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Letter to the Editor

Neuroprotective Effects of Pulsed Electromagnetic Fields in Acute Stroke

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Dear Sir:

Currently, treatments for acute ischemic stroke (AIS) are based on reperfusion therapies; however, the time window for these interventions is limited to the first few hours after stroke onset. Therefore, alternative neuroprotective therapies are urgently required.

Acute blood flow disruption leads to early neuronal death in the core of the ischemic area, later, secondary processes, such as inflammation, excitotoxicity, and oxidative stress enlarge the infarct core into the surrounding tissue, the "penumbra,"[1] which represents the perfect target for neuroprotective strategies. Adenosine receptors (ARs) have recently emerged as potential therapeutic targets in brain ischemia,[2] granting AR agonists a role to prevent "penumbra" evolution.[3-5] Grant et al.[6] showed that pulsed electromagnetic fields (PEMFs) promote significant neuroprotection in a rabbit model of transient focal ischemia. This effect is explained by the selective agonist activity for A_{2A} AR of PEMFs, first described by Varani et al.[7]

Based on preclinical and early clinical experiences (phase 1 and 2 studies),[8-10] we designed a double-blind, placebo-controlled, randomized study to assess whether PEMF exposure was effective in reducing cerebral ischemic volume (primary endpoint) and promoting functional recovery (secondary endpoint) (I-NIC study, NCT02767778). I-NIC clinical trial protocol is provided as Supplementary Materials.

One hundred and sixty-eight patients were screened, and 37 patients with ischemic stroke in the middle cerebral artery (MCA) territory were enrolled: 16 patients were assigned to the active group and 21 to the placebo group (sham device) (Figure 1). The detailed methodology is described in the Supplementary Methods. There were no significant differences in the demographic and clinical characteristics at baseline between the groups (Supplementary Table 1). Magnetic resonance imaging (MRI) was conducted at baseline and 7 and 45 days after stroke onset (Supplementary Table 2). The average lesion volume decreased from 23.3±25.3 cm^3 to 11.9±12.8 cm^3 in the active group (P=0.023), and from 12.1±17.7 cm^3 to 7.3±7.5 cm^3 in the placebo group (P=0.065) (Figure 2A and B, Supplementary Figure 1).

Clinical scores, Barthel Index (BI), modified Rankin Scale (mRS) scores, and National Institutes of Health Stroke Scale (NIHSS) scores showed significant improvements at 7, 45, and 90 days compared with baseline in both groups (Figure 2C-E and Supplementary Table 3). Excellent outcome at 90 days (defined as a

score on the mRS of 0 or 1) was achieved in 10 out of 11 patients (90.9%) in the active group and in 12 out of 18 patients (66.7%) in the placebo group (Figure 2C).

Twenty patients underwent reperfusion therapies: 12 in the placebo group and eight in the active group. PEMF treatment resulted in significant MRI volume reduction compared with baseline in the active group only (P=0.04) (Figure 3); and the average normalized MRI volume reduction at 45 days over baseline was 50.0% in the active group and 22.7% in the placebo group, with a significant difference between the groups (P=0.04).

The clinical scores at 7, 45, and 90 days improved from baseline in both groups. Notably, the improvements in clinical scores were larger and earlier in the active group than in the placebo group, in line with the volumetric changes in the lesions. MRI volume reduction and BI clinical score improvement were the largest in the active group in the first 7 days after AIS, that is, when PEMF treatment was delivered. Moreover, the BI score reached the widest recovery as early as 45 days in the active group compared to 90 days in the placebo group.

Among patients who underwent reperfusion treatment, the BI score improved from 42.5±30.9 at baseline to 92.9±16.8 (P<0.01) at 90 days in the active group; whilst in the placebo group, BI increased from 54.2±37.2 at baseline to 82.7±37.2 (P=0.10) at 90 days.

The safety of PEMFs was carefully monitored during treatment; no severe adverse events that would require treatment interruption occurred (Supplementary Table 4). At follow-up visits, the patients did not report any side effects that could be attributed to the PEMF treatment.

Our results show that PEMF treatment is safe, well tolerated, and efficiently deployed in stroke units. It is performed at the patient's bed (Supplementary Figures 2 and 3), does not require dedicated infrastructure or specialized personnel, does not extend the length of hospital stay, and costs are expected to be contained.

In summary, the instrumental and clinical results of the I-NIC study showed that PEMF treatment protects the central nervous system following ischemic stroke. At 45 days, the area of neu-

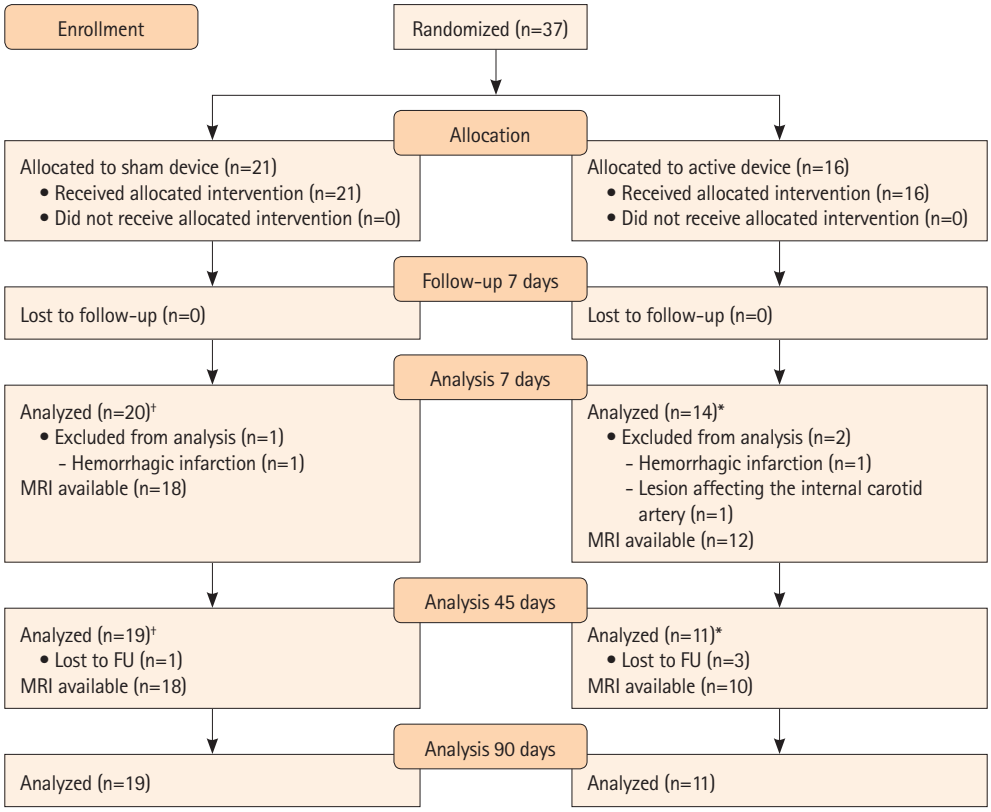

Figure 1. CONSORT study flow diagram. CONSORT, Consolidated Standards of Reporting Trials; FU, follow-up; MRI, magnetic resonance imaging. *Of the 16 patients in the active group, 14 were available for analysis at the 7-day FU: two patients were excluded from the analysis because they did not meet the inclusion criteria "2" and "3" (Supplementary Methods); 11 patients were available for analysis at the 45-day and 90-day FUs: three patients did not return for follow-up visit during the COVID-19 pandemic, but when interviewed by telephone, they were in good health and did not mention any negative effect related to the treatment; †Of the 21 patients in the placebo group, 20 were available for analysis at the 7-day FU: one patient was excluded from the analysis because he did not meet the inclusion criteria "3" (Supplementary Methods), 19 patients were available for analysis at the 45-day and 90-day FUs: one patient died from causes unrelated to the treatment.

ronal sufferance identified by MRI was significantly reduced over baseline in the actively treated patients only (P=0.02), whereas no statistically significant reduction was observed in the placebo group (Figure 2B and Supplementary Figure 1).

Among patients receiving reperfusion therapy, PEMF treatment favored MRI volume reduction and clinical amelioration, showing that the treatments can be successfully combined to prevent the enlargement of structural damage.

The present study has limitations: (1) PEMF treatment was restricted to patients with lesions located in the MCA territory; (2) the difference in average lesion volume at baseline between the groups was wide; and (3) the low number of patients included, resulting from the stringent inclusion criteria and the clinical protocol request for three MRI exams over 45 days. Then, the

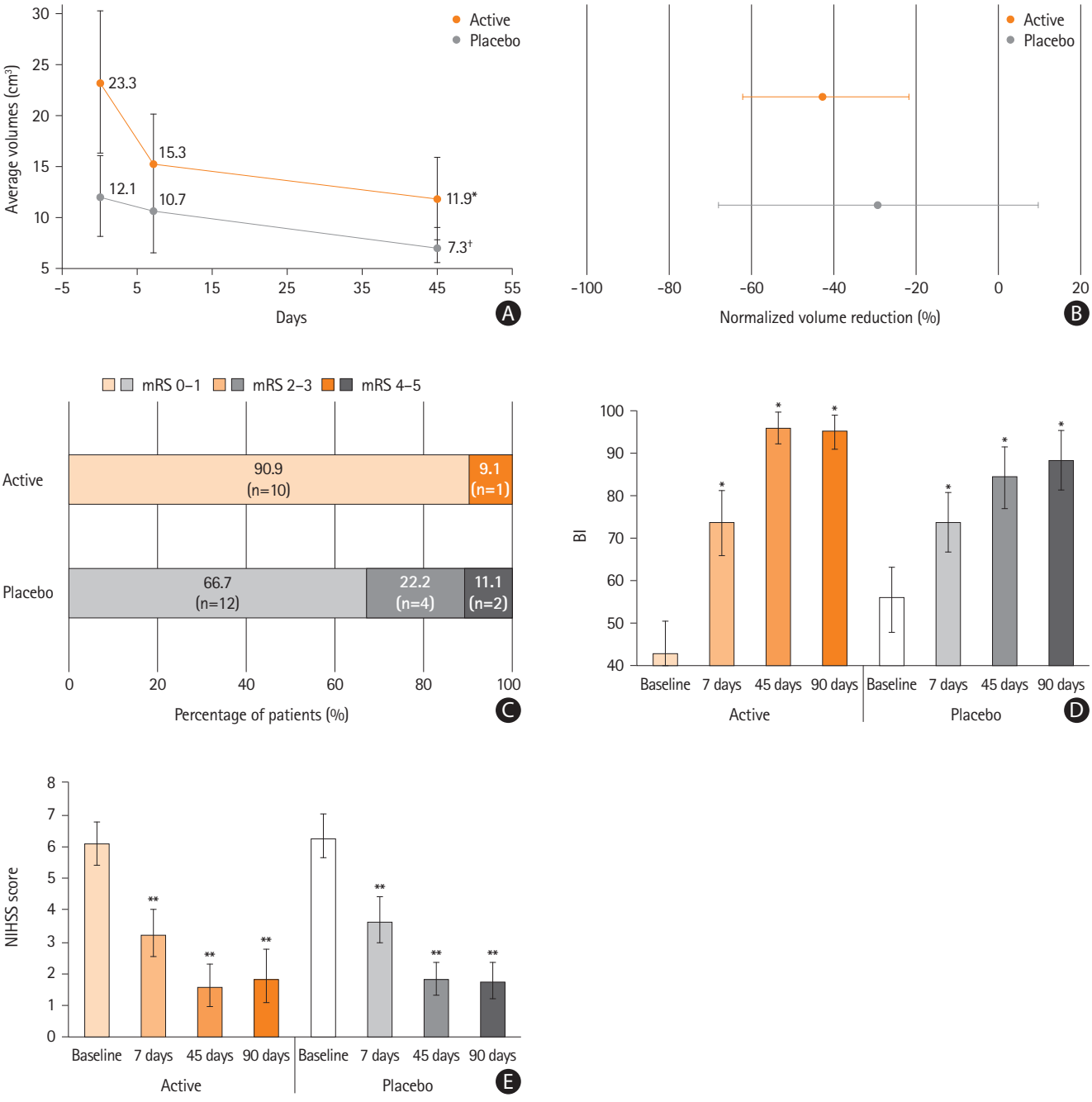

Figure 2. Primary and secondary outcomes. (A) Line graph showing mean lesion volumes (±SE) at baseline, 7 days, and 45 days in the placebo and active groups. (B) Normalized MRI volume reduction (mean±SE). (C) Distribution of scores on the mRS at 90 days. (D) BI over time in the active and placebo groups (mean±SE). (E) NIHSS over time in the active and placebo groups (mean±SE). SE, standard error; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; BI, Barthel Index; NIHSS, National Institutes of Health Stroke Scale. *P<0.05; **P<0.01; †P=nonsignificant (vs. baseline).

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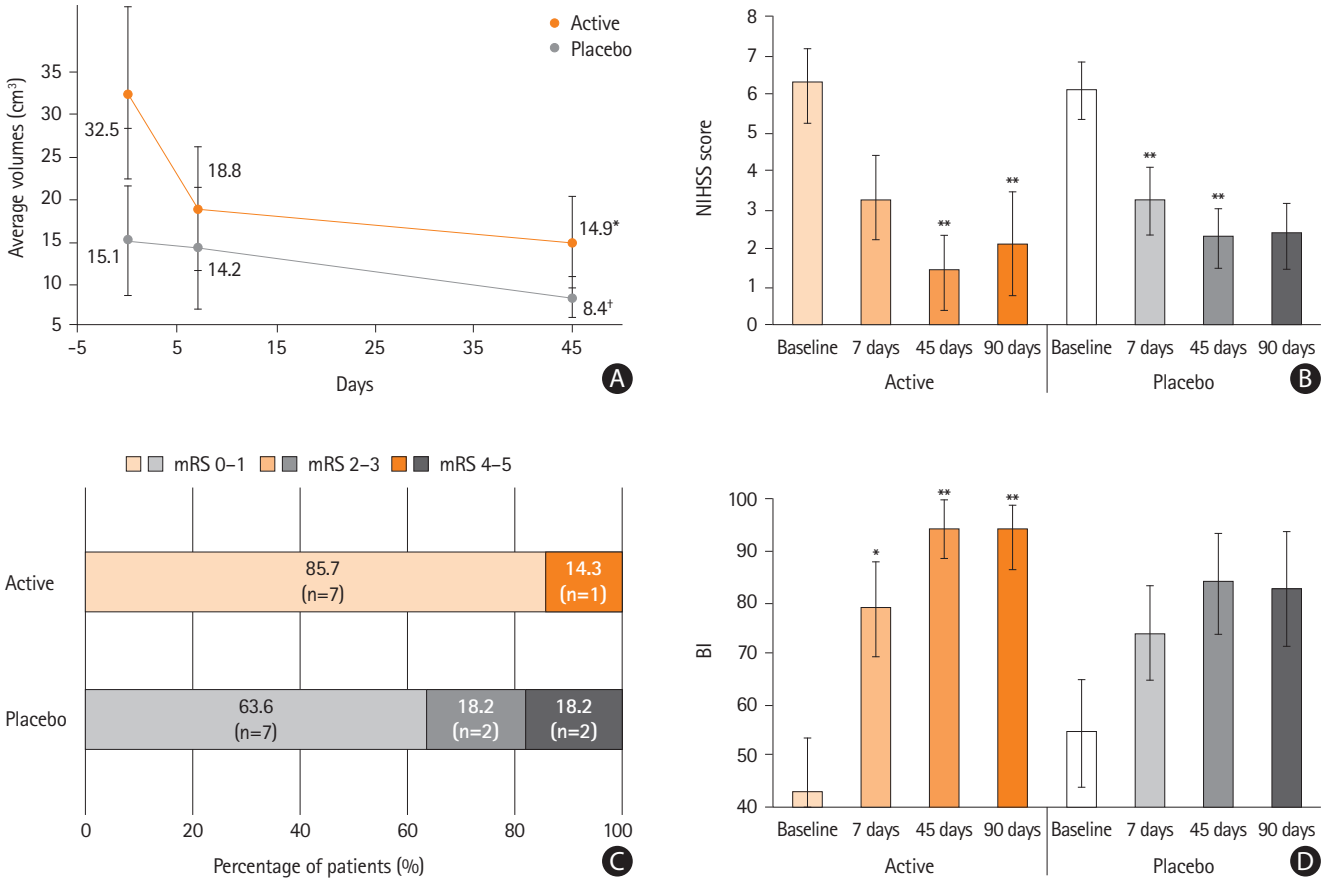

Figure 3. Subgroup analysis. (A) Line graph showing mean lesion volumes (±SE) at baseline, 7 days, and 45 days in the placebo and active groups. (B) NIHSS score (means±SE) over time in the active and placebo. NIHSS score significantly improved over baseline in both groups. (C) Distribution of scores on the mRS at 90 days. Excellent outcome at 90 days was achieved in 85.7% of patients in the active group and in 63.6% of patients in the placebo group (P=0.634). (D) BI score (means±SE) over time in the active and placebo groups. The BI score improved from 42.5±30.9 at baseline to 92.9±16.8 (P<0.01) at 90 days in the active group; whilst in the placebo group, BI increased from 54.2±37.2 at baseline to 82.7±37.2 (P=0.100) at 90 days. SE, standard error; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; BI, Barthel Index. *P<0.05; **P<0.01; †P=nonsignificant (vs. baseline).

COVID-19 pandemic further slowed enrolment and access to follow-up visits.

In the interim analysis, the primary endpoint of the study was reached and the safety and tolerability of PEMF treatment were proven; therefore, the study was interrupted.

This is the first human clinical trial to use PEMF treatment to limit neuronal damage in patients with ischemic stroke. Our results show that PEMF treatment should be considered to reduce the neuronal damage occurring in the "penumbra," offering to clinicians the opportunity to extend the time for intervention to the first week after ischemic stroke.

Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.5853/jos.2024.01529.

Funding statement

The I-NIC study was sponsored by IGEA.

Conflicts of interest

FC has received travel grants and/or speaking honoraria from Biogen, Merck, Sanofi-Genzyme, and Roche and research grants from Merck, which are not relevant to this work. AZ reported consultancies with Bayer, Astra Zeneca, Boehringer Ingelheim, and CSL Behring, which were not relevant to this work. RC owns shares in IGEA. SSa and SSe are employees of IGEA.

The other authors have no conflicting interests to declare.

Author contribution

Conceptualization: FC, RC, VDL. Study design: FC, AZ, FV, RC, VDL. Methodology: NA, MC, ML, FC, VDL. Data collection: FC,

AZ, FV, MD, VT, GC, LL, SC, SSe. Investigation: FC, AZ, FV, MD, VT, GC, LL, SC. Statistical analysis: FC, SSa, MC, ML, RC. Writing—original draft: FC, SSa, RC, VDL. Writing—review & editing: FC, AZ, SSa, RC, VDL. Funding acquisition: RC, VDL. Approval of final manuscript: all authors.

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Supplementay Materials

Low-frequency Pulsed Electromagnetic Fields (ELF-MF) as Treatment for Acute Ischemic Stroke (I-NIC)

NCT02767778

Type of Study: Multicenter
Coordinating center: Policlinico Campus Bio-Medico di Roma
PI of the coordinating center: Prof. Vincenzo Di Lazzaro
Short title: Use of ELF-MFs in acute cerebral ischemia
Study code: I-NIC
Revision 3, 13/01/2020

Trade name of the experimental medical device: I-NIC
Classification according to EN 60601-1: Class II Device - Type BF
Classification according to MDD 93/42 CEE: Class IIa Device
Intended use: Neuroprotection in Ischemic Stroke

Introduction

In developed countries, stroke is the third leading cause of death and the leading cause of permanent disability. Approximately 45% of patients with stroke have long-term residual motor deficits that result in considerable personal, social, and economic costs. In Europe, treatment costs account for approximately 4% of the total healthcare budget, whereas long-term indirect costs increase continuously as the population ages.

Thrombolysis and thrombectomy are currently the only acute-phase therapies that have proven to be effective in modifying the course of the disease with acceptable side effects; however, the use of these treatments remains limited to patients with non-hemorrhagic stroke who arrive at equipped centers within a few hours of clinical onset. Therefore, most patients with stroke do not receive specific treatments. The development of complementary or alternative therapies is therefore of great importance [1,2].

Acute occlusion of the cerebral artery leads to a reduction in blood flow to the affected region. This condition is characterized by the functional inactivation of the neuron, which is still structurally intact, and is called the ischemic penumbra. This potentially recoverable area represents a potential target for therapeutic interventions. For a greater reduction in cerebral blood flow, an absolute lack of oxygen occurs; therefore, oxidative metabolism halts, with consequent necrosis of the neuron.

The biochemical processes leading to cerebral infarction are complex. Ischemia induces necrosis by depriving cells of metabolic substrates, such as oxygen and glucose, causing the blockage of mitochondria that produce ATP. Without ATP, ionic membrane pumps cannot maintain a normal concentration of Na+ and K+ between the intra-and extracellular compartments, causing cell swelling and an increase in extracellular K+ and intracellular Ca++ concentrations. Intracellular depolarization induces the release of glutamate from presynaptic terminals, causing neurotoxicity.

A low degree of ischemia, as observed in the ischemic penumbra, can also activate the apoptotic cascade and cause cell death within days or weeks.

Transcranial magnetic stimulation (TMS) is a noninvasive method commonly used in experiments for the *in vivo* evaluation of cortical excitability through the definition of neurophysiological parameters that express the functionality of excitatory and inhibitory brain circuits.

Repetitive magnetic stimulation (rTMS) has gained immense therapeutic value. In the literature, rTMS has been used with some effectiveness in neurological and psychiatric diseases such as depression,[3] Parkinson's disease,[4] amyotrophic lateral sclerosis [5] and ischemic stroke [6]. rTMS promotes (or inhibits) phenomena of neuronal plasticity by delivering magnetic stimuli at different frequencies and intensities.

In recent years, there has been considerable interest in the biological effects of low-frequency and low-intensity magnetic fields (ELF-MFs).

In vitro studies have shown that ELF-MFs can act on neuronal cells by modifying gene expression,[7] promoting neurite growth, [8] reducing apoptosis [9] and promoting neuronal differentiation of stem cells [10]. In addition, recent studies [11] have shown that exposure to ELF-MFs increases the production of brain-derived neurotrophic factor (BDNF), a neurotrophin that appears to play a crucial role in brain plasticity and neuroprotection [12].

Several exposure systems have been developed to explore the biological effects of ELF-MFs and their potential therapeutic applications.

In particular, pulsed electromagnetic fields (PEMF) are characterized by a constant variation in the magnetic field amplitude over time.

From a clinical point of view, PEMFs are commonly used in the orthopedic field to promote bone regeneration after fractures [13] and to reduce pain in osteoarthritis [14]. In cardiology, studies on murine models of myocardial ischemia have shown the ability of ELF-MFs to improve ischemic myocardial function [15]. Based on these findings, the first clinical trial was initiated. Preliminary results obtained from 33 patients with ischemic cardiomyopathy who were not eligible for revascularization showed that PEMF exposure did not cause side effects and induced a significant and long-lasting improvement in angina symptoms [16].

In the field of neurology, several *in vitro* experimental studies have demonstrated the ability of PEMFs to modulate synaptic transmission through their actions on membrane proteins [17]. In particular, the modulation of neurotransmitters such as glutamate [18] and adenosine [19–22] has been demonstrated.

The adenosine receptor A_{2A} has been identified as the main cellular target of IGEA electromagnetic fields [19]. The effect of PEMFs on the A_{2A} receptor is associated with a strong anti-inflammatory and neuroprotective action that protects neuronal cells from apoptosis and inhibits the formation of free oxygen radicals induced by hypoxia [23]. PEMFs significantly reduce the recruitment of microglial cells to the damaged region and the release of pro-inflammatory cytokines [24], which are crucial steps in the exacerbation of stroke brain damage.

The neuroprotective action of PEMFs against brain ischemia was experimentally demonstrated in rabbits in a study conducted at Stanford University by Grant et al. [25]. Exposure lasting several hours results in a significant reduction (65-70%) in the size of the ischemic area, as assessed by magnetic resonance imaging (MRI) and histological examination. These results were recently confirmed by Pena-Philippides et al. [26] who evaluated the effect of PEMFs on ischemic lesion size and inflammatory parameters in mice.

The effect of PEMFs on human brain tissue was evaluated in healthy subjects in 2009 [27]. Twenty-two healthy volunteers (9 men and 13 women, average age 27.6 ± 9 years) underwent magnetic stimulation with PEMFs for 45 consecutive minutes. Magnetic field exposure was well-tolerated, and no adverse events were reported. To study the possible mechanisms of action of PEMF, subjects underwent an evaluation of cortical excitability by transcranial magnetic stimulation (TMS) before and after PEMF stimulation. TMS is a safe and non-invasive technique that allows the in vivo study of the functioning of various brain circuits, particularly those dependent on neurotransmitters such as glutamate, GABA, and acetylcholine. In particular, the following parameters were measured: i) resting motor threshold (RMT) and active motor threshold (AMT); ii) short-latency afferent inhibition (SAI), expression of the activity of cholinergic and GABAergic circuits; iii) short-latency intracortical inhibition (SICI), expression of the activity of GABAergic circuits; and iv) intracortical facilitation (ICF), which depends mainly on the activity of the glutamatergic circuits.

The study reported a significant variation in the ICF parameters in the group under real stimulation compared to that in the group under placebo stimulation. In particular, after 45 min of PEMF stimulation, the ICF increased by approximately 20% compared with the initial value. No other parameters (RMT, AMT, SAI, and SICI) showed significant changes. This study demonstrated that PEMF brain stimulation is safe and well-tolerated in healthy subjects and can significantly enhance intracortical facilitation, suggesting that PEMFs may promote cortical excitatory neurotransmission.

Based on these results, an "early feasibility study" was designed to evaluate the effect of daily exposure to PEMFs therapy on the MRI (Nuclear Magnetic Resonance Imaging) evolution of neurological lesions in patients with acute ischemic stroke (clinicaltrials.gov NCT01941147). Six patients underwent brain stimulation with PEMFs, five of whom completed the study (follow-up at 12 months); one patient was lost to follow-up at 3 months. No patient experienced adverse events during treatment, at the end of treatment (5 days), or at follow-up. MRI analysis of the volume of the ischemic area was conducted at baseline (within 48 h of stroke, T0) and after 45 days (T45). The volume of the ischemic lesion was reduced in one patient stimulated for 45 min and in all patients stimulated for 120 min, thus suggesting that PEMFs exposure can promote the reduction of the lesion volume [28]. The treatment regimen of 120 min/day for 5 days was chosen for the next randomized clinical trial.

In conclusion, these results, together with the significant amount of preclinical data, the proven effect of these PEMFs on the intact human brain [27], and the lack of adverse events in both healthy subjects and patients with ischemic stroke, encourage further investigation of the possible application of PEMF as a neuromodulation and treatment tool for neurological disorders, such as stroke.

Therefore, the purpose of this study was to evaluate the effects of ELF-MFs delivered with the experimental medical device I-NIC on the extent of the ischemic area measured by MRI at different follow-ups.

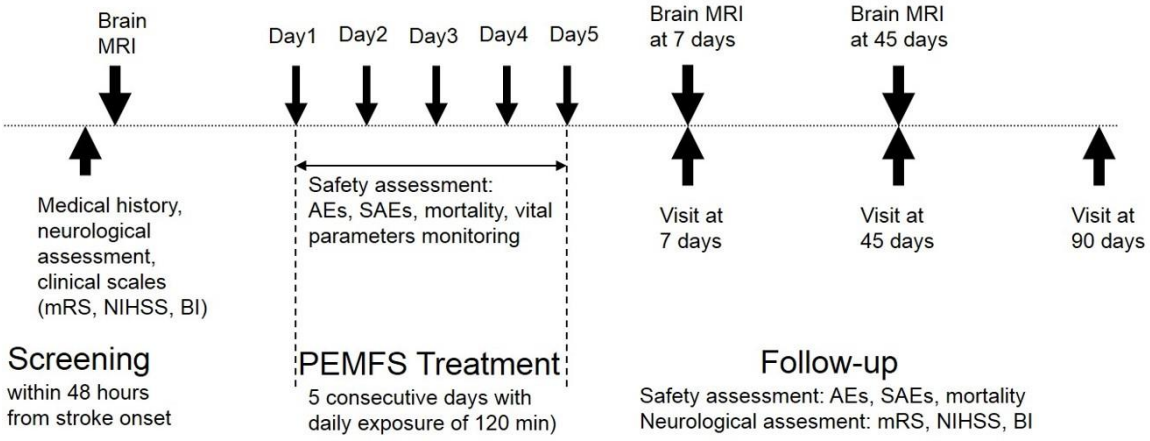
Title

Low-frequency pulsed electromagnetic fields (ELF-MFs) are used to treat acute ischemic strokes (I-NIC).

Study design

Multicenter, prospective, randomized, placebo-controlled, double-blind study.

Figure shows the flow-chart of the study.



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Aim of the study

This multicenter, prospective, randomized, double-blind, placebo-controlled study aimed to evaluate the efficacy and safety of ELF-MFs delivered in the form of PEMFs for the treatment of patients with ischemic stroke in the acute phase.

Objective of the study

Primary Objective

To evaluate the effects of ELF-MFs delivered with the experimental medical device, I-NIC, on the extent of the ischemic area measured by MRI at different follow-ups.

Secondary Objectives

1. The clinical efficacy of ELF-MFs was evaluated by scoring the following rating scales:
    - Modified Rankin Scale (mRS)
    - Barthel Index
    - National Institutes of Health Stroke Scale (NIHSS)
2. To evaluate the safety of ELF-MF by means of the following parameters:
    - any clinical worsening during the days of stimulation as measured by the NIHSS clinical scale
    - any hemorrhagic transformation of the ischaemic lesion evidenced by MRI at different follow-ups
    - Incidence of serious adverse events (AEs), serious adverse events (SAEs), mortality during the pacing period, and follow-up. Adverse events were recorded using registration reports in the forms present at each follow-up.
3. To assess the tolerability of ELF-MFs by means of:
    - ad hoc questionnaires to highlight any discomfort or feelings of distress that may lead to treatment discontinuation
    - number of patients requesting treatment discontinuation

Sample size calculation

The sample size was calculated from literature data [29] showing, in a group of subjects with cerebral ischemia and no treatment, a net increase of 95.7cm^3 in lesion size measured 7 days after onset.

Assuming that, in the active group, the increase was less than 30% (i.e., approximately 60 cm^3 from the first assessment), with a significance of 95% and a statistical power of 80%, 62 patients were required per group.

Duration of the study

Patient enrollment will only occur if the patient fulfils the inclusion and exclusion criteria for the protocol.
For each patient, the study lasts 3 months (calculated from cerebral ischemia to the last follow-up). For each center, the study will last four years from the date of recruitment of the first patient, subject to reaching the total number of patients to be enrolled.

Eligibility Criteria

Inclusion Criteria:

- age > 50 years;
- first onset, mono-hemispheric ischemic stroke in the middle cerebral artery territory;
- onset of symptoms within 48 hours;
- National Institutes of Health Stroke Scale (NIHSS) score between 4 and 25;
- signed written informed consent.

Exclusion Criteria:

- acute intracranial haemorrhage;
- previous ischemic or haemorrhagic stroke;
- lacunar stroke, defined as stroke not involving the cortex and < 2.0 cm if measured on MRI diffusion-weighted images.
- contraindications to transcranial magnetic stimulation, such as implanted metallic parts of electronic devices or other metals in the body.
- patients with cardiac pacemakers, intracranial metal clips, deep brain stimulators, and other conditions that contraindicate exposure to ELF-MFs;
- historical modified Rankin Scale (mRS) >1;
- other serious or complex disease that may confound treatment assessment;
- women known to be pregnant, lactating, or who have a positive or indeterminate pregnancy test;
- current participation in another study.

Randomization

When recruited into the study protocol, patients are divided using a block randomization program (www.randomization.com) into two homogeneous groups of 62 patients each. One group will be treated with an active stimulator (experimental group) and one group will be treated with a placebo stimulator (control group). Neither the patient nor the physician an distinguish between the real and placebo stimulations.
In order to obtain two homogeneous groups, the following patient stratification criteria were defined: age (50 ≤ age ≤65 and age > 65), sex (M/F), NIHSS score at baseline (4 ≤ NIHSS < 15 and 15 ≤ NIHSS≤25), thrombolysis (yes/no).

Concealment of the randomization list

To avoid systematic errors, the randomization center will be external and will use an interactive (web) system to allocate patients into the two groups. Clinicians identify patients, obtain consent, decide on enrolment, and enter patient characteristics (age, sex, NIHSS score at baseline, thrombolysis) into web-based software, which automatically assigns the patient to the first useful place on the list in one of the two groups. The program returns a code (A/B or 1000/2000) corresponding to the stimulator that the clinician will use to stimulate the patient. Clinicians cannot distinguish the type of stimulator (real or placebo) because of the external appearance of the stimulator, the sound generated by the stimulator, and the sensation.

Informed consent

Patient consent to participate in the study will be obtained after full information about the study is provided to the patient, paying particular attention to explaining the purpose, management, and use of the patient's data.

The patient's right to withhold consent or to withdraw it at any time during the study without explanation and without implication for the proper continuation of treatment will always be respected.

Discontinuation of the study

Patient participation in this study is completely voluntary. The patient may withdraw from the study at any time without any negative impact on the quality of healthcare provided. The date of withdrawal will be recorded along with the reasons for patient withdrawal from the study.

Similarly, the trial may be terminated if the physician notes the occurrence of undesirable effects or other conditions that make it appropriate to suspend the trial in the patient's interest. In such cases, the patient is promptly informed about further valid treatments for his or her disease, which he or she may discuss with a doctor.

Patients who withdrew from the trial may have been replaced with new patients. Subjects who withdraw after at least one stimulation session will be followed up for 3 months to assess treatment safety.

Treatment schedule and dosage

The patient will be treated according to the guidelines for the treatment of cerebral ischemia with regard to therapy. The treatment protocol to which the patient may be subjected does not replace normally available therapies to which the patient will still be subjected if indicated. The treatment proposed with the experimental medical device, I-NIC, will play a complementary role to ordinary therapies, with the intention of increasing their effectiveness. The need for new treatments stems from the fact that currently available therapies do not adequately resolve the consequences of cerebral ischemia in all cases.

To deliver magnetic stimulation, a dedicated device built by IGEA (Carpi-Italy) and already used in similar studies on healthy volunteers [27] will be used.

The experimental medical device is identified as follows:

Commercial name: I-NIC

Model: I-ONE mod. CBA-03

Classification according to EN 60601-1: Class II device - Type BF
Classification according to MDD 93/42 EEC: Class IIa device
It consists of a coil and a generator. The coil consists of a flexible, rectangular solenoid, which is placed on the patient's head (in the affected hemisphere) and held in position using a suitably designed support.
The generator that powers the coil produces a magnetic field with the following characteristics
- type signal: pulsed
- stimulus frequency: 75 ± 2 Hz
- stimulus duration:1.1 ms
- peak magnetic field strength: 1.8 ± 0.3 mT
Based on the preliminary results of the early feasibility study (clinicaltrials.gov NCT01941147), treatment with ELF-MF in this study will be carried out within 48 h of the onset of symptoms, maintained for 5 consecutive days, with a daily exposure duration of 120 min. If necessary the exposure can be divided into two sessions of 60 min each.

Storage of randomization codes, decoding and reliability of the product

Each active or placebo device is coded by means of codes that are recorded in a traceability form for devices that are produced appropriately for the study. The traceability form was filed in paper form at the Research and Development Office of IGEA SpA, in the TRIAL MASTER FILE PROJECT I-NIC, and electronically in file C:\Users\a.dorati\Documents_projects\I-NIC\110_devices_I-One_for_clinical_study_I-NIC. xlsx and is also present on the company server as a backup copy.
To test the reliability of the product, the output and input of each active or placebo device were checked by recording the delivery parameters of a low-frequency pulsed electromagnetic field (ELF-MF).

Clinical assessments at Baseline

- o Medical history
- o Neurological physical examination
- o Validated clinical scales: Barthel Index, Modified-Rankin Scale, NIHSS

Clinical assessments at 7, 45 and 90 days:

- o Neurological physical examination
- o Validated clinical scales: Barthel Index, Modified-Rankin Scale, NIHSS
- o Data collection form for adverse events

Neuroradiological evaluation at baseline, 7 and 45 days:

MRIs will be performed according to the following protocol:

Baseline MRI (within 48 h): DWI (multiple b), ARM intra, FLAIR, GET2* o SWI, PWI (DSC), T1post Gd
- o RMN 7 days: DWI (b1000), FLAIR, T1, ARM intra-, T2* SWI
- o RMN 45 days: FLAIR, T1, GET2* or SWI

Safety

Informed consent must be obtained from all study participants. No sampling of biological materials or drug administration is planned. The safety of the treatment will be assessed by clinical monitoring, instrumental monitoring of vital parameters during treatment, assessment of mortality and incidence of adverse events during treatment up to 3 months after the end of treatment, and MRI evaluation for possible hemorrhagic transformation of the ischemic lesion at different follow-ups.

Statistical analysis plan

The collected data will be statistically evaluated by an independent observer using Student's t-test, repeated measures analysis of variance (ANOVA), Pearson's chi-squared test, and a generalized linear mixed effects model.
As required by the Ministry of Health, the study will be conducted in two phases. The first phase involves the enrollment and data analysis of half of the sample (62 patients divided into experimental and control groups). The efficacy and safety results will be reported in a report prepared by the principal investigator and submitted to the Ministry of Health for evaluation; only after a possible non-objection by the Ministry and the Ethics Committees, the second phase of the investigation can be started to complete the enrollment of the 124 patients.
Particular attention will be paid to obtaining a complete follow-up for all patients.
An "intention-to-treat" analysis is not planned, but only a "protocol" analysis in which all patients who undergo MRI and clinical follow-up at 1 month will be included.
Any deviations from the original statistical plan are described and appropriately justified in the final report.

Monitor

A monitoring activity managed by IGEA is foreseen at the centers participating in the study, aimed at monitoring patient compliance, checking the progress of patient recruitment in the study, the correct implementation of the study, and providing any technical support to the researchers.

Data Collection and Management and Storage of Documentation

Patients will be encrypted using the appropriate codes. Patient data will be collected at baseline and at different follow-ups in electronic Case Report Forms (CRFs) on a web platform, in a dedicated server, and processed in accordance with Legislative Decree no. 196/2003 (Privacy Code) Articles 11-12-13 on the protection of persons with regard to the processing of personal data and will be used exclusively for scientific research purposes.

Ethical aspects

The study will be conducted according to Good Clinical Practice (ICH/GCP Annex 1 of the Ministerial Decree of 15.7.1997) in compliance with the ethical principles of the Declaration of Helsinki and the Oviedo Convention.
Before the trial begins, the Policlinico Campus Bio-Medico of Rome–UOC Neurology will obtain approval for the study protocol from the relevant Ethics Committee and the Ministry of Health, with particular regard to the information sheet and informed consent form.

Costs

All costs associated with the trial will be covered by the sponsor as stipulated in the agreement. No additional costs will be charged.

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Supplementary Methods

The study protocol was reviewed and approved by the Italian Ministry of Health, which recommended an interim analysis in consideration of the limited number of patients included in the previous dose-finding phase 2 study. Ethics approval was obtained from all participating institutions, and the research was completed in accordance with the Helsinki Declaration. All participants gave written informed consent to participate. The data that support the findings of the I-NIC study are available from the corresponding author upon reasonable request. This manuscript was prepared following the Consolidated Standards of Reporting Trials (CONSORT) guidelines for randomized controlled trials.

Study design and participants

This is a prospective, multicenter, randomized, placebo-controlled double-blind study conducted in seven Italian centers between April 2016 and September 2022.

Patients were eligible to participate in the trial if they met the following criteria: (1) age between 50 and 85, (2) diagnosis of first onset, mono-hemispheric ischemic stroke in the middle cerebral artery (MCA) territory; (3) within 48 hours from stroke onset; and (4) having National Institutes of Health Stroke Scale (NIHSS) score between 4 and 25. The exclusion criteria were: (1) acute intracranial hemorrhage; previous ischemic or hemorrhagic stroke; (2) lacunar stroke, defined as not involving the cortex and <2.0 cm in diameter on magnetic resonance imaging (MRI) diffusion-weighted images; (3) contraindications to transcranial magnetic stimulation such as implanted metallic parts of implanted electronic devices or other metal in the body; (4) historical modified Rankin Scale (mRS) >1; (5) other serious or complex disease that may confound treatment assessment; (6) women known to be pregnant, lactating, or having a positive or indeterminate pregnancy test; and (7) simultaneous participation in another study.

Randomization and study intervention

Once enrolled, patients were randomly assigned in a 1:1 ratio to the active or placebo group. The clinician informed the patient about the possibility of being recruited into a two-arm trial. At enrollment, the patient accepted to be referred to either of the two groups throughout the duration of the study. The assignment of the patient to an active or placebo group was performed using a web-based computer program (www.randomization.com). It provided random sequences that were applied in each center and in each subgroup built on the randomization criteria: age (50≤age≤65 and age>65), sex (M/F), NIHSS score at baseline (4≤NIHSS<15 and 15≤NIHSS≤25), and thrombolysis/thrombectomy (yes/no).

Patients in the active group received real pulsed electromagnetic field (PEMF) treatment and the standard of care for acute ischemic stroke (AIS) according to current guidelines.[1] Within 48 hours from the onset of the stroke, the patients in the active group underwent 120 minutes, of daily, PEMF treatment for 5 consecutive days, during their hospital stay. PEMF treatment was delivered using a disposable rectangular (120×160 mm), flexible coil, positioned on the ischemic hemisphere and connected to the pulse generator (CBA-03; IGEA, Carpi, Italy) (Supplementary Figure 2) producing a single-pulsed signal at 75±2 Hz, with a pulse duration of 1.3 ms and a magnetic field peak intensity of 1.8±0.3 mT measured by a gaussmeter (Model 425 gaussmeter; Lake Shore Cryotronics, Inc., Westerville, OH, USA).[2] The coil was held in place by a helmet positioned on the patient head. Patients were bedridden during the treatment but were free to move around without compromising the correct positioning of the coil. Data from the dose finding study previously published by our group[3,4] showed that the minimum peak value of the magnetic field was always above 1 mT in the infarct area located in the MCA territory (Supplementary Figure 3). Patients enrolled in the placebo group received a sham treatment through a coil that does not deliver magnetic stimulation since it is electrically disconnected from the pulse generator (CBA-03 sham device; IGEA) and the standard of care for AIS according to current guidelines.

Investigators, caregivers, outcome assessors, and all participants were blinded to the randomization group. Patients received either the active or the sham device based on the randomization list. The device for real exposure produces no auditory signals and is identical to the device for sham exposure, which does not generate the magnetic field. Investigators and caregivers received proper training for the positioning of the coil and the delivery of the PEMF treatment. The procedure was kept standard over time and across centers through site visits from a certified clinical research associate (CRA).

Outcomes

The primary outcome of this trial was the effect of PEMF treatment on the extent of ischemic lesion volume, measured by MRI at baseline (within 48 hours from the onset of the stroke) and 45 days from the onset of the AIS.

The secondary outcomes were as follows:

(1) To evaluate the clinical efficacy of PEMFs by calculating the changes from baseline to day 7, day 45, and day 90 of the following clinical scores: mRS, Barthel Index (BI), and the NIHSS. All clinical investigators were trained and certified in the assess-

ment of the clinical scores.

(2) To evaluate the safety of PEMF treatment. Safety was monitored as follows: (i) clinical evaluation during the days of PEMF exposure measured by the NIHSS clinical scale; (ii) hemorrhagic transformation of the ischemic lesion was monitored by MRI at the different follow-ups; (iii) incidence of adverse events (AEs) and serious adverse events (SAEs); and (iv) mortality during the days of PEMF exposure and follow-up. Moreover, during PEMF exposure, patients were constantly monitored by a multimodal monitor that simultaneously assesses and displays electrocardiogram (ECG) and relevant vital parameters (respiratory rate, heart rate, blood pressure, pulse oximetry).

(3) To evaluate the tolerability of PEMF treatment through: (i) *ad hoc* questionnaires to highlight any discomfort or distress that could lead to a discontinuation of treatment, and the (ii) number of patients completing the full treatment period.

MRI evaluation and lesion volume calculation

MRI images were obtained with a 1.5-T scanner. MRI protocol included T1-weighted spin-echo (SE) sequence in axial plane, T2-weighted turbo spin-echo (TSE) sequence in sagittal and coronal plane; diffusion-weighted imaging (DWI) sequence in axial plane; T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence in axial plane and T2*-weighted imaging in axial plane. Details for each sequence are reported in Supplementary Table 2.

DWI was obtained at three b values magnitude from 0 to 1,000 s/mm^2. A quantitative measure of volumetric lesion area was extracted from MRI image segmentation, at 48 hours from the insult (T0), at the 7 days (T7), and at the 45 (T45) days follow-ups. Lesions at T0 and T7 were segmented from DWI sequences, as they provide an estimation of the ischemic volume that might progress to permanent damage,[5] whereas chronic lesions were segmented from FLAIR sequences, as typical for follow-up evaluations.[6] Co-registration of DWI and FLAIR sequences and segmentations were performed within the software 3D Slicer (National Alliance for Medical Image Computing [NA-MIC], Grant U54 EB005149), using automatic tools of thresholding and level tracing.

DWI positivity was defined as an area of high signal with b= 1,000 s/mm^2, while the corresponding diffusion coefficient image showed a low signal; T2-FLAIR positivity was defined as the presence of an area of high signal in the region corresponding to the DWI-positive lesion.

Subgroup analysis

Within the study population, a subgroup of patients who received reperfusion therapy (thrombolysis only, thrombectomy only, or thrombolysis and thrombectomy combined) in addition to PEMF treatment (either active or sham device) was identified. The primary and secondary outcomes foreseen by the study protocol were analyzed in this subgroup of patients following the same criteria described for the total population.

Statistical analysis

The sample size was calculated considering literature data[7] and the experience gained during the dose-finding study[3] that showed an average reduction in lesion size in PEMF-treated patients at 30 days equal to 5.7 cm^3 with a standard deviation equal to 13. Based on this premise, group sample sizes of 62 for each group achieve 80% power to reject the null hypothesis of equal means when the population mean difference is $\mu 1-\mu 2=15.0-9.3=5.7$ with standard deviations of 13.0 for group 1 and 12.0 for group 2, and with a significance level (alpha) of 0.050 using a one-sided two-sample unequal-variance t-test.

In the descriptive analysis, quantitative variables are reported as mean and standard deviation, and qualitative variables as absolute counts and percentages.

Volume changes were normalized to baseline volume for each patient. Normalized data are expressed as percentage of volume reduction over baseline (normalized volume reduction).

Quantitative variables are analyzed with *post hoc* paired analysis for variables with Gaussian distribution, and Wilcoxon h-test for variables not normally distributed. Bonferroni correction is applied to all tests.

Comparisons between two groups are performed with heteroscedastic two-tailed Student t-test for quantitative variables with Gaussian distribution, heteroscedastic two-tailed Mann-Whitney test for variables not normally distributed, contingency tables, and with two-tailed chi-square test with Fisher correction for qualitative variables.

A *P*-value of 0.05 is considered as statistically significant. Statistical analyses are performed with NCSS 9 Statistical Software (NCSS, LLC., Kaysville, UT, USA; https://www.ncss.com/software/ncss/).

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Supplementary Table 1. Patient characteristics at baseline

Characteristics	Active (n=14)	Placebo (n=20)	*P*
Age (yr)	70.6±12.7	71.4±10.1	0.846
Male sex	8 (57.0)	10 (50.0)	0.738
Hypertension	3 (21.4)	9 (45.0)	0.275
Diabetes	1 (7.1)	3 (15.0)	0.627
Treatment			0.737
Thrombolysis only	2 (14.3)	6 (30.0)	
Thrombectomy only	3 (21.4)	3 (15.0)	
Thrombolysis and thrombectomy	3 (21.4)	3 (15.0)	
No treatment	6 (42.9)	8 (40.0)	
BI at baseline	43.2±27.0	55.5±34.7	0.255
mRS at baseline	3.4±1.3	3.2±1.4	0.672
NIHSS at baseline	6.1±2.4	6.4±3.2	0.757

Values are presented as mean±standard deviation or n (%).
BI, Barthel Index; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale.

Supplementary Table 2. MR sequence parameters

	T2-weighted TSE	T2-weighted FLAIR	DWI	T1-weighted (SE)	T2*-weighted
Slice thickness (mm)	1–2	1–5	4–5	5	3
Repetition time (s)	4,800–5,000	4,800–11,100	3,000–8,200	580	1,160–1,255
Echo time (s)	335–382	125–382	65–89	12	23–24
Inversion time (s)	-	1,800–2,800	-	-	-
Acquisition matrix	248–256 × 231–256	248–308 × 191–256	116–192 × 94–192	272 × 206	256 × 206
Flip angle (degree)	90–120	90–120	90	70	18
Pixel spacing (mm×mm)	1–1.016 × 1–1.1016	0.5–1.016 × 0.5–1.016	0.575–1.875 × 0.575–1.875	0.685 × 0.685	0.898 × 0898
Spacing between slices (mm)	0–2	0–6	5–6	6	3

MR, magnetic resonance; TSE, turbo spin echo; FLAIR, fluid-attenuated inversion recovery; DWI, diffusion-weighted imaging; SE, spin echo.

Supplementary Table 3. BI score values

	BI score		*P*
	Active	Placebo	
Baseline	43.2±27.0	55.5±34.7	0.255
7 days	73.6±28.9	73.8±31.6	0.986
45 days	95.9±12.0	84.2±31.8	0.163
90 days	95.0±13.4	88.3±29.6	0.416

Values are presented as mean±standard deviation.
BI, Barthel Index.

Supplementary Table 4. Adverse events during the 5 days of treatment

Adverse events	Active (n=14)	Placebo (n=20)	*P* (chi-square)
Nausea	0	1	
Headache	1	2	
Heat sensation	1	3	
Heat sensation at the lower limb	1	0	
Headache and heat sensation	0	1	
Total events	3	7	0.467

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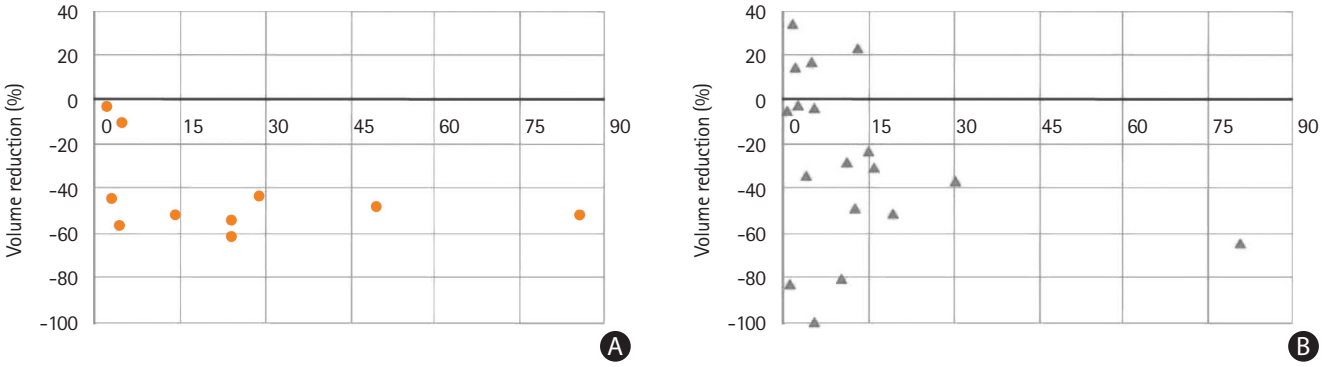
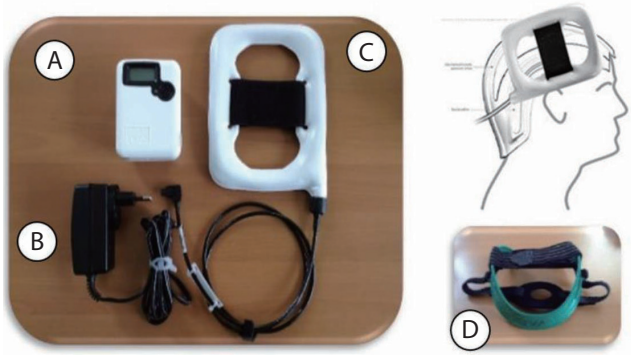
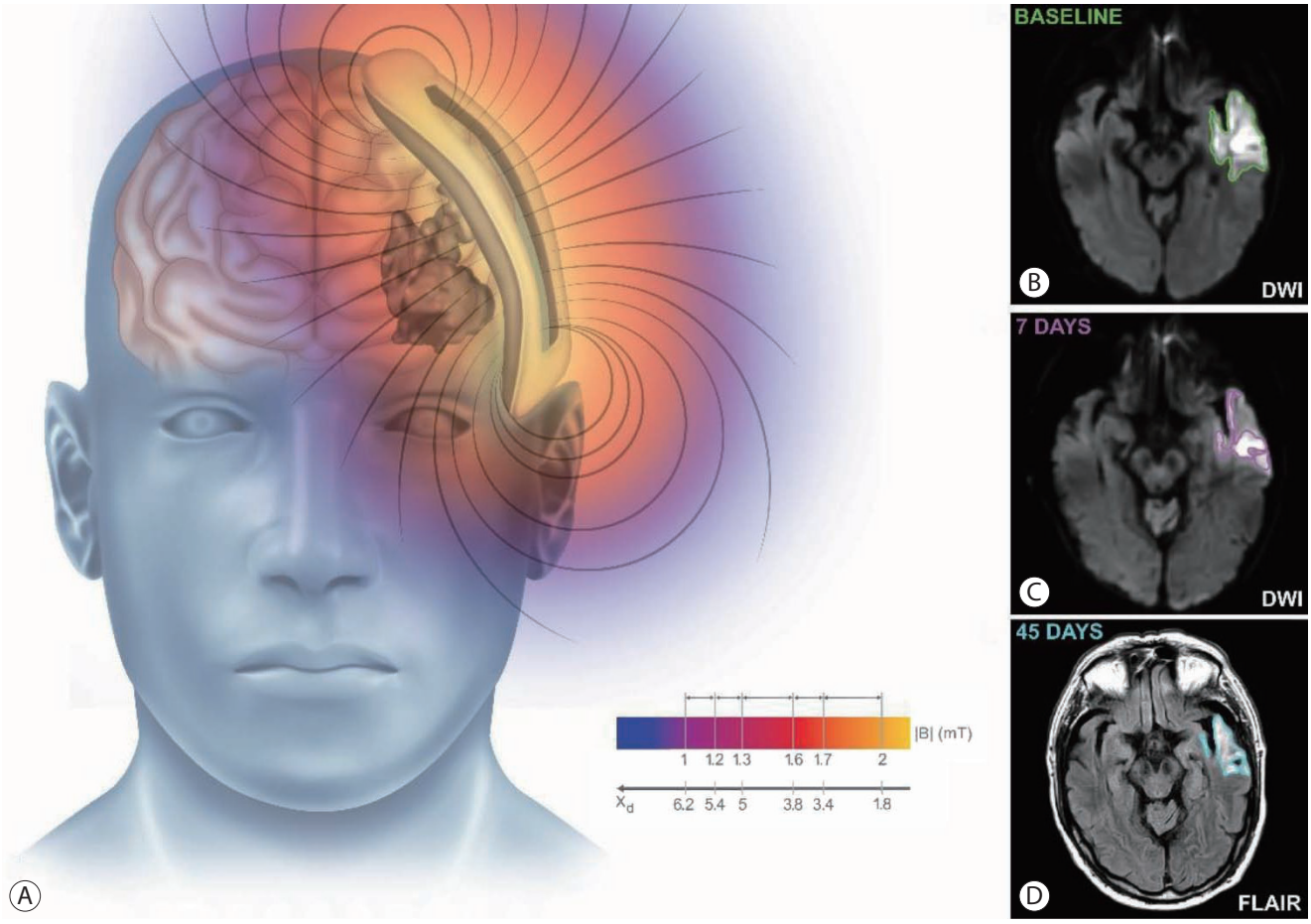


Supplementary Figure 1. Normalized volume reduction at 45 days in (A) individual active patients and (B) individual placebo patients.



Supplementary Figure 2. I-NIC device. The figure shows the components of the I-NIC device: (A) generator, (B) power supply, (C) coil, and (D) helmet to keep the solenoid in place. pulsed electromagnetic field (PEMF) treatment was delivered for 5 consecutive days after stroke, for 2 hours every day.



Supplemetary Figure 3. Magnetic field distribution in the brain. (A) Distribution of the magnetic field in the infarct area: a representative patient is shown. In this clinical trial, the magnetic field amplitude was set to expose the brain areas suffering the ischemic event to at least 1 mT. MRI images of a representative active patient at (B) baseline, (C) 7 days, and (D) 45 days. MRI, magnetic resonance imaging; DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery.

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