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A randomized controlled cross-over double blind study protocol on THC/CBD oromucosal spray as an add-on therapy for post-stroke spasticity

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ABSTRACT

Introduction: Stroke is the most disabling neurological disorder and often causes spasticity. Transmucosal cannabinoids (THC:CBD, Sativex) is currently available to treat spasticity-associated symptoms in patients with multiple sclerosis. Cannabinoids are being considered useful also in the treatment of pain, nausea and epilepsy, but may bear and increased risk for cardiovascular events. Spasticity is often assessed with subjective and clinical rating scales, which are unable to measure the increased excitability of the monosynaptic reflex, considered the hallmark of spasticity. The neurophysiological assessment of the stretch reflex provides a precise and objective method to measure spasticity. We propose a novel study to understand if Sativex could be useful in reducing spasticity in stroke survivors and investigating tolerability and safety by accurate cardiovascular monitoring.

Methods and analysis: We will recruit 50 patients with spasticity following stroke to take THC:CBD in a double blind placebo-controlled crossover study. Spasticity will be assessed with a numeric rating scale for spasticity, the modified Ashworth scale and with the electromyographic recording of the stretch reflex. The cardiovascular risk will be assessed prior to inclusion. Blood pressure, heart rate, number of daily spasms, bladder function, sleep disruption and adverse events will be monitored throughout the study. A mixed-model ANOVA will be used to compare the stretch reflex amplitude between the time points; semi-quantitative measures will be compared using the Mann-Whitney test (THC:CBD vs placebo) and Wilcoxon test (baseline vs treatment).

Ethics and dissemination: The study was registered on the EudraCT database with number 2016-001034-10 and approved by both the Italian Medicines Agency (Agenzia Italiana del Farmaco – AIFA) and local Ethics Committee. Data will be made anonymous and uploaded to a open access repository. Results will be disseminated by presentations at national and international conferences and by publication in journals of clinical neuroscience and neurology.

Strengths and limitations of this study:

- First study on Sativex to treat post-stroke spasticity
- Electromyographic recording of the stretch reflex to precisely measure spasticity
- Assessment of cannabinoids tolerability in stroke survivors
- Limited number of patients in relation to a monocentric pilot study

Keywords: THC:CBD, Sativex, stretch reflex, spasticity, stroke, cannabinoids

INTRODUCTION

Stroke is one of the most disabling neurological disease and frequently determines important chronic consequences such as spasticity. Prevalence of post-stroke spasticity ranges from 4% to 42.6%, with the prevalence of disabling spasticity ranging from 2% to 13%[1]. Treatment of post-stroke spasticity is based on rehabilitation, local injection of botulinum toxin (BoNT) in the affected muscles for focal spasticity and/or use of classic oral drugs such as tizanidine, baclofen, thiocolchicoside and benzodiazepines, which are not always effective and have a good number of possible side effects.

The transmucosal administration of delta-9-tetrahydrocannabinol and cannabidiol (THC and CBD at 1:1 ratio oromucosal spray, Sativex®) is able to reduce spasticity acting on endocannabinoid receptors CB_1 and CB_2. This novel drug has been licensed after an extensive clinical trials program[2–4] in adult patients with multiple sclerosis who have shown no significant benefit from other antispasmodic drugs. More than 45000 patient/years of exposure since its approval in more than 15 EU countries support their antispasticity effectiveness and safety profile in this indication[5]. Besides improving spasticity, cannabinoids can be beneficial in reducing pain, chemotherapy-induced nausea and vomiting, moreover, they contribute to reducing seizures and to lowering eye pressure in glaucoma[6]. Cannabinoids can also exert psychological effects by lowering anxiety levels and inducing sedation or euphoria. Marijuana, which is the main source of cannabinoids, is declared illegal in many countries mostly because the risk of abuse, dependence and withdrawal syndrome, related to the effect of its high amounts of THC. Several reports support an increased ischemic stroke risk related to relevant abuse of smoked marijuana[7–17] as well as synthetic cannabinoids[18–20]. Ischemic stroke following cannabis involves more frequently basal ganglia and cerebellum where CB_1 and CB_2 receptors show a higher expression[13].

The “French Association of the Regional Abuse and Dependence Monitoring Centres Working Group on Cannabis Complications” warns about the increased cardiovascular risk related to the use of herbal cannabis, mostly consisting of acute coronary syndromes and peripheral arteriopathies, potentially leading to life-threatening conditions[21]. The detrimental consequences of cannabinoids could be attributed to the increase in hearth rate[22] as well as arterial spasms also in the context of a reversible cerebral vasoconstriction syndrome[23], but also vasculitis, postural hypotension and cardioembolism[24].

On the other side, some studies support a beneficial effect on stroke evolution of cannabinoid receptors stimulation. In fact, cannabinoid mediated activation of CB_1 and CB_2 receptors reduces inflammation and neuronal injury in acute ischemic stroke[25]. Activation of CB_2 receptors shows protective effects after ischemic injury[26] and inhibits atherosclerotic plaque progression[27,28].

To our knowledge no correlations have been reported between haemorrhagic stroke and

cannabinoids intake. In our opinion, the modification of blood pressure is the most important cannabinoid effect that should be taken into account in patients with a previous haemorrhagic stroke or predisposed to intracranial bleeding. Cannabinoids are indeed capable of inducing blood pressure fluctuations in a specific triphasic pattern (low-high-low) potentially harmful if the patient with bleeding risk[29]. Ischemic disease is not included among THC/CBD oromucosal spray contraindications. However, considering that to our knowledge no study has been performed with THC/CBD oromucosal spray on post-stroke spasticity, we believe that a particular caution should be used in stroke patients.

A major issue in studies involving spasticity, is the decision of which method of measure consider as endpoint. The definition of spasticity provided by Lance is one of the most precise and reliable, focusing on the stretch reflex as the neurophysiological equivalent of spasticity[30]. Probably because of technical complexity and required expertise, neurophysiological approaches are rarely adopted. Clinical rating scales such as the modified Ashworth scale (MAS)[31] or subjective scores such as the numeric rating scale (NRS) for spasticity are being widely used[32,33]. Recent evidence supports the idea that MAS and NRS are indeed useful to quickly rate spasticity in a clinical setting, however provide a very variable and imprecise assessment of many symptoms related to spasticity, but where spasticity itself is probably only a common factor[34]. The adoption of stretch reflex as the most appropriate neurophysiological measure of spasticity increases the specificity and reduces the variability of the endpoint and is particularly suitable for clinical trials.

Our proposal is therefore to assess the efficacy of THC/CBD oromucosal spray in patients with spasticity following stroke as add-on to first line antispasticity medications with an experimental pilot randomized placebo controlled crossover clinical trial using the stretch reflex as primary outcome measure. Prior to inclusion in the study, we propose strict selection criteria in order to reduce the risk of relevant side effects.

METHODS AND ANALYSIS

Subjects

At the Department of Neuroscience, IRCCS AOU San Martino - IST , University of Genova, we will recruit 50 patients with spasticity secondary to stroke occurred at least 3 months earlier. Both naïve to BoNT or BoNT treated patients will be recruited, however those treated with BoNT will enter the study at least 4 months after the last injection in order to allow a reasonable wash-out. The study will last 2 years.

Inclusion criteria are:

- presence of spasticity rated between 1 and 3 at the Ashworth Modified Scale (MAS) in at least one of the following segments: flexor muscles of the wrist, flexor muscles of the forearm, extensor muscles of the leg, and/or foot plantiflexors;

- absence of significant peripheral nervous system pathology detectable on clinical basis;

- absence of concomitant parkinsonism;

- acceptable cardiovascular ischemic risk following a cardiological evaluation along with the laboratory and instrumental exams requested by the cardiologist, as well as a CHA_2DS_2VASc score less than 7;

- absence of a demonstrated stenosis higher than 50% at intracranial main arteries (mean cerebral, basilar, internal carotid, vertebral) or at cervical tracts of carotid and vertebral arteries;

- absence of significant cognitive impairment hampering patients' capability of understanding the study protocol and signing the consent form;

Apart from the criteria listed above, there will be no limitations related to age, sex and degree of disability.

The following sociodemographic data will be collected: gender, age, time of acute lesion, years with spasticity, areas affected with spasticity.

Study protocol

Patients will enter a crossover study paradigm after a cardiac-cerebral-vascular risk assessment performed by a cardiologist and by a vascular neurologist; those with an acceptable cardiac-cerebral-vascular risk will be randomly assigned (in a 1:1 ratio) to one of the two following treatment sequences (**Figure 1**):

• THC:CBD oromucosal spray – Placebo (i.e. THC:CBD oromucosal spray during Period I and Placebo during Period II)

• Placebo - THC:CBD oromucosal spray (i.e. Placebo during Period I and THC:CBD oromucosal spray during Period II)

The Randomization List will be generated through a validated SAS® program, in permuted blocks of reasonable size in order to guarantee the treatment balance.

After the first (T0) baseline evaluation visit, during one month period the patients will titrate their medication to reach the optimal number of puffs/day and then evaluated again (T1). A 2-weeks washout time will allow patients taking THC/CBD oromucosal spray (or placebo) to reach their baseline spasticity condition and then patients will switch arms and perform another month the other treatment. After while the final (T2) assessment will be performed. During the whole study, the patients will have to maintain the same therapy apart from the study drug, in order to minimize

other conditions that could affect the level of spasticity. During T1 and T2 evaluation, additional information about the number of sprays/day will be collected, along with all possible adverse events. During the 10 weeks of the study, patients not responding to the treatment and unable to complete the study untreated will undergo a rescue treatment (e.g. BoNT) and exit the study.

This is a double-blind study. All individuals involved in the study conduct, including patients, investigator staff, persons performing the assessments and data analysts will remain blinded to the identity of the treatment from the time of randomization until database lock.

Randomization data will be kept strictly confidential until the time of unblinding and will not be accessible to anyone involved in the conduct of the study. The identity of the treatments will be concealed by the use of study drugs (THC:CBD oromucosal spray and matching Placebo) that are identical in packaging, labeling, schedule of administration and in appearance.

At the conclusion of the study, when the database has been locked, the assigned blinded treatment codes will be broken and made available for the final statistical analysis.

Individual patient unblinding during the course of the trial will only be allowed in the event of patient emergencies upon request from the investigator.

Experimental procedure

Preliminary setting and clinical evaluation

After the selection process and informed consent signature, all subjects will undergo a complete physical and neurological examination. The range of motion and MAS of the following segments will be assessed on both sides: elbow flexors, forearm pronators, wrist flexors, finger flexors, leg extensors, foot plantiflexors. The subjective amount of pain and muscle rigidity, quality of sleep and bladder dysfunction will be assessed using a 0-10 Numeric Rating Scale (NRS), as well as the amount of spasms using the Daily Spasm Score (DSS). The amount of disability related to hygiene, dressing, limb position and pain will be also assessed by mean of the Disability Assessment Scale (DAS)[35].

Blood pressure, heart rate will also be evaluated during each scheduled visits and patient's diary will be checked. The participants will be instructed about the medication dose up-titration process, patients' diary fulfilment and next visit date. All patients will be given a portable blood pressure device and instructed to record daily blood pressure values at home.

Possible adverse events will be monitored during the entire duration of the study. The detailed schedule of assessments and procedures is reported in **Table 1**.

Stretch reflex technical setup

The Biopac MP150 data acquisition system connected to a TSD130B twin-axis electronic goniometer (Biopac Systems Inc, USA) will be used for data acquisition. The goniometer will be

placed across the joint in order to optimally record the angle during the displacements with a sampling rate of 2KHz. EMG activity produced by the stretched muscle will be recorded by surface electrodes (TSD150B, Biopac Systems Inc, USA) placed over the muscle belly. Movement timing will be paced with a software emulated metronome.

The subjects will be evaluated in a quiet room with a temperature between 21 and 23°C. A limb segment presenting with spasticity between 1 and 3 at the MAS will be selected among flexor muscles of the wrist, flexor muscles of the forearm, extensor muscles of the leg, foot plantiflexors. If spasticity is detectable in more segments, in order to cause less discomfort to patients and examiners and prefer joints with a higher range of motion (allowing more accurate passive movement timing), the segments will be selected in the following order: wrist flexors, elbow flexors, leg estensors, foot plantiflexors (defined “selected segment” and undergoing the stretch reflex procedure). The subject will be seated if the selected segment is flexor muscles of the wrist, flexor muscles of the forearm, otherwise the subject will be lying on a comfortable examination table in a supine (extensor muscles of the leg) position with head and shoulders slightly elevated, or prone (foot plantiflexors) with the feet protruding from the examination table in order to allow a full ankle range of movement.

Stretch reflex procedure

The method was described in details in the first validating work[36]. The method consists in moving the selected segment throughout the full range of motion in the time corresponding to the interval between two consecutive metronome tones. The increase or decrease of tone frequency (beats per minute - BPM) determines a parallel change in the time required to perform a complete passive flexion or extension movement, so that given a certain range of motion, the mean movement velocity will be similarly modulated. The choice of the BPM was done taking into account that low values could not be able to elicit a stretch reflex (especially in subjects with a low degree of spasticity), while high values could produce discomfort to the subject and excessive fatigue in the examiner. Continuous flexion and extension movements in a "sinusoidal" way may be unsuitable to elicit and measure spasticity because of the possibility to trigger post-activation depression[37] or paratonia[38]. However, the method allows performing discontinuous (or "linear") movements as well, by interposing a few tones interval between movements.

The subjects were instructed to remain relaxed and to avoid resisting or facilitating the movements performed by the examiner. The stretch reflexes will be measured during movements determining elongation of the spastic muscle. At least 15 discontinuous stretch reflexes will be acquired during each experimental session. The recordings will be performed at each time point (T0, T1, T2) using the same metronome BPM. In order to obtain a reproducible electrode

positioning between sessions, a picture of the electrode and its relation with nearby anatomical landmarks will be taken in each subject at T0. In order to reduce variability, the examiner performing the passive movements will be the same in all time points for each patient[36].

Data analysis

The main endpoints of the study will be to assess the effect of THC/CBD oromucosal spray on spasticity measured with the stretch reflex and NRS for spasticity. To analyse the stretch reflex, the electromyographic recordings will be filtered, rectified and the average amplitude of the bursts will be calculated (meanEMG) using the dedicated AcqKnowledge analysis software (Biopac Systems Inc, USA). A mixed-model ANOVA with GROUP (THC/CBD oromucosal spray, placebo) as between-subjects factor and TIME (T0, T1, T2) as within-subjects factor will be used to compare meanEMG values.

All semi-quantitative rating scales scores will be compared between THC/CBD oromucosal spray/placebo groups using the Mann-Whitney test at each evaluation (T0, T1, T2). A comparison between T0 and T1 as well as between T0 and T2 will also be performed in each group using Wilcoxon test.

Since this is a pilot study, no formal sample size calculation con be done. It must be noticed, however, that in our previous study on patients with multiple sclerosis, we detected a significant reduction of the stretch reflex analysing 36 subjects[34].

ETHICS AND DISSEMINATION

The study was registered on the EU Clinical Trials Register (EudraCT) database with number 2016-001034-10 and named “SativexStroke”. We received the approval from the Italian Medicines Agency (Agenzia Italiana del Farmaco – AIFA) on September 23rd 2016, the approval from the local Ethics Committee “Comitato Etico Regionale della Liguria” on December 14th 2016 (protocol version 4.2) and the authorization from “ IRCCS AOU San Martino - IST” hospital on March 3rd 2017 (decree number 227).

We plan to start patients recruitment at spring 2017, after drug has been produced and labelling completed. The data collected in the study will be made anonymous and uploaded on a open access data repository. The analysis will be probably completed by the end of 2019, after the 2-years study period. The data will be disseminated by presentation at national and international conferences and by publication in journals of clinical neuroscience and neurology.

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AUTHORS' CONTRIBUTIONS

Lucio Marinelli: principal investigator, study conception, manuscript drafting

Maurizio Balestrino, Carlo Serrati, Carlo Gandolfo: patients recruitment, evaluation of the cerebral vascular risk profile, manuscript review

Laura Mori, Luca Puce: co-investigators, technical setup, data acquisition

Laura Giorello: study monitoring, adverse events tracking, administrative support

Antonio Currà, Francesco Fattapposta, Giovanni Abbruzzese, Carlo Trompetto: clinical consultant and manuscript review

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COMPETING INTERESTS STATEMENT

None declared.

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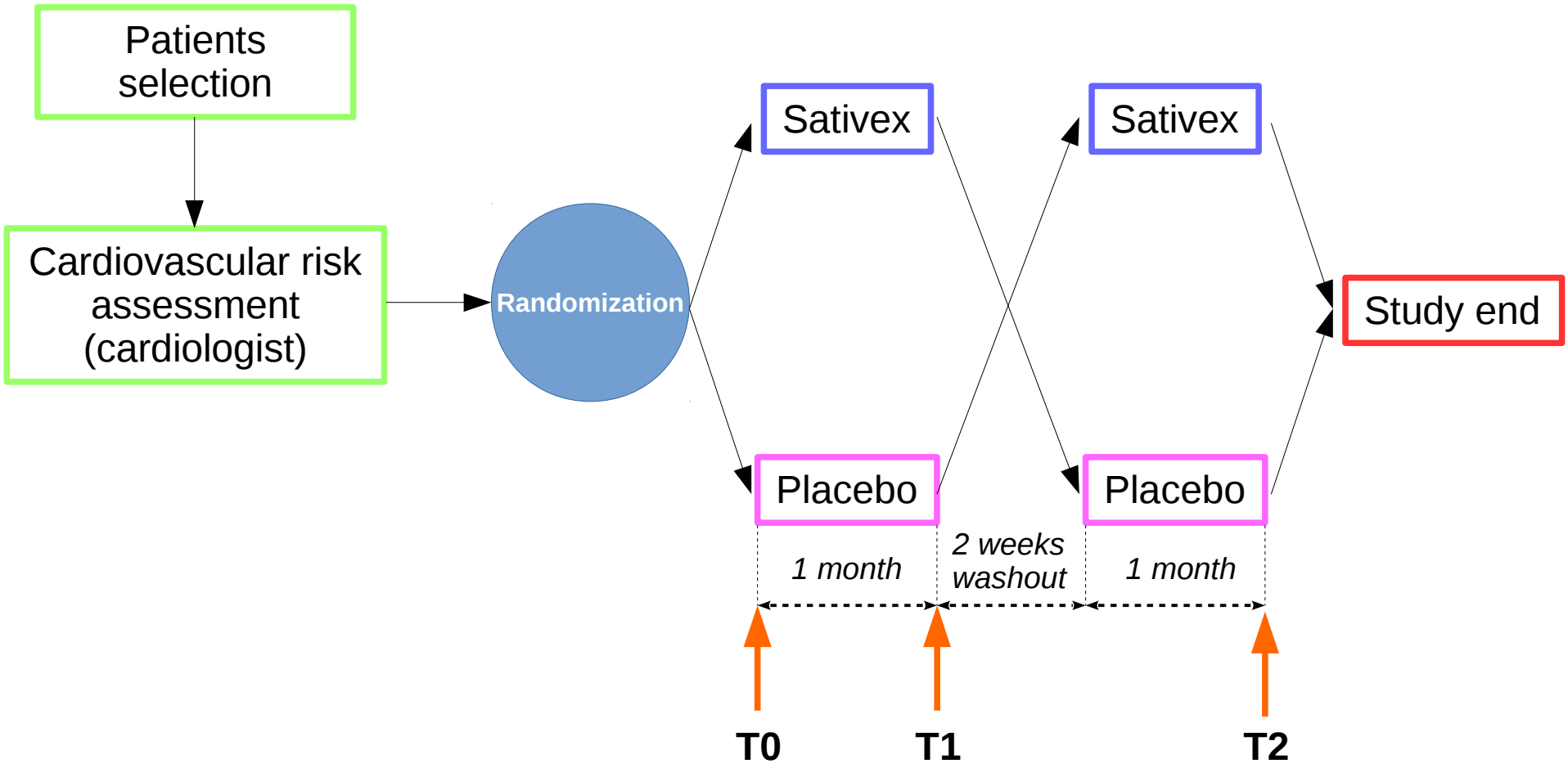
Table 1: Schedule of assessments and procedures

Study Period	**Screening and randomization**	**Period 1**	**Wash-out**	**Period 2**
Visit	**1 (T0)**	**2 (T1)**	**3**	**4 (T2)**
Informed consent	X			
Demography	X			
Medical and treatment history	X			
Physical and neurological examination	X	X	X	X
Full cardio evaluation	X			
Cardio consultation “as needed”		X	X	X
Vital signs	X	X	X	X
Inclusion & exclusion criteria	X			
Modified Ashworth scale	X	X		X
Spasticity NRS	X	X		X
Stretch reflex evaluation	X	X		X
Check patients diary		X		X
Spasticity NRS, Pain NRS, Bladder dysfunction NRS		*X*		*X*
Spasms number		*X*		*X*
Sleep quality NRS		*X*		*X*
Blood pressure and heart rate		*X*		*X*
Dispense study drug	X		X	
Check of returned study drug		X		X
Adverse events		X	X	X
Concomitant medications	X	X	X	X

NRS=numerical rating scale


Patients selection
Cardiovascular risk assessment (cardiologist)
Randomization
Sativex
Placebo
Sativex
Placebo
Study end
1 month
2 weeks washout
1 month
T0
T1
T2

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	8
	2b	All items from the World Health Organization Trial Registration Data Set	8
Protocol version	3	Date and version identifier	8
Funding	4	Sources and types of financial, material, and other support	8-9
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1; 9
	5b	Name and contact information for the trial sponsor	N/A
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
	6b	Explanation for choice of comparators	3-4
Objectives	7	Specific objectives or hypotheses	4

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5-6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	5-6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5-6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5-6
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	N/A
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	5-6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5-6

Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5-6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	5-6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	5-6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	5
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	6
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	6
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A

Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	N/A
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	9
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	8
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	8
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	8
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Attached as needed
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

A randomized controlled cross-over double blind pilot study protocol on THC:CBD oromucosal spray efficacy as an add-on therapy for post-stroke spasticity

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Primary Subject Heading:	Neurology
Secondary Subject Heading:	Rehabilitation medicine, Cardiovascular medicine, Diagnostics
Keywords:	THC:CBD, Sativex, stretch reflex, spasticity, Stroke < NEUROLOGY, cannabinoids

A randomized controlled cross-over double blind pilot study protocol on THC:CBD oromucosal spray efficacy as an add-on therapy for post-stroke spasticity

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ABSTRACT

Introduction: Stroke is the most disabling neurological disorder and often causes spasticity. Transmucosal cannabinoids (THC:CBD, Sativex) is currently available to treat spasticity-associated symptoms in patients with multiple sclerosis. Cannabinoids are being considered useful also in the treatment of pain, nausea and epilepsy, but may bear and increased risk for cardiovascular events. Spasticity is often assessed with subjective and clinical rating scales, which are unable to measure the increased excitability of the monosynaptic reflex, considered the hallmark of spasticity. The neurophysiological assessment of the stretch reflex provides a precise and objective method to measure spasticity. We propose a novel study to understand if Sativex could be useful in reducing spasticity in stroke survivors and investigating tolerability and safety by accurate cardiovascular monitoring.

Methods and analysis: We will recruit 50 patients with spasticity following stroke to take THC:CBD in a double blind placebo-controlled crossover study. Spasticity will be assessed with a numeric rating scale for spasticity, the modified Ashworth scale and with the electromyographic recording of the stretch reflex. The cardiovascular risk will be assessed prior to inclusion. Blood pressure, heart rate, number of daily spasms, bladder function, sleep disruption and adverse events will be monitored throughout the study. A mixed-model ANOVA will be used to compare the stretch reflex amplitude between the time points; semi-quantitative measures will be compared using the Mann-Whitney test (THC:CBD vs placebo) and Wilcoxon test (baseline vs treatment).

Ethics and dissemination: The study was registered on the EudraCT database with number 2016-001034-10 and approved by both the Italian Medicines Agency (Agenzia Italiana del Farmaco – AIFA) and local Ethics Committee "Comitato Etico Regionale della Liguria". Data will be made anonymous and uploaded to a open access repository. Results will be disseminated by presentations at national and international conferences and by publication in journals of clinical neuroscience and neurology.

Strengths and limitations of this study:

- First study on Sativex to treat post-stroke spasticity
- Electromyographic recording of the stretch reflex to precisely measure spasticity
- Assessment of cannabinoids tolerability in stroke survivors
- Limited number of patients in relation to a monocentric pilot study

Keywords: THC:CBD, Sativex, stretch reflex, spasticity, stroke, cannabinoids

INTRODUCTION

Stroke is one of the most disabling neurological disease and frequently determines important chronic consequences such as spasticity. Prevalence of post-stroke spasticity ranges from 4% to 42.6%, with the prevalence of disabling spasticity ranging from 2% to 13%[1]. Treatment of post-stroke spasticity is based on rehabilitation, local injection of botulinum toxin (BoNT) in the affected muscles for focal spasticity and/or use of classic oral drugs such as tizanidine, baclofen, thiocolchicoside and benzodiazepines, which are not always effective and have a good number of possible side effects.

The transmucosal administration of delta-9-tetrahydrocannabinol and cannabidiol (THC and CBD at 1:1 ratio oromucosal spray, Sativex®) is able to reduce spasticity acting on endocannabinoid receptors CB_1 and CB_2. This novel drug has been licensed after an extensive clinical trials program[2–4] in adult patients with multiple sclerosis who have shown no significant benefit from other antispasmodic drugs. More than 45000 patient/years of exposure since its approval in more than 15 EU countries support their antispasticity effectiveness and safety profile in this indication[5]. Besides improving spasticity, cannabinoids can be beneficial in reducing pain, chemotherapy-induced nausea and vomiting, moreover, they contribute to reducing seizures and to lowering eye pressure in glaucoma[6]. Cannabinoids can also exert psychological effects by lowering anxiety levels and inducing sedation or euphoria. Marijuana, which is the main source of cannabinoids, is declared illegal in many countries mostly because the risk of abuse, dependence and withdrawal syndrome, related to the effect of its high amounts of THC. Several reports support an increased ischemic stroke risk related to relevant abuse of smoked marijuana[7–17] as well as synthetic cannabinoids[18–20]. Ischemic stroke following cannabis involves more frequently basal ganglia and cerebellum where CB_1 and CB_2 receptors show a higher expression[13].

The “French Association of the Regional Abuse and Dependence Monitoring Centres Working Group on Cannabis Complications” warns about the increased cardiovascular risk related to the use of herbal cannabis, mostly consisting of acute coronary syndromes and peripheral arteriopathies, potentially leading to life-threatening conditions[21]. The detrimental consequences of cannabinoids could be attributed to the increase in hearth rate[22] as well as arterial spasms also in the context of a reversible cerebral vasoconstriction syndrome[23], but also vasculitis, postural hypotension and cardioembolism[24].

On the other side, some studies support a beneficial effect on stroke evolution of cannabinoid receptors stimulation. In fact, cannabinoid mediated activation of CB_1 and CB_2 receptors reduces inflammation and neuronal injury in acute ischemic stroke[25]. Activation of CB_2 receptors shows protective effects after ischemic injury[26] and inhibits atherosclerotic plaque progression[27,28].

To our knowledge, no correlations have been reported between haemorrhagic stroke and

cannabinoids intake. In our opinion, the modification of blood pressure is the most important cannabinoid effect that should be taken into account in patients with a previous haemorrhagic stroke or predisposed to intracranial bleeding. Cannabinoids are indeed capable of inducing blood pressure fluctuations in a specific triphasic pattern (low-high-low) potentially harmful if the patient with bleeding risk[29]. Ischemic disease is not included among THC:CBD oromucosal spray contraindications. However, considering that to our knowledge no study has been performed with THC:CBD oromucosal spray on post-stroke spasticity, we believe that a particular caution should be used in stroke patients.

The decision of which method of measure consider as endpoint is a major issue in studies involving spasticity. The definition of spasticity provided by Lance is one of the most precise and reliable, focusing on the stretch reflex as the neurophysiological equivalent of spasticity[30]. Probably because of technical complexity and required expertise, neurophysiological approaches are rarely adopted. Clinical rating scales such as the modified Ashworth scale (MAS)[31] or subjective scores such as the numeric rating scale (NRS) for spasticity are being widely used[32,33]. Recent evidence supports the idea that MAS and NRS are indeed useful to quickly rate spasticity in a clinical setting, however provide a very variable and imprecise assessment of many symptoms related to spasticity, but where spasticity itself is probably only a common factor[34]. The adoption of stretch reflex as the most appropriate neurophysiological measure of spasticity increases the specificity and reduces the variability of the endpoint and is particularly suitable for clinical trials.

Our proposal is therefore to assess the efficacy of THC:CBD oromucosal spray in patients with spasticity following stroke as add-on to first line antispasticity medications with an experimental pilot randomized placebo controlled crossover clinical trial using the stretch reflex as primary outcome measure. Prior to inclusion in the study, we propose strict selection criteria in order to reduce the risk of relevant side effects.

METHODS AND ANALYSIS

Subjects

At the Department of Neuroscience, of the “Ospedale Policlinico San Martino”, we will recruit 50 patients with spasticity secondary to stroke occurred at least 3 months earlier. Both naïve to BoNT or BoNT-treated patients will be recruited, however those treated with BoNT will enter the study at least 4 months after the last injection in order to allow a reasonable wash-out. The study will last 2 years.

Inclusion criteria are:

- presence of spasticity rated between 1 and 3 at the MAS in at least one of the following segments: flexor muscles of the wrist, flexor muscles of the forearm, extensor muscles of the leg, and/or foot plantar flexors;

- absence of significant peripheral nervous system pathology detectable on clinical basis;

- absence of concomitant parkinsonism;

- acceptable cardiovascular ischemic risk following a cardiological evaluation along with the laboratory and instrumental exams requested by the cardiologist, as well as a CHA_2DS_2VASc score less than 7;

- absence of a demonstrated stenosis higher than 50% at intracranial main arteries (mean cerebral, basilar, internal carotid, vertebral) or at cervical tracts of carotid and vertebral arteries;

- absence of significant cognitive impairment hampering patients' capability of understanding the study protocol and signing the consent form;

Apart from the criteria listed above, there will be no limitations related to age, sex and degree of disability.

The following sociodemographic data will be collected: gender, age, time of acute lesion, years with spasticity, areas affected with spasticity.

Study protocol

Patients will enter a crossover study paradigm after a cardiac-cerebral-vascular risk assessment performed by a cardiologist and by a vascular neurologist; those with an acceptable cardiac-cerebral-vascular risk will be randomly assigned (in a 1:1 ratio) to one of the two following treatment sequences (**Figure 1**):

• THC:CBD oromucosal spray – Placebo (i.e. THC:CBD oromucosal spray during Period I and Placebo during Period II)

• Placebo - THC:CBD oromucosal spray (i.e. Placebo during Period I and THC:CBD oromucosal spray during Period II)

The Randomization List will be generated through a validated SAS® program, in permuted blocks of reasonable size in order to guarantee the treatment balance.

After the first (T0) baseline evaluation visit, during one month period the patients will titrate their medication to reach the optimal number of puffs/day and then evaluated again (T1). A 2-weeks washout time will allow patients taking THC:CBD oromucosal spray (or placebo) to reach their baseline spasticity condition and then patients will switch arms and perform another month the other treatment. After while the final (T2) assessment will be performed. During the whole study, the patients will have to maintain the same therapy apart from the study drug, in order to minimize

other conditions that could affect the level of spasticity. For the same reason, patients are required not to undergo any physical and rehabilitative treatment during the study. During T1 and T2 evaluation, additional information about the number of sprays/day will be collected, along with all possible adverse events. During the 10 weeks of the study, patients not responding to the treatment and unable to complete the study untreated will undergo a rescue treatment (e.g. BoNT) and exit the study. All patients will be monitored by a neurological clinical follow-up at least for 1 year upon study termination.

This is a double-blind study. All individuals involved in the study conduct, including patients, investigator staff, persons performing the assessments and data analysts will remain blinded to the identity of the treatment from the time of randomization until database lock.

Randomization data will be kept strictly confidential until the time of unblinding and will not be accessible to anyone involved in the conduct of the study. The identity of the treatments will be concealed by the use of study drugs (THC:CBD oromucosal spray and matching Placebo) that are identical in packaging, labeling, schedule of administration and in appearance.

At the conclusion of the study, when the database has been locked, the assigned blinded treatment codes will be broken and made available for the final statistical analysis.

Individual patient unblinding during the course of the trial will only be allowed in the event of patient emergencies upon request from the investigator.

Experimental procedure

Preliminary setting and clinical evaluation

After the selection process and informed consent signature, all subjects will undergo a complete physical and neurological examination. The range of motion and MAS of the following segments will be assessed on both sides: elbow flexors, forearm pronators, wrist flexors, finger flexors, leg extensors, foot plantar flexor. The subjective amount of pain and muscle rigidity, quality of sleep and bladder dysfunction will be assessed using a 0-10 Numeric Rating Scale (NRS), as well as the number of daily spasms. The amount of disability related to hygiene, dressing, limb position and pain will be also assessed by mean of the Disability Assessment Scale (DAS)[35].

Blood pressure, heart rate will also be evaluated during each scheduled visits and patient's diary will be checked. The participants will be instructed about the medication dose up-titration process, patients' diary fulfilment and next visit date. All patients will be given a portable blood pressure device and instructed to record daily blood pressure values at home.

Possible adverse events will be monitored during the entire duration of the study. The detailed schedule of assessments and procedures is reported in **Table 1**.

Stretch reflex technical setup

The Biopac MP150 data acquisition system connected to a TSD130B twin-axis electronic goniometer (Biopac Systems Inc, USA) will be used for data acquisition. The goniometer will be placed across the joint in order to optimally record the angle during the displacements with a sampling rate of 2KHz. EMG activity produced by the stretched muscle will be recorded by surface electrodes (TSD150B, Biopac Systems Inc, USA) placed over the muscle belly. Movement timing will be paced by a software emulated metronome.

The subjects will be evaluated in a quiet room with a temperature between 21 and 23°C. A limb segment presenting with spasticity between 1 and 3 at the MAS will be selected among flexor muscles of the wrist, flexor muscles of the forearm, extensor muscles of the leg, foot plantar flexor. If spasticity is detectable in more segments, in order to cause less discomfort to patients and examiners and prefer joints with a higher range of motion (allowing more accurate passive movement timing), the segments will be selected in the following order: wrist flexors, elbow flexors, leg estensors, foot plantar flexors (defined "selected segment" and undergoing the stretch reflex procedure). For example, if a patient is affected by spasticity on both wrist flexors (MAS=2) and leg extensors (MAS=3), the stretch reflex procedure will be performed on wrist flexors. The subject will be seated if the selected segment is flexor muscles of the wrist, flexor muscles of the forearm, otherwise the subject will be lying on a comfortable examination table in a supine (extensor muscles of the leg) position with head and shoulders slightly elevated, or prone (foot plantar flexors) with the feet protruding from the examination table in order to allow a full ankle range of movement.

Stretch reflex procedure

The method was described in details in the first validating work[36]. The method consists in moving the selected segment throughout the full range of motion in the time corresponding to the interval between two consecutive metronome tones. The increase or decrease of tone frequency (beats per minute - BPM) determines a parallel change in the time required to perform a complete passive flexion or extension movement, so that given a certain range of motion, the mean movement velocity will be similarly modulated. The choice of the BPM will be done taking into account that low values could not be able to elicit a stretch reflex (especially in subjects with a low degree of spasticity), while high values could produce discomfort to the subject and excessive fatigue in the examiner. Continuous flexion and extension movements in a "sinusoidal" way may be unsuitable to elicit and measure spasticity because of the possibility to trigger post-activation depression[37] or paratonia[38]. However, the method allows performing discontinuous (or "linear") movements as well, by interposing a few tones interval between movements.

The subjects will be instructed to remain relaxed and to avoid resisting or facilitating the

movements performed by the examiner. The stretch reflexes will be measured during movements determining elongation of the spastic muscle. At least 15 discontinuous stretch reflexes will be acquired during each experimental session. The recordings will be performed at each time point (T0, T1, T2) using the same metronome BPM. In order to obtain a reproducible electrode positioning between sessions, a picture of the electrode and its relation with nearby anatomical landmarks will be taken in each subject at T0. In order to reduce variability, the examiner performing the passive movements will be the same in all time points for each patient[36].

Data analysis

The main endpoints of the study will be to assess the effect of THC:CBD oromucosal spray on spasticity measured with the stretch reflex and NRS for spasticity. To analyse the stretch reflex, the electromyographic recordings will be filtered, rectified and the average amplitude of the bursts will be calculated (meanEMG) using the dedicated AcqKnowledge analysis software (Biopac Systems Inc, USA). A mixed-model ANOVA with GROUP (THC:CBD oromucosal spray, placebo) as between-subjects factor and TIME (T0, T1, T2) as within-subjects factor will be used to compare meanEMG values.

All semi-quantitative rating scales scores will be compared between THC:CBD oromucosal spray/placebo groups using the Mann-Whitney test at each evaluation (T0, T1, T2). A comparison between T0 and T1 as well as between T0 and T2 will also be performed in each group using Wilcoxon test.

Since this is a pilot study, no formal sample size calculation can be done. It must be noticed, however, that in our previous study on patients with multiple sclerosis, we detected a significant reduction of the stretch reflex analysing 36 subjects[34].

ETHICS AND DISSEMINATION

The study was registered on the EU Clinical Trials Register (EudraCT) database with number 2016-001034-10 and named “SativexStroke”. We received the approval from the Italian Medicines Agency (Agenzia Italiana del Farmaco – AIFA) on September 23rd 2016, the approval from the local Ethics Committee “Comitato Etico Regionale della Liguria” on December 14th 2016 (protocol version 4.2) and the authorization from “ IRCCS AOU San Martino - IST” hospital on March 3rd 2017 (decree number 227).

We plan to start patients recruitment in September 2017, after drug has been produced and labelling completed. All study-related information will be stored securely at the study site and all participant information will be stored in locked file cabinets in areas with limited access. Electromyographic recordings and all the study-related files will be stored on a password-protected personal computer.

During the study only the principal investigator will have access to the full dataset. The final trial dataset will be blinded of any identifying participant information and uploaded to a open access data repository. The analysis will be probably completed by the end of 2019, after the 2-years study period. The data will be disseminated by presentation at national and international conferences and by publication in journals of clinical neuroscience and neurology.

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AUTHORS' CONTRIBUTIONS

Lucio Marinelli: principal investigator, study conception, manuscript drafting

Maurizio Balestrino, Carlo Serrati, Carlo Gandolfo: patients recruitment, evaluation of the cerebral vascular risk profile, manuscript review

Laura Mori, Luca Puce: co-investigators, technical setup, data acquisition

Gianmarco Rosa: cardiological evaluations and cardiovascular risk assessment

Laura Giorello: study monitoring, adverse events tracking, administrative support

Antonio Currà, Francesco Fattapposta, Giovanni Abbruzzese, Carlo Trompetto: clinical consultant and manuscript review

FUNDING STATEMENT

This work will be partly supported by Almirall Italia. The study drugs (Sativex and Sativex placebo) will be provided by GW Pharmaceuticals without cost for the patient and for the Italian public health system.

COMPETING INTERESTS STATEMENT

None declared.

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Figure legends

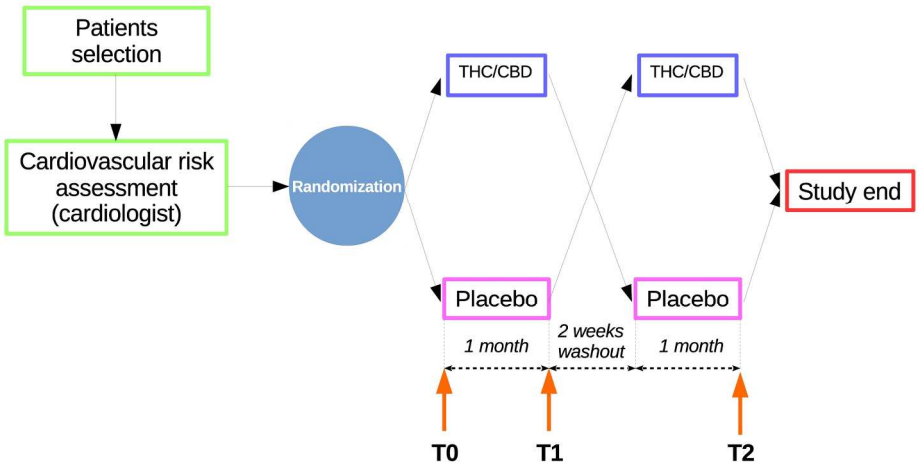
Figure 1

Graphical representation of the study protocol, particularly depicting the crossover design and the time points.

Table 1: Schedule of assessments and procedures

Study Period	Screening and randomization	Period 1	Wash-out	Period 2
Visit	**1 (T0)**	**2 (T1)**	**3**	**4 (T2)**
Informed consent	X			
Demography	X			
Medical and treatment history	X			
Physical and neurological examination	X	X	X	X
Full cardio evaluation	X			
Cardio consultation "as needed"		X	X	X
Vital signs	X	X	X	X
Inclusion & exclusion criteria	X			
Modified Ashworth scale	X	X		X
Spasticity NRS	X	X		X
Stretch reflex evaluation	X	X		X
Check patients diary		X		X
Spasticity NRS, Pain NRS, Bladder dysfunction NRS		*X*		*X*
Spasms number		*X*		*X*
Sleep quality NRS		*X*		*X*
Blood pressure and heart rate		*X*		*X*
Dispense study drug	X		X	
Check of returned study drug		X		X
Adverse events		X	X	X
Concomitant medications	X	X	X	X

NRS=numerical rating scale



Graphical representation of the study protocol, particularly depicting the crossover design and the time points.

209x148mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	8
	2b	All items from the World Health Organization Trial Registration Data Set	8
Protocol version	3	Date and version identifier	8
Funding	4	Sources and types of financial, material, and other support	8-9
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1; 9
	5b	Name and contact information for the trial sponsor	N/A
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
	6b	Explanation for choice of comparators	3-4
Objectives	7	Specific objectives or hypotheses	4

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5-6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	5-6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5-6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5-6
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	N/A
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	5-6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5-6

Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5-6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	5-6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	5-6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	5
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	6
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	6
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A

Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	N/A
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	9
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	8
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	8
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	8
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Attached as needed
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.