06-12-2016 Journalnummer: H-15006836

PROTOCOL

The cluster headache inducing abilities of calcitonin gene-related peptide

Version 1.2

Anne Luise Vollesen, MD, PhD student
Agneta Henriette Snoer, MD, PhD student
Song Guo, MD, PhD
Jan Hoffman. MD, PhD
Anja Sofie Petersen, medical student, research assistant
Rigmor Jensen, DrMSc, prof
Messoud Ashina, DrMSc, prof

Study investigator and site

Anne Luise Vollesen, MD, PhD student
Neurologisk afdeling N, Dansk Hovedpinecenter
Glostrup Hospital
Nordre Ringvej
2600 Glostrup
Phone.: 25 73 22 84
E-mail: luisevollesen@gmail.com

Table of contents

Title: The cluster headache inducing abilities of calcitonin gene-related peptide

1. Purpose

To investigate possible incidence of headache and cluster headache after administration of calcitonin-related peptide (CGRP) or placebo.

1.1 End points

Primary end points are differences in the occurrence of cluster headache attacks within 60 minutes after administration of CGRP over 20 minutes (1.5μg/min) versus placebo.

2. Background

Cluster headache is a primary headache disorder characterized by short but severe, almost unbearable pain attacks [1]. The pain is localized retro- and periorbitally and is almost always unilateral. The disorder is considered to be the most painful form of headache. Consequently, the disease is a major burden on patients and society, as it results in prolonged periods of illness, reduced quality of life and high healthcare costs. Thus, there are major social costs for the patient and their surroundings [2]

The headache usually lasts between 15 minutes and three hours and occurs between once every other day to eight times a day. The pain attacks are accompanied by prominent autonomic symptoms in the form of tearing, nasal discharge, eye redness, ptosis and motor disorder. These symptoms are ipsilateral to the pain [3]. The basis for the interconnection between the autonomic symptoms and the attack pain is still poorly understood. It has been observed that the autonomic symptoms in some patients may occasionally occur without accompanying pain attacks - and vice versa. This indicates that the underlying pathways for pain and autonomic symptoms respectively may be separate - possibly only partially. The threshold for initiation of the pain as well as autonomic symptoms may also be different. [4]

Thus, no explanation unifies the unilateral pains of the cluster headache, which corresponds to the fifth cranial nerve (n. Trigeminus) propagation, with the ipsilateral autonomic symptoms [4]. Consequently, the treatment options for this patient group are based on empiricism rather than on a real understanding of the disease mechanisms.

It is still debatable whether the pain evolves centrally or peripherally. Efferent parasympathetic fibers from the sphenopalatine ganglion innervate cranial vessels [3-6] and regulate vascular tone [7,8]. It has been suggested that efferent outflow from parasympathetic nerve fibers with neuropeptide release may activate perivascular nociceptors during cluster headache attacks [9]. It has previously been shown that nitroglycerine provokes cluster headache attacks in episodic cluster

headache patients (ECH patients) in active cluster, but not in remission phase [10] [11]. A similar pattern applies to known cluster headache attack “triggers”, such as alcohol. During the active phase, alcohol intake induces an attack in some patients, unlike in remission phase. A possible explanation may be that a disturbed interaction between certain areas of the brain causes a "permissive state" during which attacks may occur. [4]

CGRP is a neuropeptide, known from a number of both physiological and pathological processes and occurs naturally in the body. It has been shown that plasma concentration of CGRP is significantly increased during cluster headache attacks but not before and after attacks [11]. Attacks occur approximately 20-40 min after nitroglycerine infusion with vasodilation. During this latency period before the attack, no increase in plasma concentration of CGRP [10] [11] is seen.

Provocation studies with CGRP has been performed in migraine patients in a number of studies at the Danish Headache Center with no serious side effects noted [12] [14] [15]. CGRP induces migraine attacks in 65% of migraine patients [12], and CGRP antagonists and monoclonal antibodies are effective as attack and preventive migraine treatment [13]. A number of drug trials testing CGRP antagonists in cluster headache patients are underway.

It is unknown whether CGRP provokes attacks in patients with cluster headache. In order to understand the mechanisms of any response, we will take regular blood samples before and during the study to measure biochemical markers for parasympathetic activation and activation of glial cells in the trigeminal ganglion (VIP, PACAP, CGRP, NSE and S100B). The current study could help reveal crucial and unexplained mechanisms behind cluster headache attacks as well as possibly predict the role of CGRP antagonists in cluster headache treatment. It could shed light on the possibility of more targeted treatment of this severe pain disorder.

3. Hypotheses

Based on the above, we set out the following hypotheses:

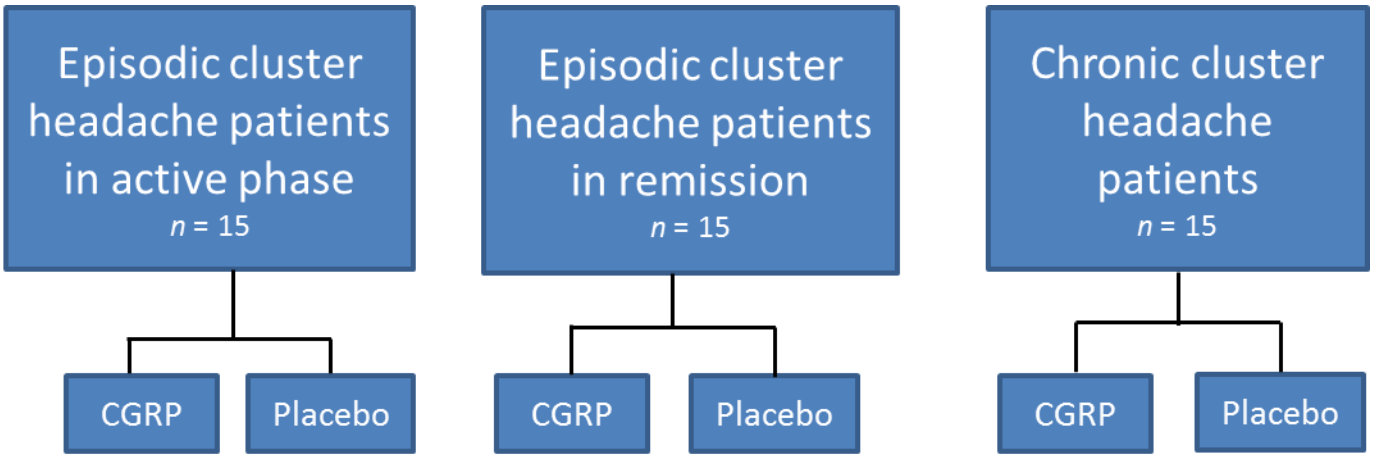
- Provocation with CGRP of episodic cluster headache patients in active phase triggers cluster headache attacks
- 2. Provocation with CGRP of episodic cluster headache patients in remission phase does not trigger cluster headache attacks
- Provocation with CGRP of chronic cluster headache patients triggers cluster headache attacks more often than in episodic cluster headache patients.

4. Design and study day

4.1 Design

It is a randomized, double-blind, placebo-controlled two-way crossover study. A total of 2x45 study days are planned with 45 participants. These are divided into three groups as follows:

Figure 1



Participants attend study days two times with at least one week intervals. The study is a double-blind cross-over design with placebo vs. CGRP.

4.2 Meassured parametres

Headache response: Characteristics of headache, autonomic symptoms, accompanying symptoms and other side effects are recorded using a schedule every 10 minutes in the first hour (Appendix 4). Pulse and blood pressure are measured prior to intake of the study drug and at the end of the trial (1 hour after). At the end of the trial, the headache response of the trial participant is recorded every hour for the following 24 hours (Appendix 5).

4.3 Randomization

Randomization is conducted so that there are approximately as many trial participants who receive active drug on the first and second study days. The trial leader and other participants in the trial do not have access to the randomization code. It is stored where the study is carried out in a light-sealed envelope and may be broken in cases where it is deemed necessary for the safety of the participants. There will be an envelope for each subject so that the overall randomization code is not revealed to other subjects in case an envelope is broken.

4.4 Participants

Cluster headache patients diagnosed with episodic and chronic cluster headache according to the International Classification of Headache Disorders 3rd Beta Edition [16].

Trial participants are recruited by telephoning suitable cluster headache patients who are current or former patients at the Danish Headache Center, who have expressed their interest in participating in clinical trials. Recruitment is also done via advertisement on the website forsøgsperson.dk and sundhed.dk (Appendix 7). Drop outs are replaced to ensure sufficient statistical material.

4.5 Inclusion criteria

- Cluster headache patients fulfilling HIS criteria for episodic and chronic cluster headache, male and female, aged 18-65, 50-100 kg.
- Fertile women must use safe contraceptive methods. Fertile women include non-hysterectomized women or women at least 2 years post-menopausal. Safe contraceptive methods include IUD, hormonal contraceptive pills or implants, or surgical sterilization.

4.6 Exclusion criteria

- All other primary headache disorders.
- Episodic headache patients in remission must be headache free 8 h before study start.
- Episodic headache patients in active phase or chronic cluster headache patients must be attack free for 3 h before study start.
- Pregnant or lactating women.
- History or signs of hyper- (systolic >150 mmHg and/or diastolic >100 mmHg) or hypotension (systolic <90 mmHg and/or diastolic <50 mmHg)
- History or signs of cardiovascular or cerebrovascular disease, or psychiatric disease or drug misuse.

4.7 Healthy controls

In order to validate the assay, independently of the experiment, blood samples of 15 healthy subjects are performed. These are recruited by advertising at Glostrup Hospital.

4.7.1 Inclustion criteria

- Healthy subjects of both sexes between 18-65 years, 50-100 kg

4.7.2 Exclusion criteria

- Migraine and cluster headache according to the IHS criteria [1].

- Headache less than 8 hours before blood sampling

4.7 Study day

Prior to the screening visit, the participants will receive an approved participant information and the "Before You Decide" folder published by DNVK (Authority of the Ministry of Health and Prevention). The attending physician or a person to whom the responsibility is delegated will inform the participant thoroughly at the subsequent meeting or telephone call that it is a request for participation in a scientific study and will provide detailed information about the purpose, course and possible risks of the trial. By telephoning the participant we will plan a screening visit at Ringvejsblokken, Glostrup Hospital, at least one day later, and during the phone call we will inform participants about the right to further reporting time (24 hours) after the oral information and the possibility to bring an assessor to the visit. Since the participants are already well-informed on the basis of the telephone conversation, the written participant information and the newly submitted oral information on the purpose, course and possible risks are obtained by oral and written consent before the actual medical examination, unless the participants wish for further reporting time. The screening visit takes place in undisturbed room behind closed doors, the room being marked as "busy".

A medical chart is recorded and a general medical examination is conducted. ECG is measured and blood pressure is measured. This is done to uncover any underlying risk factors and to ensure that the participants meet the inclusion criteria and do not meet the exclusion criteria. The participants will also be informed of the right to withdraw from the study without having to argue for this, and that this will not affect any future treatment.

4.8 Study day 1 & 2

Participants meet at 9.30 AM. Women of childbearing potential will submit to a pregnancy test (urine HCG). After arrival, baseline values of headache intensity are first performed using a 0-10 Verbal Rating Scale (VRS) as well as heart rate and blood pressure. The trial person meets 2 times (in addition to the screening visit) and receives infusion of CGRP once and placebo the second time.

Before the start of the trial, the subject is placed in a bed, and an intravenous cannula is placed in the right and left elbow veins used for infusion of CGRP. There will be a medical doctor available throughout the trial. After 20 minutes rest, baseline values for pulse and blood pressure are noted. After this, the infusion of CGRP (1.5 μg / min)/placebo is started for 20 minutes. During the test,

blood samples are taken 4 times (time 0, 20, 30 and 90min). If the patient develops a cluster headache attack, further blood tests may be necessary: At start of the attack and 15 minutes and 30 minutes after attack start. Pulse and blood pressure recording and possible headache intensity and compulsive symptoms are performed every 10 minutes during and up to 90 minutes after the start of infusion. If the patient develops a cluster headache attack, separate seizure registration will be performed every 10 minutes. This may mean an extension of the total registration period after the infusion for a maximum of 120 minutes. The participant lies in bed throughout the experiment. After the test, the subject will return home if he/she feels ready for it.

The participant is given a questionnaire for registration at home of any headache and other symptoms in the following 12 hours. If the subject develops a cluster headache attack during the trial, treatment will be offered in the form of oxygen or attack medicine Sumatriptan injection 6 mg s.c, Sumatriptan tablet 50 mg or 100 mg or Sumatriptan nasal spray.

5. Methods

5.1 Number of participants

The main objective of the study is to test the difference between the number of patients who develop headache after taking CGRP or placebo. We determine the risk of a type 1 error of 5% and want a strength of 80% and thus a risk of type 2 error of 20%. Our hypothesis is that CGRP induces headache in at least 70% of patients and headache in less than 20% of the placebo group. We have therefore calculated that 15 people in each group should be included. Calculations are made through statistics programs on the following website: http://statpages.org/

5.2 Calculations and statistical methods

Primary end points are differences in the occurrence of cluster headache attacks, the area under the headache curve (AUC headache) (headache intensity x duration) and time to maximum headache score after CGRP versus placebo. For comparison of: the occurrence of cluster headache attacks uses McNemar test; AUC headache and maximum headache score between two experimental days assessed with non-parametric test (Wilcoxon signed rank sum test). Secondary end points are changes over time in blood pressure and heart rate, and ANOVA is used to test the difference between two experimental days.

5.3 Headache registration

Headache is rated verbally on a verbal rating scale (VRS) 0-10. 0 represents no pain, 1 an "altered, pressing or throbbing but not really painful feeling", 5 moderate headache and 10 the worst possible headache. Headache response is classified according to IHS criteria [16]. Other effects are recorded qualitatively using the questionnaire (Appendix 4). Registration: Headache is recorded every 10 minutes in time 0-60 minutes of the investigator. Starting from one hour after hospitalization, headache data is collected using patient-filled headache questionnaire for the following 12 hours (see Appendix x). Once filled out, it will be returned in an attached envelope to the Danish Headache Center. The questionnaire clarifies characteristics of any headache response.

5.4 Blood samples

During the study blood samples will be taken 4 times (time 0, 20, 30 and 90 min) from intravenous cannula to measure serum concentrations of VIP, PACAP38, S100B, NSE and CGRP. Four x 30 mL of blood is taken over 90 minutes, ie totaled 120 mL of blood. If the patient develops a cluster headache attack, it may be necessary to take further blood samples, a maximum of 3 x 30 mL, ie a total of approximately 210 mL of blood.

In order to validate the assay, independently of the experiment, blood samples of 15 healthy subjects will be taken. In these blood samples, serum concentrations of VIP, PACAP38, S100B, NSE and CGRP are also measured.

Blood samples are stored for approx. 3 months before being submitted for analysis. After analysis, blood samples are destroyed. Blood samples will be analyzed at the biochemical department Gentofte Hospital and Glostrup Hospital.

6. Risk assessment

CGRP infusion: Insertion of intravenous cannula may cause subcutaneous hematoma that disappears after a few days. Our research group has used the CGRP infusion model for several years and has not observed any side effects other than redness, palpitations, warmth sensation, and some developed a transient blood pressure drop and consequently complain of transient dizziness and nausea (17-19). Blood pressure and the clinical situation stabilized quickly upon interruption of infusion and placement in Trendelenburg's position.

Sumatriptan: Sumatriptan has long been used as a medicine against cluster headaches. Sumatriptan will be used as rescue medicine in the event of induction of cluster headache and will give the participants immediate pain relief. The side effects of Sumatriptan injection are considered modest.

According to the Pharmaceutical Catalogue, the most common side effects are transient redness, fatigue and drowsiness.
Insertion of intravenous cannula may cause a subcutaneous hematoma that disappears after a few days and, in very rare cases, infection. There are no known side effects, risks or significant discomfort associated with pulse and blood pressure recording.

7. Unintended events

All unintended events during the trial are recorded and if serious unintended events which are presumed to be related to the drug occur, the Danish Medicinal Agency will be notified immediately pursuant to the Medicines Act section 89 (2).
In this context, an unintended event is defined as: Any undesired event that is temporarily related to the administration of the trial medicine, whether or not this accidental event is considered to be associated with the trial drug. If an unintended event occurs more than 14 days after the last administration of the trial medicine, and there is no apparent causal link or association with the trial medicine, this is not considered an unintended event.
In this context, a serious accidental incident means any medical case regardless of dose which

- results in death
- is life threatening
- involves hospitalization or extension of existing hospitalization
- results in persistent or significant disability / incapacity or
- is a congenital anomaly / malformation

If the subject develops a cluster headache attack during the study, treatment will be offered in the form of oxygen or seizure medicine Sumatriptan injection 6 mg s.c, Sumatriptan tablet 50 mg or 100 mg or Sumatriptan nasal spray. Participants are offered attack medication to takehome home in case of later developed headache. All tests are performed in the presence of a medical doctor with the possibility of calling for necessary assistance when needed. The department has access to O2, resuscitation equipment, medicine and infusion fluids for the treatment of acute medical conditions.
This protocol shall be submitted to the Scientific Ethics Committee for the Capital Region and the Data Inspectorate in accordance with applicable rules.

8. Time frame

The experiment is scheduled to take place in summer / autumn 2016.

9. Publication

The paper will be sent to a peer reviews international journal. Both positive, negative, and inconclusive results will be published. The study is expected to result in a scientific paper with the following authors: Anne Luise Vollesen, Song Guo, Jan Hoffman, Anja Sofie Petersen, Rigmor Jensen and Messoud Ashina.

10. Economy

Disability allowance for subjects is DKK 200 per person per hour and totals approximately 7 x 200 kr. = 1400 kr. per subject (taxable). The trial does not receive support from pharmaceutical companies. The costs of the trial are covered by the Headache Research Fund, Glostrup Hospital, which includes funds under public audit. There will be ongoing research funding for the project from research funds, but implementation of the project is not dependent on obtaining such support. If such support is obtained, the committee will be informed and the participant information updated. The researcher, Song Guo, PhD student, is employed by the Danish Headache Center. Initiator is the Danish Headache Center with Song Guo as contact person.

11. Insurance

If during the trial or as a result of it any injury or complications for the trial participants occurs, they are covered by Glostrup Hospital Patient Insurance.

12. Approvals

The protocol shall be submitted to the Scientific Ethics Committee for the Capital Region and the Data Inspectorate. Our research group has previously done CGRP trials with the same dose in migraine patients, where it was used as a tool [15] [14]. It is therefore also considered as a tool in this experiment. Therefore, it is not considered to be subject to the notification obligation to the Danish Medicines Agency, and thus this is not done (Act on Medicinal Products Section 24, see the Danish Medicines Agency's guidance on the notification of clinical trials of medicinal products on humans). The study is also reported to the Data Inspectorate in accordance with applicable rules.

13. Ethics

The study will be conducted according to the Helsinki Declaration of 1984, modified at the 41st World Congress in Hong Kong in 1989. The study must be approved by the Local Science Ethics Committee. Attendees will only be included after full written and oral information and written acceptance. The study participants may withdraw from the trial at any time without justification and without it affecting future treatment. Based on our previous experience with CGRP, the side effects are mild and transient. Should unwanted events such as allergic reactions occur contrary to our expectations, there is a relevant action plan for observation and treatment of the participant. The study will help to clarify where cluster headache pain initiates. This is of great interest in future research, as such knowledge is an important prerequisite for the development of better cluster headache medicine with fewer systemic side effects in the short and long term. It is our opinion that the disadvantages, discomfort and risk of trial participants are proportional to the significance of the expected results. Processing of personal data will be complied with in accordance with the law and data protection rules. All data collected will be treated confidentially and only published in anonymized form. Council data and randomization codes are stored under safe conditions for 15 years after the end of the trial in anonymized form. If information from patient records is required, consent is obtained from the patient in accordance with section 43 (3) of the Health Act. 1. The information will be used to screen the patient for the inclusion and exclusion criteria for the experiment.

14. Department responsible for the study

Neurological dept. N, Danish Headache Center, Glostrup Hospital, Ndr. Ringvej 57, 2600 Glostrup

15. Study investigators

Anne Luise Vollesen, MD, PhD student

Song Guo, MD, PhD

Rigmor Jensen, DrMSc, prof

Messoud Ashina, DrMSc, prof

The study is performed and participant information is provided by medical doctor Song Guo, or by specially trained staff under the supervision of the study medical doctors.

1. The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia. 2013 Jul;33(9):629–808.

2. Jensen RM, Lyngberg a., Jensen RH. Burden of cluster headache. Cephalalgia. 2007;27(6):535–41.

3. Ekbom K, Hardebo JE. Aetiology , Diagnosis and Management. 2002;62(1):61–9.

4. Leone M, Bussone G. Pathophysiology of trigeminal autonomic cephalalgias. Lancet Neurol. Elsevier Ltd; 2009;8(8):755–64.

5. Hara H, Zhang QJ, Kuroyanagi T, Kobayashi S. Parasympathetic cerebrovascular innervation: an anterograde tracing from the sphenopalatine ganglion in the rat. Neurosurgery. 1993 May;32(5):822–7; discussion 827.

6. Uddman R, Edvinsson L, Hara H. Axonal tracing of autonomic nerve fibers to the superficial temporal artery in the rat. Cell Tissue Res. 1989 Jun;256(3):559–65.

7. Edvinsson L, Hara H, Uddman R. Retrograde tracing of nerve fibers to the rat middle cerebral artery with true blue: colocalization with different peptides. J Cereb Blood Flow Metab. 1989 Apr;9(2):212–8.

8. Uddman R, Hara H, Edvinsson L. Neuronal pathways to the rat middle meningeal artery revealed by retrograde tracing and immunocytochemistry. J Auton Nerv Syst. 1989 Feb;26(1):69–75.

9. Hara H, Jansen I, Ekman R, Hamel E, MacKenzie ET, Uddman R, et al. Acetylcholine and vasoactive intestinal peptide in cerebral blood vessels: effect of extirpation of the sphenopalatine ganglion. J Cereb Blood Flow Metab. 1989 Apr;9(2):204–11.

10. Seylaz J, Hara H, Pinard E, Mraovitch S, MacKenzie ET, Edvinsson L. Effect of stimulation of the sphenopalatine ganglion on cortical blood flow in the rat. J Cereb Blood Flow Metab. 1988 Dec;8(6):875–8.

11. Burstein R, Jakubowski M. Unitary hypothesis for multiple triggers of the pain and strain of migraine. J Comp Neurol. 2005 Dec;493(1):9–14.

12. Ekbom K. Nitroglycerine as a Provocative Agent in Cluster Headache. 1968.

13. Fanciullacci M, Alessandri M, Sicuteri R, Marabini S. Responsiveness of the trigeminovascular system to nitroglycerine in cluster headache patients. Brain. 1997;120:283–8.

14. Lassen L, Haderslev P, Jacobsen V, Iversen H, Sperling B, Olesen J. CGRP may play a causative role in migraine. Cephalalgia. 2002 Feb;22(1):54–61.

15. Kaiser E a., Russo AF. CGRP and migraine: Could PACAP play a role too? Neuropeptides. Elsevier Ltd; 2013;47(6):451–61.

16. Hansen JM, Hauge AW, Olesen J, Ashina M. Calcitonin gene-related peptide triggers migraine-like attacks in patients with migraine with aura. Cephalalgia. 2010;30(10):1179–86.

17. Hansen JM, Thomsen LL, Olesen J, Ashina M. Calcitonin gene-related peptide does not cause migraine attacks in patients with familial hemiplegic migraine. Headache. 2011;51(4):544–53.

18. The International Classification of Headache Disorders: 2nd edition. Cephalalgia. 2004 Jan;24 Suppl 1:9–160.

Amendment #1

06.12.16

1) Postponement of the deadline for completion of the trial until 01.01.2018.
2) Change of study investigator from Dr. Song Guo to Dr. Anne Luise Vollesen

1) The completion of the experiments is taking longer than initially assumed.
2) Dr. Song Guo wishes to transfer the investigator responsibility to Dr. Anne Luise Vollesen, as she will be fully responsible for the trial from now on.

Amendment #2

02.05.17

The following exclusion criteria regarding all subjects will be changed from

• Must be headache free for at least 8 hours before trial start

to

• Episodic cluster headache patients in remission must be headache free for at least 8 hours before trial start
• Episodic cluster headache patients in cluster and chronic cluster headache patients should be attack free for 3 hours

Since episodic cluster headache patients in cluster and chronic cluster headache patients can have up to 8 attacks daily, there is no reason to suspect that they need to have been attack free for longer than 3 hours before an attack can be provoked. Therefore, the time interval is changed for this group of patients with disease activity, whereas the time interval is maintained for episodic cluster headache patients in remission. The change will increase the feasibility of the experiment.