly reversed by the addition of EDTA together with 10 Hz/1 mT EMF exposure (Fig. 7a). Similar to the ROS/RNS results, additive effects of EDTA on the protection of MSC from OGD-

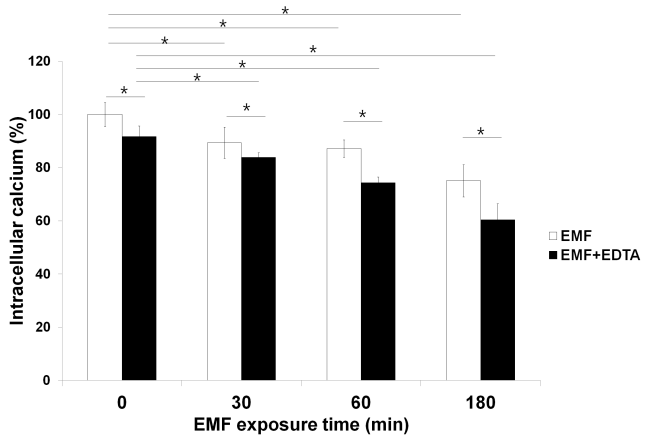

Fig. 6. EDTA promotes the suppressive effects of EMF against OGD-induced rise in intracellular calcium. Fig. 5 Bar graphs represented as mean ± S.D. of two independent experiments ($n = 9$). $^*p < 0.05$.

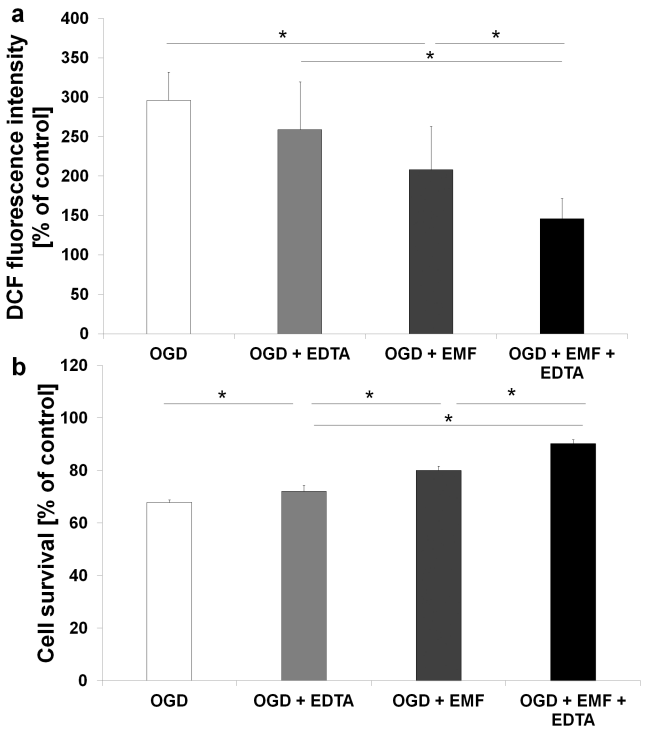

Fig. 7. EDTA promotes the suppressive effects of EMF against OGD-induced ROS/RNS production (a) and cell death (b). Bar graphs represented as mean ± S.D. of two (a) or three (b) independent experiments ($n = 8$ or 9). $^*p < 0.05$ compared with the control.

induced cell death by 10 Hz/1 mT EMF exposure were observed (Fig. 7b).

Discussion

The major findings of this study were 10 Hz/1 mT EMF exposure inhibits OGD-induced cell death of human MSC and reduces OGD-induced elevation of intracellular calcium and ROS/RNS levels. Although we cannot rule out the possibility that combinations of frequencies and intensities of the field other than 10 Hz/1 mT also have protective effect, our recent studies using *in vitro* OGD- or hypoxia/re-oxygenation (H/R) models revealed that only two combinations of frequencies and intensities such as 10 Hz/1 mT (Duong and Kim, 2016; Lim et al., 2015) and/or 50 Hz/1 mT of EMF (Duong and Kim, 2016) are protective against OGD- or H/R-induced cell death among total nine different combinations of frequencies and intensities tested in these studies, suggesting that there is some frequency- and intensity-selectivity.

The main mechanism of OGD-induced cell death is an increase in intracellular calcium and/or ROS levels. Previous studies suggested that ischemia-induced neuronal injury is caused by intracellular calcium overload, which activates enzymes that mediate destruction of proteins, lipids, and nucleic acids through a cascade of cytotoxic events (Choi, 1995, 1988; Siesjo and Bengtsson, 1989). Similarly, studies using an OGD model demonstrated an increase in intracellular Ca^{2+} levels mediated by calcium influx *via* voltage-gated Ca^{2+} channels that was a key event triggering OGD-induced cell death (Nikonenko et al., 2005; Pisani et al., 1998). Therefore, calcium-channel blocking drugs effectively protected neuronal cells from OGD-induced cell injury (Kornekov et al., 2000; Nikonenko et al., 2005; Rahbar-Roshandel et al., 2007).

Since EMF exposure is a physical non-invasive treatment with a low risk of side effects and no problems with drug interactions, our finding that EMF exposure suppressed the OGD-induced rise in intracellular Ca^{2+} are noteworthy. However, our results are contrary to previous findings that 50 or 60 Hz EMF induced an increase in intracellular Ca^{2+} levels through voltage-gated calcium channel-mediated Ca^{2+} influx in various cell types (Grassi et al., 2004; Lindstrom et al., 1995; Lisi et al., 2006; Morgado-Valle et al., 1998; Piacentini et al., 2008; Zhang et al., 2010). These finding suggested that EMF exposure changes the electrical voltage-gradient across the plasma membrane and may therefore stimulate voltage-gated calcium channels through their voltage-gated properties (Pall, 2013). However, recent study reported that 50 Hz EMF does not affect intracellular calcium levels in human neutrophils (Golbach et al., 2015). The reason for the discrepancy between the previous results and ours is currently unclear; however, it is possible that it occurred due to differences in the experimental conditions. All studies mentioned above engaged 50 or 60 Hz EMF, which is a commonly used frequency of electric current. Indeed, many studies utilized 50 or 60 Hz EMF since the relationship between cancer and EMF was first suggested (Wertheimer and Leeper, 1979), while few studies have been conducted using other frequencies of the fields. We investigated different combinations of the frequencies and intensities of the fields and only found protective effects when 10 Hz and 1 mT of EMF were used. In addition, we applied EMF to cells cultured under OGD conditions, which is known to induce a rise in intracellular Ca^{2+} levels, while the above studies used normal culture conditions. Similar to our study, EMF reduced intracellular Ca^{2+} levels increased by phorbol-12-myristate-13-acetate (Conti et al., 1985) and concanavalin A (Walleczek and Liburdy, 1990) that are known to induce calcium influx.

OGD-induced intracellular ROS production causes neuronal cell death (Domoki et al., 2009; Guo et al., 2011; Shi and Liu, 2006), and three enzymes (or enzyme complexes) have been suggested as ROS sources in the OGD model. The NADPH oxidase complex is known to be involved in ROS production during I/R, (Walder et al., 1997; Wang et al., 2006) and in the OGD model (Abramov et al., 2007; Beske and Jackson, 2012; Suh et al., 2008). The second source of cellular ROS during I/R injury is xanthine oxidase (Harrison, 2004; Manning et al., 1984). The last source is mitochondrial enzyme complex, although its significance as a ROS generator is lower than the other two sources because of its transient ROS production (Abramov et al., 2007). Activities of NADPH oxidase and xanthine oxidase are known to depend on cellular Ca^{2+} concentrations (Bánfi et al., 2001; Dykens et al., 1987). Given the intimate relationship between intracellular Ca^{2+} levels and the activities of these ROS-producing enzymes, the EMF exposure-induced reduction of intracellular Ca^{2+} is thought to be a decisive factor for the protection of MSC from OGD insults. Further supporting this notion is our result showing inhibitory effects of EDTA on the OGD-induced increase in intracellular Ca^{2+} levels and cell death. Such additive protective effects of EDTA against OGD insults have been reported in other studies (Kotani et al., 2008). Our results are also in good agreement with previous suggestions that OGD-induced Ca^{2+} influx is a key step in OGD-induced cell death (Duong and Kim, 2016; Lim et al., 2015).

In the present study, we have used a DCFH-DA which is commonly used for detecting intracellular ROS. However, some limitations of this dye to detect ROS, such as its lack of direct interaction with H_2O_2 and its reactivity with reactive nitrogen species, have been suggested in a recent review article (Kalyanaraman et al., 2012). Therefore, it is required to confirm our experiments by use of different types of probes such as boronate-based fluorescent probes (Miller et al., 2005) in future studies. Furthermore, it is required to elucidate the exact sequence of events related to redox balance that takes place in the cells in future studies, although the magnitude of changes in DCF signal is high enough to conclude that oxidative stress is implicated in OGD

toxicity and that this effect is ameliorated to some extent by 10 Hz/1 mT EMF.

Although we demonstrated the protective effects of 10 Hz/1 mT EMF against OGD-induced MSC death *in vitro*, it is unknown whether these cells are functional. Therefore, *in vitro* studies to characterize the EMF-exposed cells at the phenotypic and functional levels and further studies using an animal model of ischemic brain injury are needed to be conducted.

Conclusions

Our results suggest that 10 Hz/1 mT EMF exposure inhibits OGD-induced cell death of human MSC *via* suppression of intracellular levels of Ca^{2+} and ROS.

Acknowledgements

This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2009-0082941) and was supported by the Gachon University research fund of 2015 (GCU-2015-0098).

References

Abramov, A.Y., Scorziello, A., Duchen, M.R., 2007. Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. J. Neurosci. 27, 1129–1138.

Aggarwal, S., Pittenger, M.F., 2005. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105, 1815–1822.

Bánfi, B., Molnár, G., Maturana, A., Steger, K., HegedÛs, B., Demaurex, N., Krause, K.H., 2001. A Ca^{2+}-activated NADPH oxidase in testis, spleen, and lymph nodes. J. Biol. Chem. 276, 37594–37601.

Beck, J., Lenart, B., Kintner, D.B., Sun, D., 2003. Na-K-Cl cotransporter contributes to glutamate-mediated excitotoxicity. J. Neurosci. 23, 5061–5068.

Beske, P.H., Jackson, D.A., 2012. NADPH oxidase mediates the oxygen-glucose deprivation/reperfusion-induced increase in the tyrosine phosphorylation of the N-methyl-daspartate receptor NR2A subunit in retinoic acid differentiated SH-SY5Y Cells. J. Mol. Signal. 7, 15.

Caplan, A.I., Dennis, J.E., 2006. Mesenchymal stem cells as trophic mediators. J. Cell. Biochem. 98, 1076–1084.

Chen, J., Li, Y., Wang, L., Zhang, Z., Lu, D., Lu, M., Chopp, M., 2001. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 32, 1005–1011.

Chen, X., Li, Y., Wang, L., Katakowski, M., Zhang, L., Chen, J., Xu, Y., Gautam, S.C., Chopp, M., 2002. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. Neuropathology 22, 275–279.

Chen, J., Li, Y., Katakowski, M., Chen, X., Wang, L., Lu, D., Lu, M., Gautam, S.C., Chopp, M., 2003. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J. Neurosci. Res. 73, 778–786.

Chen, X., Armstrong, M.A., Li, G., 2006. Mesenchymal stem cells in immunoregulation. Immunol. Cell Biol. 84, 413–421.

Choi, D.W., 1988. Calcium-mediated neurotoxicity: relationship specific channel types and role in ischemic damage. Trends Neurosci. 11, 465–469.

Choi, D.W., 1995. Calcium: still center-stage in hypoxic-ischemic neuronal death. Trends Neurosci. 18, 58–60.

Conti, P., Gigante, G.E., Alesse, E., Cifone, M.G., Fieschi, C., Reale, M., Angeletti, P.U., 1985. A role for calcium in the effect of very low frequency electromagnetic field on the blastogenesis of human lymphocytes. FEBS Lett. 181, 28–32.

Crigler, L., Robey, R.C., Asawachaicharn, A., Gaupp, D., Phinney, D.G., 2006. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. Exp. Neurol. 198, 54–64.

Domoki, F., Kis, B., Gáspár, T., Snipes, J.A., Parks, J.S., Bari, F., Bushija, D.W., 2009. Rosuvastatin induces delayed preconditioning against oxygen-glucose deprivation in cultured cortical neurons. Am. J. Physiol. Cell Physiol. 296, 97–105.

Duong, C.N., Kim, J.Y., 2016. Exposure to electromagnetic field attenuates oxygen-glucose deprivation-induced microglial cell death by reducing intracellular Ca^{2+} and ROS. Int. J. Radiat. Biol. 92, 195–201.

Dykens, J.A., Stern, A., Trenkner, E., 1987. Mechanism of kainate toxicity to cerebellar neurons *in vitro* is analogous to reperfusion tissue injury. J. Neurochem. 49, 1222–1228.

George, I., Geddis, M.S., Lill, Z., Lin, H., Gomez, T., Blank, M., Oz, M.C., Goodman, R., 2008. Myocardial function improved by electromagnetic field induction of stress protein hsp70. J. Cell. Physiol. 216, 816–823.

Golbach, L.A., Philippi, J.G.M., Cuppen, J.J.M., Savelkoul, H.F.J., LidyVerburg-van Kemenade, B.M., 2015. Calcium signaling in human neutrophil cell lines is not affected by low-frequency electromagnetic fields. Bioelectromagnetics 36, 430–443.

Gomes, A., Fernandes, E., Lima, J.L.F.C., 2005. Fluorescence probes used for detection of reactive oxygen species. J. Biochem. Biophys. Methods 65, 45–80.

Grassi, C., D'Ascenzo, M., Torsello, A., Martinotti, G., Wolf, F., Cittadini, A., Azzena, G. B., 2004. Effects of 50 Hz electromagnetic fields on voltage-gated Ca^{2+} channels and their role in modulation of neuroendocrine cell proliferation and death. Cell Calcium 35, 307–315.

Guo, H., Hu, L.M., Wang, S.X., Wang, Y.L., Shi, F., Li, H., Liu, Y., Kang, L.Y., Gao, X.M., 2011. Neuroprotective effects of scutellarin against hypoxic-ischemic-induced cerebral injury via augmentation of antioxidant defense capacity. Chin. J. Physiol. 54, 399–405.

Harrison, R., 2004. Physiological roles of xanthine oxidoreductase. Drug Metab. Rev. 36, 363–375.

Henrykowska, G., Jankowski, W., Pacholski, K., Lewicka, M., Smigielski, J., Dziedziczak-Buczyńska, M., Buczyński, A., 2009. The effect of 50 Hz magnetic field of different shape on oxygen metabolism in blood platelets: in vitro studies. Int. J. Occup. Med. Environ. Health 22, 269–276.

Holzwarth, C., Vaegler, M., Gieseke, F., Pfister, S.M., Handgretinger, R., Kerst, G., Müller, I., 2010. Low physiologic oxygen tensions reduce proliferation and differentiation of human multipotent mesenchymal stromal cells. BMC Cell Biol. 11, 11.

Howard, R.S., Holmes, P.A., Koutroumanidis, M.A., 2011. Hypoxic-ischaemic brain injury. Pract. Neurol. 11, 4–18.

Kalyanaraman, B., Darley-Usmar, V., Davies, K.J., Dennery, P.A., Forman, H.J., Grisham, M.B., Mann, G.E., Moore, K., Roberts 2nd, L.J., Ischiropoulos, H., 2012. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. Free Radic. Biol. Med. 52, 1–6.

Kornekov, A.L., Pahnake, J., Frei, K., Warzok, R., Schroder, H.W., Frick, R., Muljana, L., Piek, J., Yonekawa, Y., Gaab, M.R., 2000. Treatment with nimodipine and mannitol reduces programed cell and infarct size following focal cerebral ischemia. Neurosurg. Rev. 23, 145–150.

Kotani, Y., Nakajima, Y., Hasegawa, T., Satoh, M., Nagase, H., Shimazawa, M., Yoshimura, S., Iwama, T., Hara, H., 2008. Propofol exerts greater neuroprotection with disodium edetate than without it. J. Cereb. Blood Flow Metab. 28, 354–366.

Kurozumi, K., Nakamura, K., Tamiya, T., Kawano, Y., Ishii, K., Kobune, M., Hirai, S., Uchida, H., Sasaki, K., Ito, Y., Kato, K., Honmou, O., Date, I., Hamada, H., 2005. Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. Mol. Ther. 11, 96–104.

Lee, R.H., Pulin, A.A., Seo, M.J., Kota, D.J., Ylostalo, J., Larson, B.L., Semprun-Prieto, L., Delafontaine, P., Prockop, D.J., 2009. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 5, 54–63.

Lim, S., Kim, S.C., Kim, J.Y., 2015. Protective effect of 10-Hz, 1-mT electromagnetic field exposure against hypoxia/reoxygenation injury in HK-2 cells. Biomed. Environ. Sci. 28, 231–234.

Lindstrom, E., Lindstrom, P., Berglund, A., Lundgren, E., Mild, K.H., 1995. Intracellular calcium oscillations in a T-cell line after exposure to extremely-low-frequency magnetic fields with variable frequencies and flux densities. Bioelectromagnetics 16, 41–47.

Lisi, A., Ledda, M., Rosola, E., Pozzi, D., D'Emilia, E., Giuliani, L., Foletti, A., Modesti, A., Morris, S.J., Grimaldi, S., 2006. Extremely low frequency electromagnetic field exposure promotes differentiation of pituitary corticotrope-derived AtT20 D16V cells. Bioelectromagnetics 27, 641–651.

Manning, A.S., Coltart, D.J., Hearse, D.J., 1984. Ischemia and reperfusion induced arrhythmias in the rat. Effects of xanthine oxidase inhibition with allopurinol. Circ. Res. 55, 545–548.

Miller, E.W., Albers, A.E., Pralle, A., Isacoff, E.Y., Chang, C.J., 2005. Boronate-based fluorescent probes for imaging cellular hydrogen peroxide. J. Am. Chem. Soc. 127, 16652–16659.

Morgado-Valle, C., Verdugo-Díaz, L., García, D.E., Morales-Orozco, C., Drucker-Colín, R., 1998. The role of voltage-gated Ca^{2+} channels in neurite growth of cultured chromaffin cells induced by extremely low frequency (ELF) magnetic field stimulation. Cell Tissue Res. 291, 217–230.

Nikonenko, I., Bancila, M., Bloc, A., Muller, D., Bijlenga, P., 2005. Inhibition of T-type calcium channels protects neurons from delayed ischemia-induced damage. Mol. Pharmacol. 68, 84–89.

Nomura, T., Honmou, O., Harada, K., Houkin, K., Hamada, H., Kocsis, J.D., 2005. I.V. infusion of brain-derived neurotrophic factor gene modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. Neuroscience 136, 161–169.

Pall, M.L., 2013. Electromagnetic fields act *via* activation of voltage-gated calcium channels to produce beneficial or adverse effects. J. Cell. Mol. Med. 17, 958–965.

Parekkadan, B., van Poll, D., Suganuma, K., Carter, E.A., Berthiaume, F., Tilles, A.W., Yarmush, M.L., 2007. Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure. PLoS One 2, e941.

Park, J.E., Seo, Y.K., Yoon, H.H., Kim, C.W., Park, J.K., Jeon, S., 2013. Electromagnetic fields induce neural differentiation of human bone marrow derived mesenchymal stem cells via ROS mediated EGFR activation. Neurochem. Int. 62, 418–424.

Pesce, M., Patruno, A., Speranza, L., Reale, M., 2013. Extremely low frequency electromagnetic field and wound healing: implication of cytokines as biological mediators. Eur. Cytokine Netw. 24, 1–10.

Piacentini, R., Ripoli, C., Mezzogori, D., Azzena, G.B., Grassi, C., 2008. Extremely low-frequency electromagnetic fields promote in vitro neurogenesis via upregulation of Ca(v)1-channel activity. J. Cell. Physiol. 215, 129–139.
Pisani, A., Calabresi, P., Tozzi, A., D'Angelo, V., Bernardi, G., 1998. L-type Ca^{2+} channel blockers attenuate electrical changes and Ca^{2+} rise induced by oxygen/glucose deprivation in cortical neurons. Stroke 29, 196–201.
Poniedzialek, B., Rzymski, P., Nawrocka-Bogusz, H., Jaroszyk, F., Wiktorowicz, K., 2013. The effect of electromagnetic field on reactive oxygen species production in human neutrophils in vitro. Electromagn. Biol. Med. 32, 333–341.
Rahbar-Roshandel, N., Razavi, L., TavakoliI-Far, B., 2007. Nimodipine protects PC12 cells against oxygen-glucose deprivation. Iran. J. Pharm. Ther. 6, 51–54.
Reale, M., Kamal, M.A., Patruno, A., Costantini, E., D'Angelo, C., Pesce, M., Greig, N.H., 2014. Neuronal cellular responses to extremely low frequency electromagnetic field exposure: implications regarding oxidative stress and neurodegeneration. PLoS One 9, e104973.
Scheibe, F., Klein, O., Klose, J., Priller, J., 2012. Mesenchymal stromal cells rescue cortical neurons from apoptotic cell death in an *in vitro* model of cerebral ischemia. Cell. Mol. Neurobiol. 32, 567–576.
Scuteri, A., Cassetti, A., Tredici, G., 2006. Adult mesenchymal stem cells rescue dorsal root ganglia neurons from dying. Brain Res. 1116, 75–81.
Shi, H., Liu, K.J., 2006. Effects of glucose concentration on redox status in rat primary cortical neurons under hypoxia. Neurosci. Lett. 410, 57–61.
Siesjo, B.K., Bengtsson, F., 1989. Calcium fluxes, calcium antagonists, and calcium related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. J. Cereb. Blood Flow Metab. 9, 127–140.
Suh, S.W., Shin, B.S., Ma, H., Van Hoecke, M., Brennan, A.M., Yenari, M.A., Swanson, R. A., 2008. Glucose and NADPH oxidase drive neuronal superoxide formation in stroke. Ann. Neurol. 64, 654–663.
Wagner, W., Roderburg, C., Wein, F., Diehlmann, A., Frankhauser, M., Schubert, R., Eckstein, V., Ho, A.D., 2007. Molecular and secretory profiles of human mesenchymal stromal cells and their abilities to maintain primitive hematopoietic progenitors. Stem Cells 25, 2638–2647.
Walder, C.E., Green, S.P., Darbonne, W.C., Mathias, J., Rae, J., Dinauer, M.C., Curnutte, J.T., Thomas, G.R., 1997. Ischemic stroke injury is reduced in mice lacking a functional NADPH oxidase. Stroke 28, 2252–2258.
Walleczek, J., Liburdy, R.P., 1990. Nonthermal 60-Hz sinusoidal magnetic-field exposure enhances $^{45}Ca^{2+}$ uptake in rat thymocytes: dependence on mitogen activation. FEBS Lett. 271, 157–160.
Wang, Q., Tompkins, K.D., Simonyi, A., Korthuis, R.J., Sun, A.Y., Sun, G.Y., 2006. Apocynin protects against global cerebral ischemia-reperfusion induced oxidative stress and injury in the gerbil hippocampus. Brain Res. 1090, 182–189.
Wertheimer, N., Leeper, E., 1979. Electrical wiring configurations and childhood cancer. Am. J. Epidemiol. 109, 273–284.
Wilkins, A., Kemp, K., Ginty, M., Hares, K., Mallam, E., Scolding, N., 2009. Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival *in vitro*. Stem Cell Res. 3, 63–70.
Yang, D.C., Yang, M.H., Tsai, C.C., Huang, T.F., Chen, Y.H., Hung, S.C., 2011. Hypoxia inhibits osteogenesis in human mesenchymal stem cells through direct regulation of RUNX2 by TWIST. PLoS One 6, e23965.
Zhang, X., Liu, X., Pan, L., Lee, I., 2010. Magnetic fields at extremely low-frequency (50 Hz, 0. 8 mT) can induce the uptake of intracellular calcium levels in osteoblasts. Biochem. Biophys. Res. Commun. 396, 662–666.
Zhao, M.Z., Nonoguchi, N., Ikeda, N., Watanabe, T., Furutama, D., Miyazawa, D., Funakoshi, H., Kajimoto, Y., Nakamura, T., Dezawa, M., Shibata, M.A., Otsuki, Y., Coffin, R.S., Liu, W.D., Kuroiwa, T., Miyatake, S., 2006. Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and ex vivo HGF gene transfer with HSV-1 vector. J. Cereb. Blood Flow Metab. 26, 1176–1188.
Zhu, W., Chen, J., Cong, X., Hu, S., Chen, X., 2006. Hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells. Stem Cells 24, 416–425.