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/).

Supplementary References

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