Received: March 1, 2021 Revised: May 18, 2021 Accepted: June 7, 2021

(onlinelibrary.wiley.com) DOI: 10.1111/ner.13489

Pulsed Electromagnetic Fields: A Novel Attractive Therapeutic Opportunity for Neuroprotection After Acute Cerebral Ischemia

Fioravante Capone, MD[1]; Simona Salati, PhD[2]; Fabrizio Vincenzi, PhD[3]; Micaela Liberti, PhD[4]; Giorgio Aicardi, MSc[5]; Francesca Apollonio, PhD[4]; Katia Varani, PhD[3]; Ruggero Cadossi, MD[2]; Vincenzo Di Lazzaro, MD[1]

ABSTRACT

Objectives: Acute cerebral ischemia is characterized by several pathological processes evolving during time, which contribute to the final tissue damage. Secondary processes, such as prolonged inflammatory response, impaired mitochondrial function and oxidative stress, are responsible for the progression of brain injury to the peri-infarct area, called "penumbra." Adenosine has been shown to play a crucial role in regulating the inflammatory cascade following brain ischemia. Pulsed electromagnetic fields (PEMFs) act as modulators of adenosine receptors, increasing the functionality of the endogenous adenosine. In particular, PEMF exposure induces a significant upregulation of A_{2A} and A_3 adenosine receptors in different neuronal cell types. Several lines of evidence suggest that PEMF exposure might play a neuroprotective role after ischemic damage.

Materials and Methods: This review summarizes the current knowledge on the mechanism of action of PEMFs and their biological effects on neuronal damage both in preclinical and clinical studies.

Results: PEMFs counteract hypoxia-induced apoptosis and ROS production in neuronal-like cells and exert a strong anti-inflammatory effect on microglial cells. Data from stroke animal models showed that PEMFs exposure is able to reduce the size of the infarct area and decrease the levels of pro-inflammatory mediators. In clinical studies, PEMFs stimulation proved to be safe and well tolerated. Preliminary results on acute ischemic stroke patients showed a dose-dependent reduction in the lesion size.

Conclusions: Altogether, these data demonstrate the efficacy of PEMFs against several mechanisms underlying ischemic damage and suggest that PEMFs might represent a novel noninvasive adjunctive treatment for acute ischemic stroke, providing neuroprotection and reducing functional deficits following ischemia.

Keywords: A_{2A} adenosine receptors, acute ischemic stroke, biophysical stimulation, neuroprotection, pulsed electromagnetic fields

Conflict of Interest: Ruggero Cadossi and Simona Salati are respectively President and employee at IGEA S.p.A., the company producing the PEMF exposure device used in the NCT01941147 and NCT02767778 trials; Vincenzo Di Lazzaro and Fioravante Capone were respectively PI and recruiting physician of the clinical trial NCT01941147 whose results are reported in this article; Vincenzo Di Lazzaro and Fioravante Capone are respectively PI and recruiting physician of the randomized clinical trial NCT02767778 currently ongoing. All the other authors declare that there is no conflict of interest.

Address correspondence to: Fioravante Capone, MD, Unit of Neurology, Neurophysiology, and Neurobiology, Department of Medicine, Università Campus Bio-Medico, Roma, Italy. Email: f.capone@unicampus.it

[1] Unit of Neurology, Neurophysiology, and Neurobiology, Department of Medicine, Università Campus Bio-Medico, Roma, Italy;
[2] IGEA SpA, Clinical Biophysics, Carpi, Italy;
[3] Department of Translational Medicine, University of Ferrara, Italy;
[4] Department of Information Engineering, Electronics and Telecommunications (DIET), University of Rome "La Sapienza", Roma, Italy; and
[5] Neurophysiology Research Unit, Department for Life Quality Studies, University of Bologna, Bologna, Italy

For more information on author guidelines, an explanation of our peer review process, and conflict of interest informed consent policies, please go to http://www.wiley.com/WileyCDA/Section/id-301854.html

Source(s) of financial support: This work was supported by the Italian Ministry of Health, project # CO-2013-02358697, Ricerca Finalizzata Bando 2013-Progetti Cofinanziati con l'Industria.

INTRODUCTION

Worldwide, stroke represents the third leading cause of death and the main cause of permanent disability (1). About 45% of stroke patients have long-term residual motor deficits that produce considerable personal, social and economic costs. In Europe, treatment costs represent about 4% of the total healthcare budget, while long-term indirect costs increase continuously with an aging population.

The primary goal of ischemic stroke therapy is to restore blood flow as quickly as possible by recanalizing the occluded vessel to prevent damage to the area that surrounds the infarct core. In the last decade, thrombolytic, intravenous, and intra-arterial therapies have achieved remarkable progress. However, the time window of intervention is limited to the first hours after stroke, and thus there is an urgent need for complementary or alternative therapies capable of reducing the consequences of brain ischemia beyond this short period.

During the stroke, the acute disruption of blood flow results in pronounced deprivation in oxygen and nutrient supplies leading to immediate neuronal death and irreversible damage in the core of the affected area. The blockage of the aerobic mechanism in the mitochondria causes poor production of ATP thus leading to the failure of the Na^+/K^+ ATPase pumps, cell swelling, and Ca^{2+} influx into the cells. Intracellular depolarization also causes the release of glutamate from the presynaptic terminal eliciting neurotoxicity, with consequent neuronal necrosis (2).

Later, secondary processes, such as inflammatory response, excitotoxicity, impaired mitochondrial function and oxidative stress, enlarge the area of neuronal death to the partially preserved peri-infarct area, the so-called ''penumbra'' (3). Thus, neuroprotective strategies should aim at tackling these secondary events in order to prevent the spread of irreversible damage to the ischemic penumbra (4).

In the past years, several potential neuroprotective agents showed promising results in different animal stroke models; however, these promising preclinical data failed to translate into clinical evidence (4). The search for new and effective therapies targeting the ''penumbra'' remains an important unmet clinical need. Novel therapeutic strategies, including both pharmacological and nonpharmacological treatments aimed at reducing the secondary brain damage following ischemia, should be explored.

Adenosine has been reported to be a key-player in the regulation of the inflammatory events following cerebral ischemia, controlling blood cell infiltration and activation of microglial cells.

Pulsed electromagnetic fields (PEMFs) act as modulators of adenosine receptors (ARs); in particular, PEMF stimulation induces a significant upregulation of A_{2A} and A_3 ARs in different cell types. Through their action on ARs, PEMFs have been shown to reduce inflammation, migration, and recruitment of immune cells in the central nervous system (CNS) (5) and protect neuronal cells from apoptosis and oxidative stress (6,7).

In this review, we provide an overview of the biological effects exerted by PEMF stimulation on neuroinflammation and survival of neurons and neuron-like cells exposed to hypoxic/ischemic damage and insights into the underlying molecular mechanisms. Altogether, the findings summarized in this review suggest that PEMF exposure might represent an interesting novel neuroprotective treatment for ischemic stroke patients.

MATERIALS AND METHODS

A review of the literature related to the application of PEMFs in acute ischemic stroke was conducted. The search was performed using online databases PubMed, Web of Knowledge, and Google Scholar. The last electronic search was conducted in February 2021. A comprehensive list of keywords was generated to include all synonyms of PEMFs, acute ischemic stroke, hypoxia, neuronal plasticity, neuron apoptosis, inflammation, and A_{2A} adenosine receptor.

Searches were conducted independently by two reviewers. Each reviewer read the title and abstract of articles to screen for relevance. Articles were classified as either possibly relevant or clearly irrelevant. Articles classified as clearly irrelevant by both reviewers were excluded, and articles classified as possibly relevant by both reviewers were included. Discrepancies between the two reviewers were resolved through discussion or by consulting a third reviewer. Only full-length articles available in English were eligible for inclusion. Overall, this review covers the findings from 30 original papers and 8 review articles.

Adenosine A_{2A} Receptors Modulate Neuroinflammation in Brain Ischemia

In the past decades, significant advances have been made in the understanding of the mechanisms and signaling pathways involved in the evolution of the cerebral ischemic area. It is now clear that ischemia is characterized by several events evolving during time. Minutes to hours after the onset of cerebral ischemia, a robust inflammatory response is initiated through the activation of resident immune cells (microglia) and the recruitment of circulating leukocytes, that release pro-inflammatory and pro-apoptotic molecules (8). This protracted neuroinflammation is responsible for the progression of secondary brain injury.

Immediately after ischemic insult, the extracellular concentrations of ATP and adenosine increase (9). Adenosine is an important regulator of local tissue function, particularly during stress conditions associated with impaired energy support such as ischemic conditions, where adenosine has been already reported to act mainly as a neuroprotective endogenous agent. Adenosine acts through the interaction with four membrane receptors (ARs): A_1, A_{2A}, A_{2B}, and A_3 that belong to the family of G-protein coupled receptors. The A_1 and A_{2A} ARs have high affinity for adenosine while the A_{2B} and A_3 ARs show relatively lower affinity for adenosine. A_{2A} and A_{2B} ARs are coupled to G stimulatory protein (Gs) thus activating adenylyl cyclase (AC) and inducing increase in cAMP intracellular levels, leading to the activation of protein kinase A (PKA) and the phosphorylation of the cyclic AMP response element binding protein (CREB). On the other side, the A_1 and A_3 ARs are coupled to G inhibitory protein (Gi), thus inhibiting AC activity and decreasing cAMP levels (10).

A_{2A} and A_3 ARs have recently emerged as potential therapeutic attractive targets in brain ischemia. In a mouse model of transient focal cerebral ischemia, the A_{2A} AR antagonist SCH58261 has been shown to protect from neurological deficit within the first hours following damage, while failing to maintain protection seven days after transient focal ischemia (11). On the other hand, the same authors reported that treatment with the A_{2A} AR agonist CGS21680 is able to protect from the neurological deficit by preventing leucocytes infiltration and neuroinflammation from the first day up to seven days after the stroke (12). Control of inflammation and inhibition of innate immune cell trafficking have also been described for the A_3 receptor agonist LJ529 (13). These data suggest that A_{2A} AR antagonists can provide neuroprotection in the first minutes and hours following an ischemic insult by controlling excessive excitotoxicity, while A_{2A} and A_3

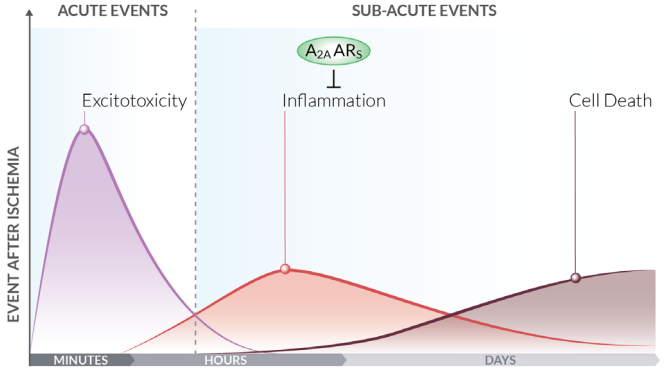

Figure 1. Time-related pathophysiological events after ischemia. [Color figure can be viewed at wileyonlinelibrary.com]

AR agonists can control massive blood cell infiltration and activation of microglial cells in the hours and days after ischemia (Fig. 1). The control of the inflammatory response following the ischemic insult grants A_{2A} and A_3 AR agonists a therapeutic window spanning from hours to few days after stroke.

Effects of PEMFs on Adenosine Receptors

In recent years, considerable interest has arisen on the biological action of extremely low-frequency (0–300 Hz) magnetic fields (ELF-MFs). PEMFs are usually low-frequency fields with a very specific waveform and amplitude and are characterized by a constant variation in the amplitude of the magnetic field over time. The pulsed magnetic field is generated by a wire coil wherein electric current circulates. Such current is responsible for the generation of the pulsed magnetic field, which in turn, induces a time-varying secondary electrical field within the exposed tissue (Fig. 2).

PEMFs are successfully used in the orthopedic field as noninvasive, safe, and cost-effective therapy to enhance bone healing and to promote early cartilage repair (14).

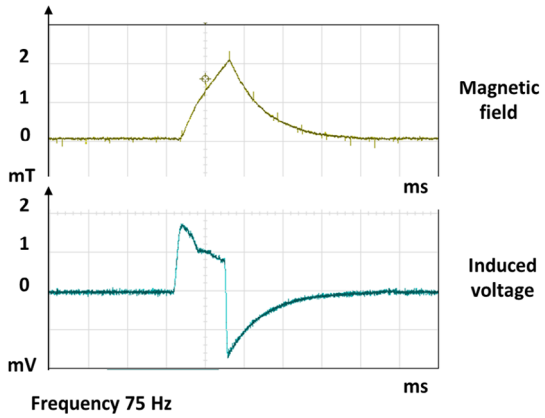

Figure 2. Signal waveform of IGEA PEMFs therapy. The peak intensity of the magnetic field is 1.5 ± 0.2 mT, detected using the Hall probe (HTD61-0608-05-T; F.W. Bell, Sypris Solutions, Louisville, KY, USA) of a gaussmeter (DG500; Laboratorio Elettrofisico, Milan, Italy) with a reading sensitivity of 0.2%. The corresponding peak amplitude of the induced electric voltage is 2.0 ± 0.5 mV, detected using a standard coil probe (50 turns, 0.5 cm internal diameter of the coil probe, 0.2 mm copper diameter) and the temporal pattern of the signal is displayed using a digital oscilloscope (Le Croy, Chestnut Ridge, NY, USA). [Color figure can be viewed at wileyonlinelibrary.com]

In 2002, Varani et al. described for the first time the interaction between PEMFs (1.5 mT, 75 Hz) and A_{2A} ARs in human neutrophils, where saturation binding data revealed a significant increase in the receptor density on the cell membrane without modification of the receptor affinity upon PEMFs treatment (15). Following that, several other reports described the effect of PEMFs (1.5 mT, 75 Hz) on adenosine receptors, specifically the A_{2A} and the A_3 subtypes, in different cell types, such as osteoblasts, chondrocytes, and synoviocytes (16,17). Notably, the effect of PEMFs is selective for the A_{2A} and the A_3 ARs, whereas A_1 and A_{2B} receptors are not influenced by PEMFs exposure.

The increase of A_{2A} ARs mediated by PEMFs (1.5 mT, 75 Hz) is associated with a significant inhibition of the NF-kB signaling pathway, leading to a decrease both in the synthesis and activation of pro-inflammatory cytokines, such as TNF-α and IL1β (16). The anti-inflammatory and tissue-preserving effects exerted by PEMFs through the specific action on A_{2A} and A_3 ARs show great potential to be exploited also to control brain inflammation and to provide neuroprotection following brain damage.

In order to assess whether PEMFs stimulation was able to act on neuronal cells by modulating adenosine receptors, Varani et al. analyzed rat cerebral cortex tissue samples, cerebral cortex membranes, and cortical neurons through saturation binding experiments (18). In intact rat cerebral cortex and cortical neurons, PEMFs stimulation induced a time and dose-dependent transient increase in A_{2A} ARs. The upregulation of A_{2A} ARs in response to PEMFs stimulation (1.5 mT, 75 Hz) on neuronal cells was further confirmed by Vincenzi et al. (19). Saturation binding assays and mRNA analysis revealed that PEMFs exposure up-regulated A_{2A} and A_3 ARs in PC12 rat adrenal pheochromocytoma and U87MG human glioblastoma cell lines (Fig. 3). Using a pharmacological approach, that is, the treatment with A_{2A} and A_3AR selective agonists, Vincenzi et al. were able to show that PEMFs stimulation enhances the effect of ARs agonists on cAMP production. This effect was abrogated by the selective A_{2A} and A_3AR antagonists confirming that the observed effect was due to the activation of ARs and not to an alteration of adenylyl cyclase (AC) functionality. These data demonstrate that PEMFs act as modulators, enhancing the activity of endogenous adenosine. Interestingly, in NGF-treated PC12 and U87MG cells, PEMFs stimulation of A_{2A} and A_3 ARs significantly reduced the activation of the pro-inflammatory NF-kB signaling pathway.

Taken together, these studies demonstrate that PEMFs modulate the expression of A_{2A} and A_3 ARs and act as a selective agonist on these ARs in neuronal cells. This mechanism of action underlies the neuroprotective effect of PEMFs stimulation on the CNS. At the cellular level, this neuroprotective effect is achieved through three main pathways: 1) protection from apoptosis, 2) inhibition of inflammation, and 3) promotion of neuronal plasticity (Fig. 4).

Protection From Apoptosis

In 2002, the stimulation of A_{2A} ARs has been reported to protect neurons from cell death triggered by the exposure to neurotoxins (20), thus the agonist effect of PEMFs exposure on A_{2A} ARs can be exploited to promote neuron survival and viability. Osera et al. found that PEMFs exposure was able to impact on the response to oxidative damage. The human neuroblastoma SH-SY5Y cell line was exposed to repeated PEMFs stimulation (2 mT, 75 Hz) for 10 min four times a week. PEMFs exposure led to a significant increase in superoxide dismutase activity and pre-

exposure to PEMFs decreased ROS production following oxidative stress challenge, suggesting that PEMFs are able to enhance cellular defense against oxidative stress (21).

Recently, PEMFs (1.5 mT, 75 Hz) have also been shown to protect rat pheochromocytoma PC12 and human neuroblastoma-derived SH-SY5Y cells from hypoxia-induced injury (6). In neuron-like cells, PEMFs exposure significantly reduced apoptosis, partially restored hypoxia inducible factor-1α (HIF-1α) activation to normoxic conditions and inhibited ROS production. These data suggest that PEMF stimulation is able to activate ROS-targeting defense mechanisms, that protect neuronal cells from hypoxia-induced cell death. In NGF-stimulated PC12 cells cultured in hypoxic conditions, PEMFs stimulation (1.5 mT, 75 Hz) induces a rapid activation of the p38 mitogen-activated protein kinase (p38 MAPK) cascade, which in turn activates the chaperone heat-shock proteins of 70 kDa (HSP70), resulting in a significant increase in the phosphorylation of the cAMP response element-binding protein (CREB) (5). CREB activation leads to increased expression of the brain-derived neurotrophic factor (BDNF) and of the anti-apoptotic protein Bcl-2 thus promoting neuronal survival. During ischemic stroke, astrocytes play an important role in protecting the brain from excitotoxicity through the release of neurotrophic and angiogenic factors (22). In the human astrocyte cell line 1321N1, PEMFs exposure (1.5 mT, 75 Hz) induced a time-dependent HIF-1α-independent release of vascular endothelial growth factor (VEGF) (7). The conditioned medium derived from PEMFs-exposed astrocytes significantly prevents the viability decrease induced by oxygen–glucose deprivation in neuron-like cells SH-SY5Y5 (7). Altogether, these results demonstrate the protective effect against apoptosis and hypoxic damage exerted by PEMFs in neuron-like cells, supporting the use of PEMFs as noninvasive therapy to promote neuroprotection after hypoxic injury.

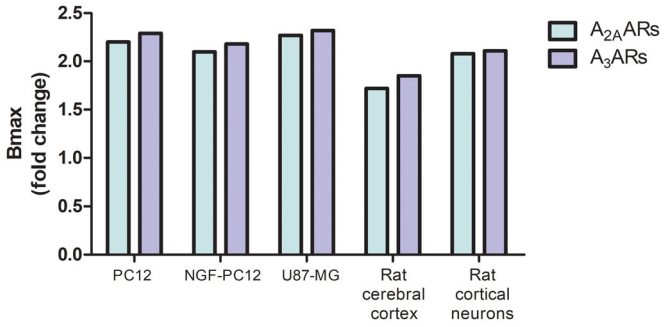

Figure 3. Fold change of A2A and A3 ARs density following PEMF exposure in PC12 cells, NGF-treated PC12 cells, U87-MG cells, rat cerebral cortex, and rat cortical neurons. [Color figure can be viewed at wileyonlinelibrary.com]

Inhibition of Inflammation

In order to assess the potential effect of PEMFs stimulation on neuroinflammation, the research group led by Varani investigated the effect of PEMFs on inflammation-induced injury in N9 microglial cells. Their results showed that PEMFs (1.5 mT, 75 Hz) significantly decrease the release of several pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-8, in LPS-activated N9 microglial cells (6). Subsequently, they further analyzed the signaling pathways regulating the anti-inflammatory effect mediated by PEMFs exposure (23). The authors reported that PEMFs (1.5 mT, 75 Hz) reduced the LPS-induced increased production of TNF-α and IL-1β in N9 cells, through JNK1/2 pathway. Moreover, PEMFs were also shown to significantly reduce crucial cell function of activated microglia, such as ROS generation, cell invasion, and phagocytosis. The recruitment of peripheral immune cells to the ischemic area is associated with the production of other inflammatory mediators, and consequent brain edema and cell death, contributing to further enlargement of the area of initial ischemic damage. Thus, PEMFs, by counteracting inflammation and inhibiting the activation of microglial cells, could represent a potential therapeutic approach to mitigate neuroinflammation upon cerebral ischemia and to prevent the spread of the ischemic damage to the penumbra.

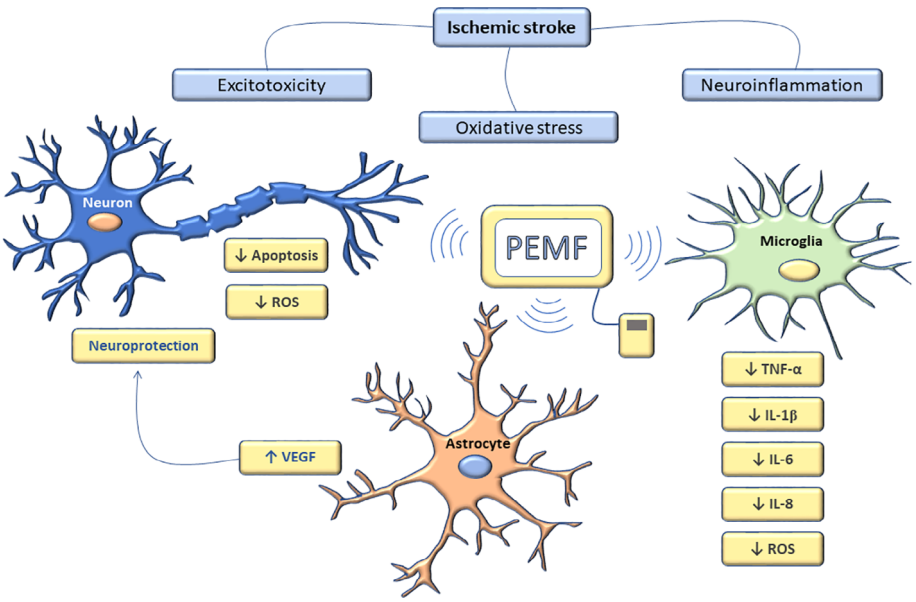

Figure 4. Proposed mechanisms of action for PEMFs stimulation. [Color figure can be viewed at wileyonlinelibrary.com]

Neuronal Plasticity

The effect of PEMFs on neuronal plasticity has been evaluated by assessing neuronal cell differentiation and neurite outgrowth in different cell lines. Zhang et al. investigated the influence of PEMFs (1.36 mT, 50 Hz) on PC12 NGF-mediated neurite outgrowth. The authors found that the pulse duty cycle significantly impacts neurite outgrowth: with low duty cycle (10%) inhibiting the number of neurite-positive cells, but at the same time increasing the average length of neurites (24). PEMF exposure (700 mT, 0.172 Hz) has also been shown to induce neuritogenesis in PC12 cells, though the activation of the MEK-ERK1/2 signaling pathway (25). The ability of PEMFs to promote neurite outgrowth has also been observed in MN9D dopaminergic neurons (26): PEMFs stimulation (5 μT, 27.12 MHz) induced neurite outgrowth and increased cell body width, indicating neuronal maturation. The authors also reported increase intracellular cAMP levels within three to five hours after treatment, suggesting cAMP formation as a potential mechanism of action. Interestingly, ARs are G-protein coupled receptors; in particular, the A_{2A} receptor is coupled with a G_S protein, which activates adenylate cyclase, inducing a significant increase in cAMP intracellular level. In this view, PEMFs stimulation through the activation of A_{2A} ARs could stimulate neurite outgrowth and neural maturation thus contributing to brain tissue remodeling after cerebral ischemia.

Moreover, Aicardi's research group analyzed the effects of PEMFs exposure on synaptic transmission in rat cortical slices under ischemic conditions. Preliminary electrophysiological results show that PEMFs stimulation (1.5 mT, 75 Hz) is able to disrupt postischemic long-term potentiation (iLTP) (unpublished data). Although a potential positive involvement in early functional recovery has been proposed, increasing evidence indicates that iLTP may contribute to excitotoxicity and neuronal cell death occurring after the ischemic insult, and may be detrimental to long-term recovery (27–29). Therefore, iLTP disruption might represent an additional mechanism for PEMFs-mediated neuroprotection.

Effects of PEMFs in Stroke Animal Models

The neuroprotective action of PEMFs in cerebral ischemia was firstly demonstrated by Grant et al. in a rabbit model of transient focal ischemia. Rabbits were exposed to PEMFs (2.8 mT, 75 Hz) for four hours starting 10 min after the onset of ischemia. PEMFs exposure resulted in a significant reduction of the extent of the ischemic area assessed through magnetic resonance imaging (MRI) and histological examination (65% and 69% reduction, respectively) (30). Similar results have also been described in an acute experimental model of myocardial infarct in rats: a significant reduction of the necrotic area was observed in the animals exposed to PEMFs (3 mT, 75 Hz) compared to nonexposed controls (31). Cerebral ischemia can be assimilated to a model of terminal vascularization, devoid of collateral circulation; in this view, results from skin free flaps experiments gain considerable significance. In these experiments, PEMFs (0.1 mT, 27.12 MHz) have been shown to stimulate angiogenesis thus promoting tissue survival (32). These results have recently been confirmed by Pena-Philippides et al., who assessed the effect of PEMFs (3 V/m, 27.12 MHz) on the size of the ischemic lesion and inflammatory parameters in a mouse model of stroke (33). The authors showed that PEMFs reduce the size of the ischemic lesion and significantly affect the expression of pro- and anti-inflammatory mediators resulting in anti-inflammatory and anti-apoptotic effects. Accordingly, in a mouse model of focal ischemia, PEMFs treatment (10 mT, 60 Hz) decreased inflammatory mediators, such as Interleukin 1 beta (IL-1β) and Matrix Metalloproteinase-9 (MMP9) and increased pro-survival molecules, via activation of the TrkB/Akt/Bad pathway (34).

The reduction of the lesion size and the modulation of neuroinflammation suggest the use of PEMFs as potential adjunctive noninvasive therapy to promote the recovery of stroke patients.

Clinical Experience

In the last years, only a few studies analyzed the influence of low frequency–low intensity PEMFs on neuronal activity in humans. In 2009, Capone et al. assessed, in 22 healthy volunteers, the effect of PEMFs on cortical excitability through transcranial magnetic stimulation (TMS) (35). TMS is a noninvasive technique that allows to obtain functional information about excitatory and inhibitory neurotransmission in circuits of the human cerebral cortex. All subjects tolerated the exposure to PEMFs well, and no adverse event was reported. The authors showed that 45 min of PEMFs exposure (1.8 mT; 75 Hz) significantly enhanced intracortical facilitation, suggesting that PEMFs may promote cortical

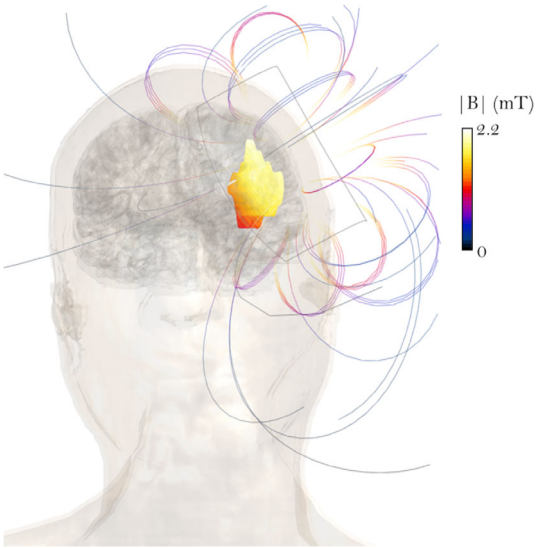

Figure 5. Streamline view of B field along the frontal plane of the model showing the magnetic field intensity on the patientspecific brain lesion. [Color figure can be viewed at wileyonlinelibrary.com]

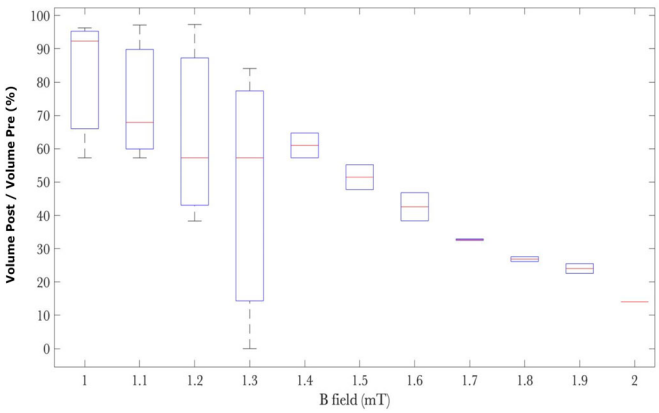

Figure 6. Box plot of the percentage quantity "ratio" with respect to increasing values of magnetic field intensity. [Color figure can be viewed at wileyonlinelibrary.com]

excitatory neurotransmission. Recently, using the same exposure device, the authors evaluated whether PEMFs could affect LTP-like plasticity in a randomized, single-blind, sham-controlled study on ten healthy subjects. LTP-like plasticity was evaluated by using intermittent theta burst stimulation (iTBS), a TMS protocol that produces a prolonged increase of cortical excitability. By measuring the changes of motor evoked potentials (MEP) amplitude before and after iTBS, the authors confirmed that whole-brain PEMFs stimulation is safe and demonstrated that such stimulation does not significantly affect LTP-like plasticity in the human motor cortex (36). On the other hand, Premi et al., in a randomized double-blind sham-controlled study, reported a persistent increase (>60%) in corticospinal excitability on ten healthy subjects exposed to high-field magnetic pulses (2 T, 7 Hz) for 15 min (37). Altogether, these studies demonstrate that PEMFs stimulation is safe and well tolerated and can modulate neurotransmission; however, further studies are needed to fully unravel the neuromodulatory activity exerted by PEMFs.

To date, few clinical studies evaluated the effect of PEMFs stimulation in stroke patients. Cichon et al. recently described the effect of PEMFs exposure (7 mT, 40 Hz, 15 min/day for four weeks) on 48 poststroke patients. Functional and mental status were evaluated using the Activity Daily Living, Geriatric Depression Scale, and Mini-Mental State Examination. The authors reported that PEMFs treatment improves patient clinical parameters, particularly cognitive and psychosomatic functions (38). The same authors also showed that PEMFs stimulation significantly increases the activity of catalase and superoxide dismutase enzymes, thus reducing oxidative stress level (39). Moreover, PEMFs stimulation was able to increase the expression level of growth factors such as BDNF and VEGF, thus favoring neuroplasticity after stroke (40). These results are in agreement with the preclinical studies published by Varani's research group, showing a significant anti-inflammatory and neuroprotective effect of PEMFs stimulation (5–7,23).

On the other hand, Cichon et al. reported that plasma levels of IL-1β (41) and the expression of the pro-apoptotic genes BAX, CASP8, TNFα, and TP53 (42) were significantly higher in the PEMFs group compared to the non-PEMFs group. The authors suggested that the increased expression of IL-1β and pro-apoptotic genes in poststroke patients upon PEMFs exposure might promote the activation of signaling pathways involved in neuroprotection and in brain plasticity; however, the underlying mechanism requires further clarification.

Using the PEMFs exposure system applied in preclinical studies (5–7,23,30) and in safety and tolerability studies on healthy volunteers (35), Capone et al. designed an open label, one arm, dose-escalation exploratory study to evaluate the safety and tolerability of PEMFs in patients with acute ischemic stroke (clinicaltrials.gov NCT01941147) (43). PEMFs treatment (1.8 mT, 75 Hz) started within 48 hours from the stroke onset and was carried out for five consecutive days. Six patients were stimulated, three for 45 min/day and three for 120 min/day. The authors reported that PEMFs stimulation was safe and well tolerated in patients affected by ischemic stroke: no severe adverse events were recorded and none of the patients experienced neurological worsening. During follow-up, all patients experienced a progressive improvement in clinical conditions. The volume of the infarct area, measured by MRI at baseline and after one month from the stroke, was reduced in one patient stimulated for 45 min and in all the patients stimulated for 120 min, thus suggesting that PEMFs exposure can promote the reduction of the lesion volume.

Using a patient semi-specific head model, where the 3D reconstruction of the ischemic lesion of the patient is placed into the head of the human body model "Duke," Colella et al. analyzed the stroke patients exposed to PEMFs for 120 min/day from the Capone study and were able to measure the magnetic flux density to which each ischemic lesion was exposed to (Fig. 5) (44). First, the authors reported that each lesion was exposed to a magnetic field intensity of at least 1 mT, which is the minimum value for which biological effects have been demonstrated in preclinical studies. Second, the dosimetric computation and volumetric analysis showed a correlation between ischemic lesion reduction and the value of magnetic field intensity experienced (Fig. 6). These data strongly suggest a dose–response effect elicited by PEMFs stimulation on the ischemic lesion. This study opened the way to a randomized, placebo-controlled, double-blind study aimed at evaluating whether PEMFs exposure is able to promote recovery in acute ischemic stroke patients (NCT02767778). This clinical trial foresees the enrolment of approximately 120 patients, and it is currently in the recruiting phase.

DISCUSSION

The characterization of the time-related development of inflammatory events responsible for the secondary brain damage following ischemic stroke opened a window of opportunity for novel treatments focused on neuroprotection. The results summarized in this review show that PEMFs induce A_{2A} and A_3 ARs upregulation in different cell types of neuronal origin. Through ARs, PEMFs are able to reduce inflammation, migration and recruitment of immune cells in the CNS (5) and protect neuronal cells from apoptosis and oxidative stress (6,7). These observations suggest that PEMFs might represent an interesting novel neuroprotective treatment for ischemic stroke patients. In particular, PEMFs enhance the endogenous physiological activity of adenosine. For this reason, the effect mediated by PEMFs through the ARs might not be followed by the desensitization and receptor downregulation events that are frequently associated with the use of drugs (45).

The application of PEMFs, that is, physical energies, for therapeutic purposes is referred as biophysical stimulation (BS) and is endowed with significant advantages compared to standard pharmacological treatments. The clinical use of a drug is based on the knowledge of its pharmacodynamics and pharmacokinetics. Pharmacodynamics defines the effect of a drug according to its dosage. Pharmacokinetics defines how the body absorbs, distributes, metabolizes and eliminates the drug. Pharmacokinetics represents the greatest obstacle to the translation into the clinics of a pharmacological treatment proven effective in preclinical studies. In 2006, Warach et al. reported the results of a phase III clinical study on Gavestinel, a selective antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, in acute ischemic stroke patients. Despite promising neuroprotective effects shown in animal models, Gavestinel failed to exert significant effects on infarct volume and patient clinical outcomes (46).

The processes of absorption, distribution, and metabolism are not involved when physical stimuli are used, thus justifying the high transferability to the clinic of the results observed in animal models. Physical signals are not altered by the surrounding tissue, nor are modified and metabolized by the body: this makes biophysical therapy particularly interesting for the treatment of conditions affecting the CNS, where the blood–brain barrier makes conventional drug distribution extremely complex. Moreover, the biophysical therapy is a local treatment, therefore maximum "concentration" and effectiveness can be easily achieved avoiding complications related to systemic side effects. In this view, BS

allows to overcome several intrinsic limitations of standard medicines.

The biological effects of physical energies are dependent on the specific signal parameters employed: frequency, amplitude, waveform of the signal, and duration of exposure, showing specificity of action equal to conventional drugs. These aspects make BS an extremely interesting example of ''soft pharmacology,'' as suggested by Borea et al. (47).

The specific action of PEMFs on A_{2A} and A_3 adenosine receptors, together with their ability to reach the CNS without being modified by the surrounding tissues, make the application of PEMFs therapy to stroke patients extremely attractive. Clinical studies performed so far in healthy volunteers and in a limited number of ischemic stroke patients provided promising preliminary results. PEMFs exposure proved to be safe and well tolerated. Moreover, data collected from ischemic patients exposed for 120 min to PEMFs treatment suggest a reduction in the lesion size measured by MRI (44). To our knowledge, no conventional drug had succeeded in achieving such a result so far. Moreover, the reduction in lesion volume attained upon PEMFs exposure is extremely relevant based on the knowledge that the decrease in lesion volume is predictive of substantial clinical improvement.

A randomized, placebo-controlled, double-blind study is currently ongoing to assess whether PEMFs exposure is able to reduce the infarct volume and to promote functional recovery in ischemic stroke patients (NCT02767778).

In conclusion, according to preclinical data and preliminary clinical results, PEMFs represent a novel promising, non-invasive, local treatment that may be able to limit ischemic damage and promote neuron survival in patients affected by ischemic stroke.

Authorship Statements

Fioravante Capone, Ruggero Cadossi, and Vincenzo Di Lazzaro were responsible for the conceptualization of the study. Simona Salati, Micaela Liberti, Giorgio Aicardi, and Katia Varani were responsible for drafting the original manuscript. Fabrizio Vincenzi and Francesca Apollonio were responsible for preparing the figures. Micaela Liberti, Katia Varani, Ruggero Cadossi, and Vincenzo Di Lazzaro were responsible for writing, reviewing, and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

How to Cite this Article:

Capone F., Salati S., Vincenzi F., Liberti M., Aicardi G., Apollonio F., Varani K., Cadossi R., Di Lazzaro V. 2021. Pulsed Electromagnetic Fields: A Novel Attractive Therapeutic Opportunity for Neuroprotection After Acute Cerebral Ischemia.
Neuromodulation 2021; E-pub ahead of print. DOI:10.1111/ner.13489

REFERENCES

1. World Health Organization. Geneva: World Health Organization. *Global Health Estimates*. 2012. http://www.who.int/healthinfo/global_burden_disease/en/.
2. Xing C, Arai K, Lo EH, Hommel M. Pathophysiologic cascades in ischemic stroke. *Int J Stroke* 2012;7:378–385. https://doi.org/10.1111/j.1747-4949.2012.00839.x.
3. Chamorro Á, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *Lancet Neurol* 2016;15:869–881. https://doi.org/10.1016/S1474-4422(16)00114-9.
4. Moretti A, Ferrari F, Villa RF. Pharmacological therapy of acute ischaemic stroke: achievements and problems. *Pharmacol Ther* 2015;153:79–89. https://doi.org/10.1016/j.pharmthera.2015.06.004.
5. Gessi S, Merighi S, Bencivenni S et al. Pulsed electromagnetic field and relief of hypoxia-induced neuronal cell death: the signaling pathway. *J Cell Physiol* 2019;234:15089–15097. https://doi.org/10.1002/jcp.28149.
6. Vincenzi F, Ravani A, Pasquini S et al. Pulsed electromagnetic field exposure reduces hypoxia and inflammation damage in neuron-like and microglial cells: PEMFs reduce hypoxia and inflammation damage. *J Cell Physiol* 2017;232:1200–1208. https://doi.org/10.1002/jcp.25606.
7. Vincenzi F, Pasquini S, Setti S et al. Pulsed electromagnetic fields stimulate HIF-1α-independent VEGF release in 1321N1 human astrocytes protecting neuron-like SH-SY5Y cells from oxygen-glucose deprivation. *Int J Mol Sci* 2020;21:8053. https://doi.org/10.3390/ijms21218053.
8. Lambertsen KL, Finsen B, Clausen BH. Post-stroke inflammation—Target or tool for therapy? *Acta Neuropathol (Berl)* 2019;137:693–714. https://doi.org/10.1007/s00401-018-1930-z.
9. Melani A, Turchi D, Vannucchi MG, Cipriani S, Gianfriddo M, Pedata F. ATP extracellular concentrations are increased in the rat striatum during in vivo ischemia. *Neurochem Int* 2005;47:442–448. https://doi.org/10.1016/j.neuint.2005.05.014.
10. Borea PA, Gessi S, Merighi S, Vincenzi F, Varani K. Pharmacology of adenosine receptors: the state of the art. *Physiol Rev* 2018;98:1591–1625. https://doi.org/10.1152/physrev.00049.2017.
11. Melani A, Dettori I, Corti F, Cellai L, Pedata F. Time-course of protection by the selective A2A receptor antagonist SCH58261 after transient focal cerebral ischemia. *Neurol Sci* 2015;36:1441–1448. https://doi.org/10.1007/s10072-015-2160-y.
12. Melani A, Corti F, Cellai L, Giuliana Vannucchi M, Pedata F. Low doses of the selective adenosine A2A receptor agonist CGS21680 are protective in a rat model of transient cerebral ischemia. *Brain Res* 2014;1551:59–72. https://doi.org/10.1016/j.brainres.2014.01.014.
13. Choi I-Y, Lee J-C, Ju C et al. A3 adenosine receptor agonist reduces brain ischemic injury and inhibits inflammatory cell migration in rats. *Am J Pathol* 2011;179:2042–2052. https://doi.org/10.1016/j.ajpath.2011.07.006.
14. Cadossi R, Massari L, Racine-Avila J, Aaron RK. Pulsed electromagnetic field stimulation of bone healing and joint preservation: cellular mechanisms of skeletal response. *JAAOS Glob Res Rev* 2020;4:e19.00155. https://doi.org/10.5435/JAAOSGlobal-D-19-00155.
15. Varani K, Gessi S, Merighi S et al. Effect of low frequency electromagnetic fields on a $_{2A}$ adenosine receptors in human neutrophils. *Br J Pharmacol* 2002;136:57–66. https://doi.org/10.1038/sj.bjp.0704695.
16. Vincenzi F, Targa M, Corciulo C et al. Pulsed electromagnetic fields increased the anti-inflammatory effect of a_{2}a and a_3 adenosine receptors in human T/C-28a2 chondrocytes and hFOB 1.19 osteoblasts. *PloS One* 2013;8:e65561. https://doi.org/10.1371/journal.pone.0065561.
17. Varani K, De Mattei M, Vincenzi F et al. Characterization of adenosine receptors in bovine chondrocytes and fibroblast-like synoviocytes exposed to low frequency low energy pulsed electromagnetic fields. *Osteoarthr Cartil* 2008;16:292–304. https://doi.org/10.1016/j.joca.2007.07.004.
18. Varani K, Vincenzi F, Targa M et al. Effect of pulsed electromagnetic field exposure on adenosine receptors in rat brain. *Bioelectromagnetics* 2012;33:279–287. https://doi.org/10.1002/bem.20704.
19. Vincenzi F, Targa M, Corciulo C et al. The anti-tumor effect of A3 adenosine receptors is potentiated by pulsed electromagnetic fields in cultured neural cancer cells. *PLoS ONE* 2012;7:e39317. https://doi.org/10.1371/journal.pone.0039317.
20. Stone TW. Purines and neuroprotection. In: Alzheimer C, editor. *Molecular and cellular biology of neuroprotection in the CNS*. Advances in Experimental Medicine and Biology. Volume 513. Boston, MA: Springer US, 2003; p. 249–280. https://doi.org/10.1007/978-1-4615-0123-7_9.
21. Osera C, Amadio M, Falone S et al. Pre-exposure of neuroblastoma cell line to pulsed electromagnetic field prevents H $_2$ O_2-induced ROS production by increasing MnSOD activity: PEMF pre-treatment increases MnSOD activity. *Bioelectromagnetics* 2015;36:219–232. https://doi.org/10.1002/bem.21900.
22. Liu Z, Chopp M. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. *Prog Neurobiol* 2016;144:103–120. https://doi.org/10.1016/j.pneurobio.2015.09.008.
23. Merighi S, Gessi S, Bencivenni S et al. Signaling pathways involved in anti-inflammatory effects of pulsed electromagnetic field in microglial cells. *Cytokine* 2020;125:154777. https://doi.org/10.1016/j.cyto.2019.154777.
24. Zhang Y, Ding J, Duan W, Fan W. Influence of pulsed electromagnetic field with different pulse duty cycles on neurite outgrowth in PC12 rat pheochromocytoma cells. *Bioelectromagnetics* 2005;26:406–411. https://doi.org/10.1002/bem.20116.
25. Kudo T, Kanetaka H, Shimizu Y et al. Induction of Neuritogenesis in PC12 cells by a pulsed electromagnetic field via MEK-ERK1/2 signaling. *Cell Struct Funct* 2013;38:15–20. https://doi.org/10.1247/csf.12030.
26. Lekhraj R, Cynamon DE, DeLuca SE, Taub ES, Pilla AA, Casper D. Pulsed electromagnetic fields potentiate neurite outgrowth in the dopaminergic MN9D cell line: PEMF signals enhance neuronal differentiation. *J Neurosci Res* 2014;92:761–771. https://doi.org/10.1002/jnr.23361.
27. Lenz M, Vlachos A, Maggio N. Ischemic long-term-potentiation (iLTP): perspectives to set the threshold of neural plasticity toward therapy. *Neural Regen Res* 2015;10:1537–1539. https://doi.org/10.4103/1673-5374.165215.

28. Stein ES, Itsekson-Hayosh Z, Aronovich A et al. Thrombin induces ischemic LTP (iLTP): Implications for synaptic plasticity in the acute phase of ischemic stroke. *Sci Rep* 2015;5:7912. https://doi.org/10.1038/srep07912.
29. Orfila JE, McKinnon N, Moreno M et al. Cardiac arrest induces ischemic long-term potentiation of hippocampal CA1 neurons that occludes physiological long-term-potentiation. *Neural Plast* 2018;2018:1–9. https://doi.org/10.1155/2018/9275239.
30. Grant G, Cadossi R, Steinberg G. Protection against focal cerebral ischemia following exposure to a pulsed electromagnetic field. *Bioelectromagnetics* 1994;15: 205–216. https://doi.org/10.1002/bem.2250150305.
31. Albertini A, Zucchini P, Noera G, Cadossi R, Pace Napoleone C, Pierangeli A. Protective effect of low frequency low energy pulsing electromagnetic fields on acute experimental myocardial infarcts in rats. *Bioelectromagnetics* 1999;20:372–377.
32. Weber RV, Navarro A, Wu JK, Yu H-L, Strauch B. Pulsed magnetic fields applied to a transferred arterial loop support the rat groin composite flap. *Plast Reconstr Surg* 2004;114:1185–1189. https://doi.org/10.1097/01.PRS.0000135857.95310.13.
33. Pena-Philippides JC, Yang Y, Bragina O, Hagberg S, Nemoto E, Roitbak T. Effect of pulsed electromagnetic field (PEMF) on infarct size and inflammation after cerebral ischemia in mice. *Transl Stroke Res* 2014;5:491–500. https://doi.org/10.1007/s12975-014-0334-1.
34. Urnukhsaikhan E, Mishig-Ochir T, Kim S-C, Park J-K, Seo Y-K. Neuroprotective effect of low frequency-pulsed electromagnetic fields in ischemic stroke. *Appl Biochem Biotechnol* 2017;181:1360–1371. https://doi.org/10.1007/s12010-016-2289-z.
35. Capone F, Dileone M, Profice P et al. Does exposure to extremely low frequency magnetic fields produce functional changes in human brain? *J Neural Transm* 2009;116:257–265. https://doi.org/10.1007/s00702-009-0184-2.
36. Capone F, Pellegrino G, Motolese F, Rossi M, Musumeci G, Di Lazzaro V. Extremely low frequency magnetic fields do not affect LTP-like plasticity in healthy humans. *Front Hum Neurosci* 2020;14:14. https://doi.org/10.3389/fnhum.2020.00014.
37. Premi E, Benussi A, La Gatta A et al. Modulation of long-term potentiation-like cortical plasticity in the healthy brain with low frequency-pulsed electromagnetic fields. *BMC Neurosci* 2018;19:34. https://doi.org/10.1186/s12868-018-0434-z.
38. Cichoń N, Czarny P, Bijak M et al. Benign effect of extremely low-frequency electromagnetic field on brain plasticity assessed by nitric oxide metabolism during Poststroke rehabilitation. *Oxid Med Cell Longev* 2017;2017:1–9. https://doi.org/10.1155/2017/2181942.
39. Cichoń N, Bijak M, Miller E, Saluk J. Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients: antioxidative effect of ELF-EMF. *Bioelectromagnetics* 2017;38:386–396. https://doi.org/10.1002/bem.22055.
40. Cichoń N, Bijak M, Czarny P et al. Increase in blood levels of growth factors involved in the neuroplasticity process by using an extremely low frequency electromagnetic field in post-stroke patients. *Front Aging Neurosci* 2018;10:294. https://doi.org/10.3389/fnagi.2018.00294.
41. Cichon N, Saluk-Bijak J, Miller E et al. Evaluation of the effects of extremely low frequency electromagnetic field on the levels of some inflammatory cytokines in post-stroke patients. *J Rehabil Med* 2019;51:854–860. https://doi.org/10.2340/16501977-2623.
42. Cichon N, Synowiec E, Miller E et al. Effect of rehabilitation with extremely low frequency electromagnetic field on molecular mechanism of apoptosis in post-stroke patients. *Brain Sci* 2020;10:266. https://doi.org/10.3390/brainsci10050266.
43. Capone F, Liberti M, Apollonio F et al. An open-label, one-arm, dose-escalation study to evaluate safety and tolerability of extremely low frequency magnetic fields in acute ischemic stroke. *Sci Rep* 2017;7:12145. https://doi.org/10.1038/s41598-017-12371-x.
44. Colella M, Camera F, Capone F et al. Patient semi-specific computational modeling of electromagnetic stimulation applied to neuroprotective treatments in acute ischemic stroke. *Sci Rep* 2020;10:2945. https://doi.org/10.1038/s41598-020-59471-9.
45. Kenakin T. Principles: receptor theory in pharmacology. *Trends Pharmacol Sci* 2004;25:186–192. https://doi.org/10.1016/j.tips.2004.02.012.
46. Warach S, Kaufman D, Chiu D et al. Effect of the glycine antagonist Gavestinel on cerebral infarcts in acute stroke patients, a randomized placebo-controlled trial: the GAIN MRI substudy. *Cerebrovasc Dis* 2006;21:106–111. https://doi.org/10.1159/000090208.
47. Borea PA, Varani K, Vincenzi F et al. The a $_3$ adenosine receptor: History and perspectives. *Pharmacol Rev* 2015;67:74–102. https://doi.org/10.1124/pr.113.008540.

COMMENTS

The idea of improving functionality of stroke patients with non-invasive electromagnetic stimulation is extremely appealing, especially considering the high prevalence of this condition, the major impact it has on the quality of life of individual patients and the societal burden associated with disability and medical needs. The authors of the review define several distinct mechanisms that may explain neuroprotective effects of the electromagnetic fields; most likely, the actual mechanism of action involves multiple pathways. It is indeed conceivable that different stimulation parameters are associated with specific underlying protective actions, and therefore translation of this research into clinical practice may include a combination of different stimulation paradigms, perhaps individually tailored based on specific stroke biomarkers.

Konstantin Slavin, MD
Chicago, IL USA

***

Stroke is a serious socioeconomic problem. Due to the increasing population of the elderly in society and the increasing incidence of cerebrovascular disease with age, the management of stroke patients should be of constant interest to doctors and researchers. Current scientific research focuses largely on the management of patients in the acute phase of stroke, including on the development of endovascular treatment techniques. However, statistics show that despite these measures, there is a consistently high rate of post-stroke failure. For this reason, it is important to include adjuvant treatment, and one of such techniques may be pulsed electromagnetic field (PEMF) therapy. This article presents the current knowledge on the biological effects of PEMF in post-stroke patients.

Natalia Cichon, PhD
Łódź, Poland

