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.

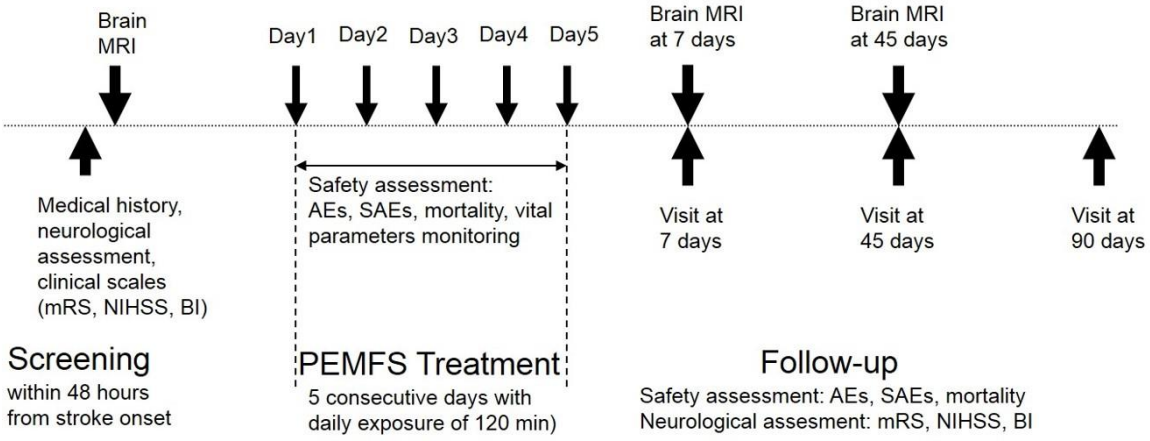
Title

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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