BIOHIT HealthCare
Innovating for Health

Research Project

Efficacy of L-cysteine in Prevention of Headache Attacks in Migraine Patients. Randomized intervention trial with a medical device (Acetium® Capsules)*

Executed by:

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SYNOPSIS

Background: Globally, 15% of the population is affected by migraines at some point in their life-time. Pprophylactic treatment of migraines is an important part of the total management of migraine patients, having twofold goals: i) to reduce the frequency, painfulness, and/or duration of migraines, and ii) to increase the effectiveness of abortive therapy. During the past several decades, a large number of optional modalities have been tested as preventive measures of migraine attacks. Not unexpectedly, the effects of any such preventive therapies are highly variable, and in many patients, the attack frequency is not under satisfactory control. Many of these drugs also have untoward side effects that offset their potential benefits.

Recently, spontaneous case testimonials were received by Biohit Oyj from migraine patients, reporting that our new medical device, Acetium® capsule (containing 100mg L-cysteine, developed for inactivation of acetaldehyde in the stomach contents after alcohol intake), proved to be highly effective against migraine attacks. Their headache attacks disappeared almost immediately after onset of Acetium administration, all of them remaining in complete remission for several months up to several years by now.

These spontaneous testimonials prompted us to formulate a novel study hypothesis that could possibly explain these dramatic effects of Acetium® capsules in migraine prevention, to be tested in this RCT. This novel hypothesis is starting from the fact that, swelling and dilatation of cerebral blood vessels is necessary to provoke the attack in this vascular-type of headache.

It is known that Nitric Oxide (NO) is the final trigger of migraine attack, operating through phosphorylated protein kinase G (PKG) and Ca2+ channels, slowing the influx of calcium into the cell, which leads to smooth muscle relaxation and vasodilation. Histamine is a potent inducer of NO Synthase, making NO available locally on the vasculature, acting through endothelial H1-receptors. Histamine is synthesized from histidine in tissue mast cells, which are ubiquitous cells and their activation e.g. in the meninges has long been suspected to be involved in generating migraine headaches. Finally, one of the potent liberators of histamine from the mast cells is acetaldehyde, which, in turn, is effectively inactivated by Acetium capsules. This led us to rational that by eliminating acetaldehyde in the stomach, Acetium capsules could block (or reduce below the threshold levels) histamine liberation from the tissue mast cells and ECL cells in the stomach, thus arresting its multitude of functions, of which vasodilatation is critically involved in the migraine attack.

Objective: To validate the novel hypothesis that daily use of Acetium® capsules is an effective means to decrease the frequency of (or completely abort) the headache attacks in migraine patients.

Study design: A double-blind, randomized placebo-controlled multi-centre trial comparing Acetium® capsules (**2 capsules, 3 times a day**) and placebo in prevention of migraine attacks during a 3-month trial period. A cohort of 200 voluntary subjects (women and men, with aural or non-aural migraine) are invited through the Finnish Migraine Association (FMA), to participate in the study. To be eligible, the subjects should: i) have the attack frequency of 2–8 times per month, ii) have had migraine for at least 1 year, iii) have the onset of their migraine before 50 years of age, iv) be between 18 and 65 years of age, and v) have a minimum of co-morbidity. They should consent to discontinue other migraine prophylactic medication prior to study entry. Before enrolment in the cohort, all subjects are requested to sign a written consent. The study protocol shall be subjected for approval by the Regional Committee on Medical Research Ethics.

Methods: A 3-month retrospective history and 1-month prospective baseline (run-in) period is used to assess the baseline attack frequency. The study setting is actually triple-blinded (participant-blind, investigator-blind, sponsor-blind). Placebo preparation with design and package identical to the test preparation will be used. Parallel group design instead of cross-over design is used. Randomization will be performed after the 1-month prospective baseline (run-in) time, using a random number generator, with blocks size of 4, and creating unique randomization codes for each subject. The need for stratified randomization is decided on the basis of the baseline data, and if needed, attack frequency is used as the stratification variable.

The treatment period in both study arms will be 3 months, followed by a 1-month post-trial observation period. The participants should use (and accurately report) their usual symptomatic or acute treatment, because not anticipated to interfere with the study medication. During the 3-month treatment period, participants will be evaluated at monthly intervals by the study coordinator. As determined by the final study compliance, data analyses might be necessary separately for i) Per Protocol (PP), and ii) Modified intention-to-treat (mITT) groups.

In addition to the baseline assessment of attack frequency, each subject will be requested to fill in a structured Questionnaire recoding their detailed migraine history and other pertinent data on potential triggers, to be used as covariates in multivariate analysis. The headache diary is the main research tool used to monitor the efficacy of the test preparations, recording all predefined assessment measures (efficacy, tolerability and safety). These diaries are submitted to the study monitor on each FU visit, to confirm the compliance.

In statistical analysis, both conventional techniques (e.g. non-parametric paired-samples and non-paired samples t-test), and more sophisticated methods will be used. The latter include i) life-table methods like Kaplan-Meier and Cox proportional hazards regression, as well as ii) generalized linear models (GEE and panel Poisson) and as a new technique in migraine RCTs, a competing risks regression, to model the natural outcomes of migraine during the intervention. This study (n=100 per study arm) is adequately powered (Type II error 0.80, type I error 0.05) to detect a true difference in attack frequency between 4 attacks/month in the placebo and 2.4 attacks/month in the Acetium arm, i.e., the difference in effect size of 1.6 attacks. Given that the study subjects are selected among patients with 2-8 monthly attacks, there figures seem reasonable estimates for the basis of these power calculations.

Specific aims: The null hypothesis of the study implicates that Acetium® capsules are no better than placebo in migraine prophylaxis during the intervention period of 3 months. Rejection or not of the null hypothesis is based on comparison of the two arms for two primary study endpoints and (to lesser extent) for a series of secondary endpoints. The **two primary study endpoints** (efficacy measures) are: a) Number of migraine attacks (NMA) per evaluation interval (1 month), and ii) Number of migraine days (NMD) per evaluation period. Potentially useful **secondary endpoints** include: i) Intensity of headache (4-tier nominal scale); ii) Attack duration in hours (potentially biased by treatment); iii) Drug consumption for symptomatic or acute treatment (NMDs treated with abortive agents and the number of drug administrations for acute therapy); iv) Patients' preferences and satisfaction; v) Responder rate (proportion of study subjects with >50% improvement in NMA or NMD, as compared to baseline values).

Study execution and time-table: Meanwhile the final protocol is under evaluation for ethical approval by HUS, preparatory measures have been taken by informing the FMA about the planned study and asking their co-operation in encouraging the interested migraine patients to contact the study coordinator. Because each study subject shall complete only a 3-month trial period, preceded by 1-month run-in time and 1-month post-trial surveillance, we expect that the study can be completed in 6 months.

Impact of the study: Given that L-cysteine is a natural (semi-essential) amino acid, converted to inert substance (MTCA) in the alimentary tract, Acetium® capsule would comprise an ideal means to conduct migraine prophylaxis for years, without concern about the side effects that are inherent to many of the current treatment modalities. If the efficacy will be proved in this formal RCT, the concept of using Acetium® capsules in prophylactic treatment of migraines would represent a major step forward in a better clinical control of these frequently intractable syndromes.

1. BACKGROUND

Migraine is a chronic neurological disorder characterized by recurrent moderate to severe headaches often in association with a number of autonomic nervous system symptoms. The word derives from the Greek hemicrania, i.e., pain on one side of the head.[1] Typically the headache is unilateral (affecting one half of the head) and pulsating in nature, lasting from 2 to 72 hours. Associated symptoms may include nausea, vomiting, photophobia (increased sensitivity to light), phonophobia (increased sensitivity to sound), and the pain is generally aggravated by physical activity.[2] Up to one-third of people with migraine headaches perceive an aura: a transient visual, sensory, language, or motor disturbance which signals that the headache will soon occur.[2] Occasionally an aura can occur with little or no headache following it.

Globally, approximately 15% of the population is affected by migraines at some point in their life-time. Migraines are believed to arise as a result of a mixture of environmental and genetic factors.[3] Importantly, some two-thirds of the cases seem to run in families.[4] Fluctuating hormone levels may also play a role: migraine affects slightly more boys than girls before puberty, but about two to three times more women than men.[5,6] Propensity for migraines usually decreases during pregnancy.[5] The basic mechanisms of migraine are still not fully understood. It is, however, believed to be a neurovascular disorder.[4] The primary theory is related to increased excitability of the cerebral cortex and abnormal control of pain neurons in the trigeminal nucleus of the brainstem.[7]

1.1.Classification of migraines

Migraines were first comprehensively classified in 1988 by Olesen et al.[9] The International Headache Society most recently updated their classification of headaches in 2004.[2] According to this classification, migraines are considered as the primary headaches along with the tension-type headaches and cluster headaches.[10] According to the International Classification of Headache Disorders (ICHD), published in 2004, migraines are divided into 7 subclasses, some of which include further subdivisions:[2]

1) Migraine without aura, or "common migraine", involves migraine headaches that are not accompanied by an aura.
2) Migraine with aura, or "classic migraine", usually involves migraine headaches accompanied by an aura. Less commonly, an aura can occur without a headache, or with a non-migraine headache. Two

other varieties are i) familial hemiplegic migraine and ii) sporadic hemiplegic migraine, in which a person has migraines with aura and with accompanying motor weakness. If a close relative has had the same condition, it is called familial, otherwise sporadic. Another variety is basilar-type migraine, where a headache and aura are accompanied by difficulty speaking, world spinning, ringing in ears, or a number of other brainstem-related symptoms, but not motor weakness. This type was initially believed to be due to spasms of the basilar artery that supplies the brainstem.[11]

3) Childhood periodic syndromes that are commonly precursors of migraine include cyclical vomiting (occasional intense periods of vomiting), abdominal migraine (abdominal pain, usually accompanied by nausea), and benign paroxysmal vertigo of childhood (occasional attacks of vertigo).

4) Retinal migraine involves migraine headaches accompanied by visual disturbances or even temporary blindness in one eye.

5) Complications of migraine describe migraine headaches and/or auras that are unusually long or unusually frequent, or associated with a seizure or brain lesion.

6) Probable migraine describes conditions that have some characteristics of migraines, but where there is not enough evidence to diagnose it as a migraine with certainty (in the presence of concurrent medication overuse).

7) Chronic migraine is a complication of migraines, and is a headache that fulfills diagnostic criteria for migraine headache and occurs for a greater time interval. Specifically, greater or equal to 15 days/month for longer than 3 months.[12]

1.2.Symptoms of migraine

Migraines typically present with self-limited, recurrent severe headache associated with autonomic symptoms.[4,13] About 15-30% of people with migraines experience migraines with an aura, [14,15] and those who have migraines with aura also frequently have attacks without aura.[9] The severity of the pain, duration of the headache, and frequency of attacks is variable.[4] A migraine lasting longer than 72 hours is termed status migrainosus.[9] There are four possible phases of a migraine attack, although not all these phases are necessarily experienced every time:[2]

1. The prodrome, which occurs hours or days before the headache.
2. The aura, which immediately precedes the headache.
3. The pain phase, also known as headache phase.
4. The postdrome, the effects experienced following the end of a migraine attack.

1.2.1.Prodrome phase

Prodromal or premonitory symptoms occur in ~60% of those with migraines[16,17] with an onset of two hours to two days before the start of pain or the aura. These symptoms may include a wide variety of phenomena, including: altered mood, irritability, depression or euphoria, fatigue, craving for certain food, stiff muscles (especially in the neck), constipation or diarrhea, and sensitivity to smells or noise.[16] This may occur in subjects who have migraine with aura or migraine without aura.

1.2.2.Aura

An aura is a transient focal neurological phenomenon that occurs before or during the headache.[17] They appear gradually over a number of minutes and generally last fewer than 60 minutes.[9] Symptoms can be visual, sensory or motor in nature and many people experience more than one. Visual effects appear most frequently, up to 99% of the cases, and in more than 50% of the subjects, these are not accompanied by sensory or motor effects. Vision disturbances often consist of a scintillating scotoma (an area of partial alteration in the field of vision which may interfere with a person's ability to read or drive).[17] These typically start near the center of vision and then spread out to the sides with zigzagging lines looking like fortifications or walls of a castle.[9] Usually the lines are in black and white but sometimes colored, and some people lose part of their field of vision known as hemianopsia while others experience blurring.

Sensory aura is the second most common type, reported by 30–40% of the people who experience auras.[9] Often a feeling of pins-and-needles begins on one side in the hand and arm and spreads to the nose-mouth area on the same side. Numbness usually occurs after the tingling has passed with a loss of position sense.[9] Other symptoms of the aura phase can include: speech or language disturbances, world spinning, and less commonly motoric problems. Such motoric symptoms indicate that this is a hemiplegic migraine, and weakness often lasts longer than one hour unlike other auras.[9] An aura rarely occurs without a subsequent headache, known as a silent migraine.

However, it is difficult to assess the frequency of such cases, because patients with symptoms not severe enough to seek treatment, may pass it off without reporting anything.

1.2.3.Pain phase

Classically the headache is unilateral, throbbing, and moderate to severe in intensity.[18] It usually comes on gradually, and is aggravated by physical activity.[2] In more than 40% of cases, however, the pain may be bilateral, and neck pain is commonly associated.[19] Bilateral pain is particularly common in those who have migraines without an aura. Less commonly pain may occur primarily in the back or top of the head.[14] The pain usually lasts from 4 to 72 hours in adults, however, in young children, it frequently lasts less than 1 hour. The frequency of attacks is variable, from a few in a life-time to several a week, with the average being about one per month.[9]

The pain is frequently accompanied by nausea, vomiting, sensitivity to light, sensitivity to sound, sensitivity to smells, fatigue and irritability. In a basilar migraine, a migraine with neurological symptoms related to the brain stem or with neurological symptoms on both sides of the body, common effects include: a sense of the world spinning, light-headedness, and confusion.[9] Nausea occurs in almost 90% of the subjects, and vomiting occurs in about one-third, making the person to seek a dark and quiet room. Other symptoms may include: blurred vision, nasal stuffiness, diarrhea, frequent urination, pallor, sweating, swelling or tenderness of the scalp, and neck stiffness.

1.2.4.Postdrome

The effects of migraine may persist for some days after the main headache has passed by, and this is called the migraine postdrome. Many victims report a sore feeling in the area where the migraine was, and some report impaired thinking for a few days after the headache has passed. The patient may feel tired or "hung over" and have head pain, cognitive difficulties, gastrointestinal symptoms, mood changes, and weakness.[20] According to one summary, "Some people feel unusually refreshed or euphoric after an attack, whereas others note depression and malaise.[21]

1.3.Causes and triggers of migraine

The underlying causes of migraines are unknown. However, they are believed to be related to a mix of environmental and genetic factors.[3] They run in families in about two-thirds of cases[4] and rarely occur due to a single gene defect.[22] A number of psychological conditions are associated, including

depression, anxiety, and bipolar disorder, as are many biological events or triggers.

1.3.1.Genetic predisposition

Studies of twins indicate a 34% to 50% genetic influence of likelihood to develop migraine headaches.[3] This genetic relationship is stronger for migraines with aura than for migraines without aura. A number of specific variants of genes increase the risk by a small to moderate amount.[22] Single gene disorders that result in migraines are rare, indeed.[22] One of these is known as familial hemiplegic migraine, a type of migraine with aura, which is inherited in an autosomal dominant fashion.[23,24] Four genes have been shown to be involved in familial hemiplegic migraine.[25] Three of these genes are involved in ion transport, while the fourth is an axonal protein associated with the exocytosis complex.

1.3.2.Triggers

Migraines may be induced by triggers, and opinions are divergent on their importance; while some report triggers being involved in a minority of cases,[4] other studies report involvement in the majority.[26] Many things have been labeled as triggers, but the strength and significance of these relationships are uncertain.[26,27] Importantly, any such trigger may be encountered up to 24 hours prior to the onset of symptoms.

1.3.2.1.Physiological aspects

Common triggers quoted are stress, hunger, and fatigue (these equally contribute to tension headaches).[26] Migraines are more likely to occur around menstruation. Other hormonal influences, such as menarche, oral contraceptive use, pregnancy, peri-menopause, and menopause, also play a role.[5] These hormonal influences seem to play a greater role in migraine without aura.[9] Migraines typically do not occur during the second and third trimesters or following menopause.[13]

1.3.2.2.Dietary aspects

Reviews on dietary triggers have disclosed that the evidence mostly relies on self-reports and as such is not rigorous enough to prove or disprove any particular triggers.[28,29] Regarding the specific agents, there does not appear to be evidence for an effect of tyramine on migraine[30] and while monosodium glutamate (MSG) is frequently reported as a dietary trigger, evidence does not consistently support this.[31]

1.3.2.3.Environmental aspects

A review on potential triggers in the indoor and outdoor environment concluded the overall evidence being of poor quality, but nevertheless suggested migraine patients to take some preventive measures related to indoor air quality and lighting. While migraine was once believed to be more common among those with high intelligence, this does not appear to be true.[9]

1.4.Diagnosis of migraines

The diagnosis of a migraine is based on signs and symptoms.[4] Imaging tests are not particularly helpful, but occasionally performed to exclude other causes of headaches. It is generally agreed that at the population level, a substantial number of people with true migraines have never been properly diagnosed.[4]

According to the International Headache Society,[2] the correct diagnosis of migraine without aura can be made according to the following criteria, known as the "**5, 4, 3, 2, 1 criteria**":

1) **Five** or more attacks: for migraine with aura, two attacks are sufficient for diagnosis.
2) **Four** hours to **three** days in duration
3) **Two** or more of the following:
    i) Unilateral (affecting half the head);
    ii) Pulsating;
    iii) Moderate or severe pain intensity;
    iv) Aggravation by or causing avoidance of routine physical activity
4) **One** or more of the following:
    i) Nausea and/or vomiting;
    ii) Sensitivity to both light (photophobia) and sound (phonophobia)

Based on a recent meta-analysis, it was concluded that if someone experiences two of the following: photophobia, nausea, or inability to work for a day, migraine diagnosis is more likely.[32] In those with four out of five of the following: i) pulsating headache, ii) duration of 4–72 hours, iii) pain on one side of the head, iv) nausea, or v) symptoms that interfere with the person's life, the probability that this is a migraine is 92%,[15] as compared to 17% among those with less than three of these symptoms.

1.5.Prevention of migraine attacks

Preventive (prophylactic) treatment of migraines is an important component of the total management of migraine patients. Such treatments can take many forms, including everything from surgery, taking certain drugs or nutritional supplements, to life-style alterations such as increased exercise and avoidance of migraine triggers. The goals of preventive therapy are twofold: i) to reduce the frequency, painfulness, and/or duration of migraines, and ii) to increase the effectiveness of abortive therapy.[33] Another reason to pursue these goals is to avoid medication overuse headache (MOH), also known as rebound headache, which is a common complaint among the migraine patients. This is believed to occur in part due to overuse of pain medications, and can result in chronic daily headache.[34] To better standardize the trials testing preventive medications, the Task Force of the International Headache Society Clinical Trials Subcommittee recently launched international guidelines.[35] During the past several decades, a large number of optional modalities have been tested as preventive measures of migraine attacks. Some of those are outdated today, but listed here to demonstrate the diversity of these approaches, and the complexity of the problem.

1.5.1.Surgery

Despite major pharmacological advances in the treatment of migraine headaches, most patients still endure symptoms until the medications take effect, and they often experience a poor quality of life despite an aggressive regimen of pharmacotherapy.[36] The most effective surgery techniques appear to be those involving the surgical cauterization of the superficial blood vessels of the scalp (the terminal branches of the external carotid artery), and the removal of muscles in areas known as "trigger sites".[37]

1.5.2.Acupuncture

Cochrane reviews have found that acupuncture is effective in the treatment of migraines.[38] The use of "true" acupuncture is not more efficient than sham acupuncture, however, both "true" and sham acupuncture appear to be more effective than routine care in the treatment of migraines, with fewer adverse effects than prophylactic drug treatment.

1.5.3.Behavioral treatments

Many physicians believe that exercise for 15–20 minutes per day is helpful for reducing the frequency of migraines. Sleep is often a good solution if a migraine is not too severe to prevent sleeping. Diet, visualization, and self-hypnosis are also alternative treatments and prevention approaches. Sexual

activity has been reported by a proportion of male and female migraine sufferers to relieve migraine pain significantly in some cases.[39]

1.5.4.Gluten-Free Diet

Studies have suggested that 4% of migraine sufferers have celiac disease, and for those who do, decreasing gluten intake may significantly reduce migraine frequency.[9] Celiac disease and gluten sensitivity may be an underlying cause of migraines in some patients, and a gluten-free diet has been demonstrated to reduce, if not completely eliminate, migraines in these individuals. There is some data that migraine patients are 10 times more likely than the general population to have celiac disease, and for those, a gluten-free diet improved blood-flow to the brain and either eliminated migraines or reduced migraine frequency, duration, and intensity.[9]

1.5.5.Herbal and nutritional supplements

1.5.5.1.Butterbur

Native butterbur contains some carcinogenic compounds, but a purified version, Petadolex, does not. A systematic review of two trials with total of 293 patients showed moderate evidence of effectiveness for a higher than the recommended dose of the proprietary Petasites root extract Petadolex in the prophylaxis of migraine.[40]

1.5.5.2.Cannabis

Cannabis was a standard treatment for migraines from 1874 to 1942.[9] It has been reported to help people through an attack by relieving the nausea and dulling the head pain, as well as possibly preventing the headache completely when used as soon as possible after the onset of pre-migraine symptoms, such as aura.[41]

1.5.5.3.Feverfew

The plant feverfew (Tanacetum parthenium) is a traditional herbal remedy believed to reduce the frequency of migraine attacks. A number of clinical trials have been carried out, but a recent Cochrane review concluded that the results have been contradictory and inconclusive.[42]

1.5.6.Manual Therapy

A systematic review stated that chiropractic manipulation, physiotherapy, massage and relaxation might be as effective as propranolol or topiramate in the prevention of migraine headaches; however, the research had some problems with methodology.[43]

1.5.7.Medical devices

1.5.7.1.Neurostimulation

Neurostimulation initially used implantable neurostimulators similar to pacemakers for the treatment of intractable chronic migraines with encouraging good results. But the need of surgery is the limiting factor in their implication, reserved for most severe cases only.[44]

1.5.7.2.Transcranial Magnetic Stimulation (TMS)

Transcranial Magnetic Stimulation (TMS) is a non-invasive technology for treating depression, obsessive compulsive disorder and tinnitus, among other ailments, and recently shown to prevent and even reduce the severity of migraines among its patients.[45] This treatment essentially disrupts the aura phase of migraines before patients develop full-blown migraines. Recently, the authors introduced a hand-held apparatus designed to apply TMS as a preemptive therapy to avert a migraine attack at the onset of the aura phase.[45]

1.5.7.3.Biofeedback

Biofeedback helps patient to become conscious of some physiological parameters to control them and try to relax. Biofeedback has been used successfully to control migraine symptoms through training and practice.[46] This method is considered to be efficient for prevention of migraine attacks, with efficacy similar to sophisticated sessions in clinics.

1.5.7.4.Hyperbaric oxygen therapy

Hyperbaric oxygen therapy has been used successfully in treating migraines,[47] suggesting that sufferers might be treated during an attack with a hyperbaric chamber similar as done in the treatment of e.g. altitude sickness.

1.5.8.Medical treatment

In their recent review, Modi and Lowder describe general guidelines concerning the proper use of drugs for prevention of migraine attacks.[33] According to these authors, factors that should prompt

consideration of preventive therapy include i) the occurrence of two or more migraines per month with disability lasting three or more days per month; ii) failure of, contraindication for, or adverse events from acute treatments; iii) use of abortive medication more than twice per week; and iv) uncommon migraine conditions (e.g., hemiplegic migraine, migraine with prolonged aura, migraine infarction).[33]

Therapy should be initiated with medications that have the highest levels of effectiveness and the lowest potential for adverse reactions. These should be started at low dosages and titrated slowly. Noteworthy, a full therapeutic trial may take two to six months. After successful therapy (e.g., reduction of migraine frequency by approximately 50% or more) has been maintained for 6 to 12 months, discontinuation of preventive therapy can be considered.[33] The most effective medications used for migraine prophylaxis include several distinct drug classes, listed in short here.

1.5.8.1.Beta blockers

A meta-analysis by the Cochrane Collaboration including 9 randomized controlled trials or cross-over studies (including 668 patients) found that propranolol had an overall relative risk of response to treatment of RR=1.94.[48]

1.5.8.2.Anticonvulsants

A recent Cochrane type of meta-analysis, comprising 10 randomized controlled trials or cross-over studies (n=1.341 patients), anticonvulsants (valproic acid and topiramate) had an 2.4 times more likely to experience a 50% or greater reduction in frequency as compared with placebo, and a number needed to treat of 3.8.[49]

1.5.8.3.Antidepressants

Tricyclic antidepressants (TCAs) such as amitriptyline and the newer selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine are sometimes prescribed. A meta-analysis by the Cochrane Collaboration found SSRIs are no more effective than placebo, however.[50]

1.5.8.4.Other drugs

A wide range of other pharmacological drugs have been evaluated for their efficacy in reducing the frequency or severity of migraine attacks.[51] Such drugs include beta-blockers, calcium antagonists,

neurostabalizers, nonsteroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), other antidepressants, and other specialized drug therapies. The US Headache Consortium lists five drugs as having medium to high efficacy: amitriptyline, divalproex, timolol, propranolol and topiramate.[51] Lower-efficacy drugs listed include aspirin, atenolol, fenoprofen, flurbiprofen, fluoxetine, gabapentin, ketoprofen, metoprolol, nadolol, naproxen, nimodipine, verapamil and Botulinum A. Additionally, most antidepressants (tricyclic, SSRIs and others such as Bupropion) are listed as "clinically efficacious based on consensus of experience" without scientific support.[51] Importantly, many of these drugs may give rise to undesirable side-effects, or may be efficacious in treating comorbid conditions, such as depression.

1.6.Histamine and headaches

Different as classical migraine and cluster headache might look like in their pathogenesis and symptom patterns, there might be a common denominator for these two conditions. As described above, both migraine and cluster headache are classified as vascular headaches. This implicates that substances inducing swelling and dilatation of the blood vessels among susceptible individuals can provoke an attack of these vascular headaches.[52] Thus, substances that cause blood vessel swelling can provoke an acute attack during a series period. Nitroglycerin or **histamine**, smoking or minimal amounts of alcohol can precipitate or increase the severity of the attacks as the sufferer's blood vessels seem to change and become susceptible to the action of these substances. The blood vessels are not sensitive to these substances during headache-free periods. Hormonal influences in women do not appear to be a factor in cluster headaches.[52]

During the past years, also the possible mechanisms responsible for the actions of histamine as a vasodilator have been elaborated.[53,54] Indeed, there are firm experimental and clinical evidence implicating that **histamine** induces the enzyme Nitric Oxide (**NO**) Synthase, making NO available to act locally on the vasculature as a vasodilator. In primates, histamine is known to activate cerebral endothelial H1-receptors leading to formation of NO. This was nicely shown by Lassen et al. (1995) who examined 20 migraine patients with pretreatment by placebo or the histamine-H1-receptor antagonist, mepyramine, in a randomized, double blind fashion, followed in both groups by i.v. histamine.[55] In patients receiving placebo, histamine administration caused immediate headache during the infusion followed by a delayed migraine attack fulfilling IHS criteria for migraine without aura.[2,9] Mepyramine pretreatment abolished both immediate headache and delayed migraine

attacks, suggesting that a migraine attack can be caused by NO formation in the endothelium of cerebral arteries.[55]

These results clearly confirm the earlier data reported by the same group.[56] Starting from the fact that nitroglycerin, which may be regarded as a prodrug for NO, induces a mild to moderate headache in healthy subjects, the authors tested, whether migraine patients are more sensitive to NO than non-migrainous subjects, using four different doses of iv nitroglycerin in a double-blind setting of 17 migraine patients, 17 age and sex matched healthy controls and 9 subjects with tension-type headache.[56] The nitroglycerin-induced headache was significantly more severe in migraine sufferers, lasted longer and fulfilled diagnostic criteria for migraine more often. This implicates that migraine patients are supersensitive to exogenous NO from nitroglycerin as well as to endothelial cell-produced NO,[55] providing additional support to the concept that NO may be partially or completely responsible for the pain of migraine attacks.

The next logical step was to assess, how histamine and its antagonists exert their effects on cerebral (dural) arteries, as elegantly studied by Ackerman et al. (2002),[53] using intra-vital microscopy to directly assess the diameter of dural arteries in sodium pentobarbitone anaesthetised rats. Histamine infusion caused immediate and reproducible dilation of **meningeal** blood vessels that could be blocked by H(1)- (mepyramine) and H(2) (famotidine)-receptor antagonists as well as a NO-synthase inhibitor (N(G)-nitro-L-arginine methylester; $P<0.05$). Neurogenic **dural** vasodilation was not inhibited by H(2)-receptor antagonists, but was significantly inhibited by a H(1)-receptor antagonist. These data lend further support to the concept that histamine is likely to activate NO synthase and promote NO production.[53-56] NO binds to guanylyl cyclase in vascular smooth muscle cells, producing cyclic GMP, which forms phosphorylated protein kinase G (PKG). PKG phosphorylates Ca2+ channels, slowing the influx of calcium into the cell, which leads to smooth muscle relaxation and vasodilation, characteristic in migraine attack.

1.7.Liberators of histamine: acetaldehyde

Most histamine in human body is generated in granules of the **mast cells** (in tissues) or in white blood cells called basophils. Mast cells are especially numerous at sites of potential injury, including the nose, mouth, **gastrointestinal tract**, skin, and **blood vessels**. Non-mast cell histamine is found in several tissues, including the brain, where it functions as a neurotransmitter. Another important

site of histamine storage and release is the enterochromaffin-like (ECL) cells of the gastric mucosa.

Mast cells play a crucial role in **hypersensitivity**, allergic, and inflammatory reactions by secreting chemical mediators, e.g. histamine, proteases, and cytokines as a response to various immunologic and non-immunologic stimuli. Given the fact that also alcohol intake causes, especially in Oriental individuals (with hereditarily deficient acetaldehyde metabolism), **hypersensitivity** symptoms that can be blocked by antihistamines, as well as urticaria and anaphylactoid reactions in Caucasian individuals as well, led Koivisto et al. (1996) to speculate the possibility that **acetaldehyde** (the first metabolite of alcohol) might enhance these reactions by directly affecting the tissue **mast cell** functions.[57] Indeed, these authors were the first to demonstrate that this is the case. Using purified rat peritoneal mast cells incubated with different concentrations of acetaldehyde and ethanol at body temperatures, they demonstrated that acetaldehyde, at relatively low concentrations (50μM), directly induces histamine release from the mast cells. Ethanol did the same, but only at molar concentrations.[57]

The authors reasoned that such acetaldehyde-induced histamine release from the mast cells may contribute to different hypersensitivity reactions caused by alcohol intake, including some alcohol-associated gastrointestinal disorders.[57] Subsequently, the validity of this concept has been confirmed also in other clinical conditions, including human bronchial mast cells.[58] In their elegant experiments, Kawano et al. (2004) demonstrated that acetaldehyde (>3 x 10^{-4} M) increased airway muscle tone, which was associated with a significant increase in the release of histamine, but not thromboxane B2 or cysteinyl-leukotrienes. A histamine (H1-receptor) antagonist completely inhibited acetaldehyde-induced bronchial smooth muscle contraction. Acetaldehyde also induced a significant histamine release from human **lung mast cells** and their degranulation. These results strongly implicate that acetaldehyde stimulates human airway mast cells to release histamine, which is involved in bronchial smooth muscle contraction e.g. following alcohol consumption.[58]

1.7.1.Acetaldehyde: Group 1 carcinogen (IARC)

Tobacco smoke contains several classes of carcinogens that include among others polycyclic aromatic hydrocarbons, aromatic amines, and nitrosamines. Tobacco smoke contains also high concentrations of toxic aldehydes.[59] The most abundant aldehyde in tobacco smoke is acetaldehyde, and its concentration in tobacco smoke is >1,000 times greater than those of polycyclic aromatic

hydrocarbons and tobacco-specific nitrosamines.[60] Acetaldehyde is also the first metabolite of ethanol oxidation. It binds to DNA, forming stable DNA adducts that are observed in alcohol consumers. Numerous epidemiological studies in alcohol drinkers with alcohol dehydrogenase (ALDH2) deficiency or low aldehyde dehydrogenase (ADH1B) activity provide the most compelling evidence for the carcinogenicity of acetaldehyde.[61] This deficiency results in the accumulation of acetaldehyde locally into the saliva during ethanol metabolism and also in markedly increased risk for many upper gastrointestinal tract cancers.

Similarly, it was recently shown that acetaldehyde from the tobacco smoke is easily dissolved into the saliva during smoking.[62] Thus, toxic aldehydes could mediate the carcinogenic effect of tobacco smoke through saliva to oral cavity and from there further on to the larynx, esophagus, and stomach. Based on firm epidemiological and toxicological documentation, IARC proclaimed (in 2009) acetaldehyde as Group I carcinogen, equivalent to asbestos, formaldehyde and others.[63]

1.7.2.L-cysteine (Acetium®) eliminates acetaldehyde in the stomach and in saliva

Cysteine is a non-essential amino acid, which was shown (almost 40 years ago) to be capable of eliminating the toxicity of acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (MTCA).[64] MTCA is an inert and non-toxic compound that is eliminated from the body through feces and urine, without being absorbed into the blood circulation. This simple principle was used in the recent innovation of **Biohit Acetium® capsule,** which contains 100mg L-cysteine.

In the proof-of-concept study, oral administration of Acetium® was confirmed to effectively bind acetaldehyde originated from ethanol metabolism in achlorhydric stomach.[65] In that setting, the mean acetaldehyde level of gastric juice was 2.6 times higher with placebo than with Acetium (13 vs. 4.7 μM, $p<0.05$), implicating that Acetium can be used to decrease acetaldehyde concentration in achlorhydric stomach during alcohol exposure.

This led the authors to examine the concept, whether it would be possible to eliminate alcohol-derived acetaldehyde also from the saliva, using L-cysteine slowly released from a special buccal (Acetium®) tablet.[66] Indeed, this was shown to be the case in tested volunteers, in whom, up to two-thirds of acetaldehyde (after alcohol intake) could be removed from the saliva with a slow-releasing

buccal L-cysteine formulation (Acetium®). This might have important implications e.g. in prevention of upper GI-tract cancers among individuals with high acetaldehyde exposure (heavy drinkers, smokers).[66]

As the logical next step, Biohit Oyj developed an Acetium sucking tablet (Acetium® lozenge) that releases L-cysteine into the oral cavity during smoking, and tested this formulation as a potential chemopreventive agent against toxicity of tobacco smoke, i.e. in harm reduction.[67] Seven volunteers smoked five cigarettes, and during every smoking period, sucked a blinded tablet containing 0, 1.25, 2.5, 5, or 10 mg of L-cysteine, followed by acetaldehyde analysis of the saliva at 0, 5, and 10 minutes from the beginning of the smoking. L-cysteine (Acetium®) reduced highly significantly the salivary acetaldehyde. In fact, carcinogenic acetaldehyde could be totally inactivated in the saliva during smoking by the sucking tablet containing 5 mg of L-cysteine (Acetium®).[67]

2.STUDY HYPOTHESIS

The striking novel hypothesis of this study is simply the following: L-cysteine administered by daily intake of **Acetium® capsules** is an effective prophylactic means to prevent the attacks of migraines or at least significantly decreasing the attack frequency.

As described above, Acetium capsules and lozenges were originally developed for inactivation of acetaldehyde in the stomach and in the saliva after alcohol intake and smoking, respectively. This novel concept on the potential prophylactic effect of Acetium® capsule against migraines (based on acetaldehyde inactivation and the consequent block of acetaldehyde-induced histamine liberation from tissue mast cells), emerged purely by chance. Importantly, the trigger to formulating this hypothesis is derived **from the patients** suffering from severe migraines (and/or intractable cluster headache), who described to us that daily intake of Acetium® capsules (for their original indications) has completely eliminated their headache attacks.[68]

2.1. Case histories of patients with migraines (and cluster headache)

Because of their pivotal role in stimulating us to formulate the novel study hypothesis, these case histories submitted to us until to date are referred to in brief here.

Case 1: A 36-year-old woman (an employee of Biohit Oyj), whose migraine deteriorated in 2006 when the attacks started to be accompanied by aura as well as other symptoms e.g. weakness in the upper extremities. She was thoroughly examined, and the condition was diagnosed as hormonal migraine. She received a specific medication (Maxalt, Migard), but with some side effects and little effect on the attacks. Attacks continued appearing in 3-4 days a week, not infrequently severe enough to prevent daily work and other activities. Different physical therapies were of no help at all, and attacks continued with the same frequency. This lead her to seek help from a wide variety of drugs, including Panadol and Panacod, with prolonged administration for protracted headache of over one week's duration. Her situation gradually escalated to the stage when continuous use of analgesics was necessary to keep the headache at least in some control to enable daily work and other normal activities.

She reporting starting the intake of Acetium capsules on December 22, 2012, with 2 capsules in the morning and 2 in the evening. After a few months, she could reduce the dose to one capsule in the morning and one in the evening. To her major relief and surprise, all headache attacks have remained practically absent. During this period, she has had a few episodes, but all associated with a specific trigger (unrelated to her migraine). She does need no painkillers any longer, and if occasionally needed, they give a good relief. Interestingly, she once forgot taking Acetium capsules for one week (due to non-availability), and the headache attacks returned immediately. She learned not to repeat that mistake, and at the time of this testimony (June 2013), she has remained attack-free for over 4 months.

Case 2: A 12-year-old girl, daughter of Case #1, who started suffering from severe headaches when 7 years old (in 2008). The attacks were characteristic to migraine, and in 2010, migraine with aura was diagnosed. A combination therapy with Ketorin, Panadol and Burana was instituted, but with little help on the frequency of the attacks. The condition was aggravated in late 2012, with attack rate increasing up to 2-3 times a week. The migraine (possibly aggravated due to hormonal changes) diagnosis was confirmed by pediatric neurologist, who administered beta-blockers for attack prophylaxis. The patient and her mother, however, decided to test the efficacy of Acetium also for the daughter, with one capsule twice a day.

Given the striking effect in her mother, the same effect was noticed also in the daughter, who has remained without a single migraine attack since the onset of Acetium intake (April 2013). She feels herself completely healthy, fully capable of attending the school with no periods of absence ever since. Both the mother and her daughter have a feeling that with the use of Acetium capsules, they have obtained a completely new life, with no concern of migraine on daily basis.

Case 3: A 41-year-old woman (from Norway), with a long-term history of severe migraine attacks who was introduced with Acetium capsules by one of the Biohit partners in Sweden. She has also a diagnosed celiac disease and follows a strict gluten-free diet. Otherwise generally healthy, with no regular medication, except occasional anti-depressants. She started the daily use of Acetium capsules as soon as they entered into the market, and after a few weeks, her migraine attacks disappeared. She has been attack-free now for a number of years, and continues taking one Acetium capsule every day for maintenance prophylaxis.

Case 4: A 48-year-old male, with cluster headache (Horton's syndrome). He provided a very detailed description of his headaches, which fulfills the criteria of a classical cluster headache, diagnosed with the name Horton's syndrome by his physician some 16 years ago. The attacks start at distinct time of the day (at 1-2 o'clock at night in his case), are unilateral and have a maximum duration of two hours.

Some 6-7 years ago, the condition was aggravated and the attack rate increased, extending to other seasonal periods that during the first few years. The cluster periods lasted up to 8 weeks, having a definite negative impact on the quality of his daily life. He also listed the most important triggers of the clusters, including stress, associated with short sleeping hours and use of even small a mounts of alcohol. During the years, he has tested several different modalities for cluster prophylaxis. The best combination, with some reduction in cluster frequency but not total disappearance, proved to be magnesium food supplement, melatonin (5mg), abundant

drinking of water before going to sleep, refrain from alcohol intake, and highly regular sleeping. As said, the cluster frequency diminished, but the attacks did not disappear.

He started taking Acetium® capsules in December 2009, following a very intense cluster. Since then, he has continued taking one capsule in the morning. Occasionally, while feeling prodromal symptoms, he has raised the dosage to 2-4 capsules per day. Some six months since initiation of Acetium, he was able to abandon his daily routines (described above), including the use of magnesium supplement, melatonin drinking abundant water before going to sleep. Interestingly, also the previous triggers of his clusters (stress, sleeplessness, alcohol), have not evoked new attacks since the onset of Acetium® prophylaxis. According to his written testimonial (July 2013), this patient has experienced not a single attack of cluster headache since December 2009, when he started the daily intake of Acetium® capsules, one capsule per day.

The above spontaneous testimonials[68] given in writing by these four persons who all have suffered from either severe migraines (3 subjects) or intractable cluster headache (Case #4) for years, prompted us to consider, whether a plausible mechanism could be discovered to explain these dramatic effects experienced by all these subjects soon after onset of Acetium® intake on daily basis. Given that L-cysteine is a natural (semi-essential) amino acid, converted to inert substance (MTCA) in the alimentary tract, it would comprise an ideal means to conduct migraine prophylaxis for years, without concern about the side effects that are inherent to many of the current treatment modalities (see above). If proved in a formal randomized controlled trial (RCT), the concept of using Acetium capsules in prophylactic treatment of migraines and cluster headache, would represent a major step forward in a better clinical control of these frequently intractable syndromes.

2.2.Components of the study hypothesis

The current study hypothesis is built up of several elements, all based on solid experimental and/or clinical evidence. These elements include both i) the known pathways involved in the provocation of a characteristic attack of migraines, as well as ii) established and postulated mechanisms, how Acetium administration could interfere with this sequence of events.

From up-stream to down-stream, the sequence of events leading to vasodilatation and migraine attach is as follows.

2.2.1.Nitric oxide (NO) is the final trigger of migraine attack

NO binds to guanylyl cyclase in vascular smooth muscle cells, which leads to the production of cyclic GMP, which in turn forms phosphorylated protein kinase G (PKG). PKG phosphorylates Ca2+ channels, slowing the influx of calcium into the cell, which leads to smooth muscle relaxation and

vasodilation, resulting in characteristic migraine attack.[54]

2.2.2.Histamine induces Nitric Oxide (NO) Synthase

There is firm experimental and clinical evidence implicating that histamine induces the enzyme Nitric Oxide (NO) Synthase, making NO available to act locally on the vasculature as a vasodilator. Histamine is known to activate cerebral endothelial H1-receptors, leading to formation of NO.[53-56]

2.2.3.Histamine is synthesized in tissue mast cells and basophils

Histamine is synthesized in tissue mast cells and basophils by histidine decarboxylase converting histidine to histamine. Another important source of histamine are **enterochromaffin-like (ECL) cells** that are abundant in **gastric (corpus) mucosa**. Histaminic cephalalgia is the old name for cluster headaches, implicating that histamine has been linked with the development of vascular headaches since their description. Mast cells are ubiquitous, and their activation (e.g. in the meninges) by migraine triggers is now believed to contribute to genesis of migraine headaches.[69]

2.2.4.Histamine is liberated from mast cells by acetaldehyde

Mast cells play a crucial role in hypersensitivity, allergic, and inflammatory reactions by secreting chemical mediators, e.g. histamine, proteases, and cytokines as a response to various immunologic and non-immunologic stimuli. One of the potent liberators of histamine from the mast cells is acetaldehyde (a Group 1 carcinogen[63]), both in the human and in experimental animals.[57,58] This could neatly explain several hypersensitivity-like reactions associated e.g. with alcohol intake and smoking, both being abundant sources of acetaldehyde.

2.2.5.Acetaldehyde in the saliva and stomach is inactivated by Acetium®

The phenomenon that L-cysteine eliminates free acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (MTCA),[64] was exploited by Biohit Oyj while developing their **Acetium® capsule** containing 100mg L-cysteine. Both the capsule and the recently introduced Acetium® lozenge (3 mg L-cysteine) effectively eliminated acetaldehyde derived from alcohol and/or cigarette smoking, both in the stomach and in saliva, respectively.

2.2.6.The efficacy of Acetium® in migraine prophylaxis?

We are convinced that the dramatic disappearance of migraine attacks and cluster headaches

immediately after regular intake of Acetium® capsules as described in the case histories[68] cannot be by chance. Instead, we believe that the mechanism must be based on the capacity of Acetium to interfere with the above deciphered sequence of events, whereby elimination of acetaldehyde in the stomach could block (or reduce below the threshold levels) histamine liberation from the tissue mast cells and ECL cells in the stomach, thus arresting its multitude of functions, of which vasodilatation is critically involved in inducing the migraine attack.[52-56]

In the nervous system, histamine acts mainly on H1- and H3-receptors.[70] While H1-receptors mediate inflammation, H3-receptors are much more sensitive to histamine and serve as negative feedback to inhibit further excessive release of histamine by C-fibers (afferent fibers of the somatic sensory system).[71] It is tempting to speculate, whether **reduction of total histamine burden** by eliminating acetaldehyde would be equivalent to so called "modulation of histamine" that has been successfully employed in the treatment of migraine and cluster headaches for several years by now.[54] Indeed, for migraine, the H3-receptor mediated negative feedback loop has been exploited by intermittent very low doses of s.c. histamine. In theory, low histamine concentrations in the body would lead to preferential stimulation of the more sensitive H3-receptors, while leaving the H1-receptors relatively untouched (see 2.2.2.), thus activating the negative feedback loop and reducing the potential of the C-fibers to become activated.[54]

The present study is designed to validate this novel hypothesis that daily use of Acetium® capsules (2 capsules 3 times a day) is an effective means to decrease the frequency of (or completely abort) the attacks of migraine, as suggested by anecdotal case testimonials of such patents, reporting complete remissions.[68]

3.STUDY DESIGN

This **multi-centre**, double-blind, placebo-controlled randomized trial (RCT) is designed to test the efficacy of intervention by Acetium® capsules (2 capsules 3 times a day) in reducing the frequency of (or completely aborting) the headache attacks among patients with migraines. The study design follows the IHS **Guidelines for Controlled Trials of Drugs in Migraine**, recently elaborated by the International Headache Society Clinical Trials Subcommittee.[72] A cohort of 200 subjects with clinically diagnosed migraines will be enrolled utilising public invitations, and randomly allocated (after 1-month baseline period) to two study arms (n=100 in each), receiving either Acetium® capsules or

placebo (2 x 3 per day). All subjects will be requested to fill in a structured questionnaire recoding their detailed headache history and other clinical data pertinent to this study. The subjects will be administered a headache diary on daily basis, submitted to the study monitor monthly for recording the compliance of each subject with the medication. The trial period will be 3 months in both study arms.[72]

3.1.Patient selection (=criteria of being eligible)

This intervention trial is designed and conducted in conformity with the current guidelines of the International Headache Society Clinical Trials Subcommittee,[72] jointly by **specialist neurologists** (experts in vascular headaches) in Hospital X (City Y), and by the Clinical Research Department of Biohit Oyj (Helsinki). These guidelines give detailed recommendations for patient selection, trial design, as well as evaluation of the results, and closely followed in the study design of this migraine RCT.

3.1.1.Definition of migraine

The subjects into the cohort will be enrolled through the Finnish Migraine Association (website and/or membership registry), a joint national organisation for patients suffering from migraine and other vascular headaches. All subjects to be enrolled have a clinically confirmed diagnosis of migraine, based on the ICHD-II classification.[2] The diagnostic criteria of the ICHD-II are highly reproducible and universally endorsed, which ensures homogeneity of the cohort and better interpretation of data.[72] Furthermore, strict adherence to the ICHD-II criteria for migraine only precludes a relatively small patient group. In this study, migraine patients with aura and those without aura are eligible, but will be analysed as subgroups, if indicated.

3.1.2.Patients with other (non-migraine) headaches

Following the recommendations of the IHS Guidelines,[72] this proof-of-concept intervention trial shall exclude the participants with other headaches.[2] The exclusion of these "non-target headaches" strengthens the scientific robustness of this trial, which is designed to address the effects of Acetium capsules on clinically established migraine-type headache only.

3.1.3.Frequency of attacks

To be eligible for the study, the subjects should report migraine attacks with the frequency of **2–8**

times per month, and with less than 15 migraine days (NMD) per month. To be calculated as a separate attack, there should be at least **48h of freedom** from headache between the two attacks of migraine.[72] The reasoning behind this selection criteria is that 48h of freedom between the attacks permits identification of individual attacks and distinction from relapse.

3.1.4.Duration of disease

Only subjects in whom migraine has been present for **at least 1 year prior** to entering into the study. This 1-year requirement helps excluding the subjects with i) probable migraine and ii) those with secondary headaches showing features of migraine. Furthermore, this minimum of 1-year course of established migraine improves homogeneity of the study population.[72]

3.1.5.Age at migraine onset

Subjects to be enrolled should report the onset of their migraine **before 50 years of age.** This is because i) migraine with onset after 50 years is rare, and ii) there is increasing uncertainty in the diagnosis of true migraine after the age of 50 years, because the prevalence of secondary headaches with migraine features (e.g. ischemic stroke) increases.[72]

3.1.6.Age at study entry

Following the general guidelines for adult migraine RCTs, our cohort will accept only participants **between 18 and 65 years of age.** This is simply because the intervention studies on paediatric and elderly migraine patients necessitate different study protocols.[72]

3.1.7.Gender

Both **male** and **female** participants shall be eligible in the present cohort. Migraine is at least three times more common among women than in men, easily resulting in gender selection bias in migraine RCTs. This is further accentuated by the higher consultation rates for migraine among women. In this study, every effort is made to minimize a gender selection bias by encouraging particularly male patients to participate.

3.1.8.Concomitant medication

The subjects enrolled, will be allowed to continue their current acute therapy for individual migraine attacks, but their use must be well documented. Because Acetium® capsules have no known

interactions with other drugs, the other drugs not taken for migraine are not contraindicated. However, **other migraine prophylactic** medication should be **discontinued one month** prior to the study entry, i.e., at the entry to the 1-month run-in period.

Excluded are the following subjects: patients who meet the ICHD-II criteria for medication overuse;[2] patients who have taken anti-psychotics or anti-depressant medications during the previous 3 months; patients who abuse alcohol or other drugs; patients resistant to all acute migraine drugs optimally prescribed; and potentially fertile and sexually active women who do not practise contraception.

3.1.9.Co-morbidity

The intention is to enrol a cohort of subjects with minimum co-morbidity. Specific co-morbid medical conditions that **exclude participation** in this trial follow the IHS recommendations for migraine RCTs,[72] and include the following categories of patients: other acute or chronic pain disorders, severe psychiatric disease, infections, malignancy, short life expectancy, cardiovascular disease, cerebrovascular disease, uncontrolled hypertension, degenerative central nervous system diseases, as well as pregnant and lactating women.

3.2.Trial design

Also the design of this multi-centre RCT follows the guidelines of the International Headache Society Clinical Trials Subcommittee,[72] with minimal deviations from the protocol.

3.2.1.Pre-trial observation period

Inherent to the special characteristics of migraine, a special pre-trial observation period is necessary to assess the key baseline characteristics of the condition. In this trial, this is accomplished by a **3-month retrospective history** and **1-month prospective baseline** period.

A detailed assessment of a 3-month retrospective history provides some assurance on the stability of migraine frequency prior to enrolment, but this should be always confirmed prospectively in a 1-month baseline (run-in) period.[72] Regularly, this 1-month prospective period provides more accurate data on migraine frequency than the 3-month retrospective period, because it minimizes the recall bias and allows for more precise determination of the mean attack frequency at baseline. In subjects

with no attacks during the 1-month prospective run-in period, the baseline attack frequency must rely on the 3-month retrospective period only.

3.2.2.Blinding

Following the most stringent recommendations of the guidelines,[72], this company-sponsored trial with Acetium® capsules can be conducted in **triple-blind fashion**; i.e., 1) participant-blind, 2) investigator-blind, and 3) sponsor-blind (=statistician evaluating the study results), to exclude the possibility of an undue bias on the results caused by data analysis.

3.2.3.Placebo control

Placebo preparation with design and package identical to the test preparation (100 mg Acetium® capsule) will be used in this trial, administered to the other study arm of randomly allocated subjects.

3.2.4.Parallel-group design

Based on careful weighting of the advantages and drawbacks between the parallel design and the cross-over design, the current trial will be conducted as a **parallel group** design. Despite the undeniable advantages of the cross-over design in study power issues, its several important drawbacks contributed to the decision in favour of the parallel group design.[72] Importantly, the fluctuating course of migraine poses similar challenges in both these study designs.

3.2.5.Randomization

Because patients are usually (not necessarily apply here) recruited to prophylactic migraine trials over extended periods, it is recommended to randomize in relatively small blocks, because participant selection may vary with time.[72] In this trial, randomization will be performed after the 1-month prospective baseline (run-in) time, using a random number generator (https://www.sealedenvelope.com/simple-randomiser/v1/lists) with **blocks size of 4**, and creating **unique randomization codes** for each subject. The latter will be used as the only identifier of each subject in all datasets. Printed list (CSV Excel) is sealed in an envelope and stored in the company safety box, until opened at the completion of the study and all data analysis.

3.2.6.Stratification

Randomization alone may not ensure full comparability between participants in the two treatment

arms, and stratified randomization is sometimes needed to remedy this potential imbalance between the two arms. Of the baseline migraine characteristics that potentially affect the efficacy outcomes, the prime candidate is the migraine attack frequency. Therefore, it would be justified to use the **frequency of attacks** as the basis for **stratification** to improve the baseline comparability, especially because attack frequency is one of the two principal outcome measures in migraine prevention RCTs. Another option would be to control for the potential confounding by the attack frequency in the primary statistical analyses, by including this variable as a covariate in the multivariate analyses (see Statistical Methods). In the final design, **4 attacks per month** was used as the cut-off for stratification of the subjects into the **low-** and **high** attack frequency groups.

3.2.7.Duration of the treatment period

Migraines are chronic conditions with protracted and fluctuating course, both posing challenges in the statistical modelling of their natural history. In migraine intervention RCTs, the primary efficacy endpoint is the attack frequency, and keeping the treatment periods relatively long is likely to provide **more stable estimates** of the attack frequency.[72] Furthermore, only the **sustained effects** are clinically relevant, and these benefits clearly outweigh the potential risks of dropouts inevitably associated with longer treatment periods.

In this trial, the **treatment period will be 3 months**, followed by a **4-week post-trial observation** period, as recommended in the IHS guidelines.[72]

3.2.8.Symptomatic (acute) treatment

The optimal treatment for acute attacks in migraine prevention RCTs is both ethically and clinically indicated. In this trial, the participants should use and accurately report their usual symptomatic or acute treatment, because not anticipated to interfere with the study medication.

3.2.9.Follow-up visits

Each study subject will be examined by the neurologist twice during the study: i) at baseline visit, and ii) after the treatment period. During the 3-month treatment period, participants will be evaluated **at monthly intervals** by the study coordinator (**a certified GCP monitor**), to review the diaries, monitor adverse events, ensure compliance and promote continued participation in the study. The follow-up will be concluded by the last visit at the end of the 4-week post-trial period.

3.2.10.Compliance

Evidence of poor compliance with migraine prophylactic RCTs is well established, and if poor enough, can be incorrectly interpreted as drug failure.[72] Therefore, it is crucial to monitor patients' compliance with the test preparations during the entire study period. One such approach is the drug or pill count at every follow-up visit, and repeated emphasis on the values of adherence to the protocol requirements. The same applies to proper completion of headache diaries.

Irrespective what measures are taken, it can be anticipated that the number of subjects lost to follow-up, those not completely adherent to the study protocol, as well as those interrupting the intervention for other reasons, will not be negligible in both study arms. It is likely that the final analyses must be run separately for two groups: **1) Per Protocol (PP)**, and 2) **Modified intention-to-treat (mITT).** The former includes all subjects who have been compliant with the study protocol, without any major violations in i) taking the test preparations, and ii) in recording their headache diaries. The latter category includes all subjects who were not fully compliant with the protocol, but who completed all the follow-up visits and provided (at least) reasonable records of their diaries.

4.METHODS

4.1.Baseline data

Before enrolment in the cohort, all subjects are requested to sign **a written consent**, after having been explained the details of the study and the commitment requested from each subject for the successful completion of their 5-month study protocol: 1-month run-in period, 3-month test period, 1-month post-trial period.

Before initiation of the 1-month prospective (run-in) baseline period, each subject will be requested to fill in a structured **Questionnaire** recoding their detailed migraine history, including the on-going medications (**ANNEX 1).** This Questionnaire also records the migraine attack frequency during the **3-month retrospective** period, immediately preceding the enrolment in the cohort, to be used as the baseline data in case that the 1-month run-in period does not yield any attacks.

4.2.Headache diary

The headache diary (**ANNEX 2**) is the main research tool used to monitor the efficacy of the test preparations on the natural history of the migraine on daily basis throughout the entire study period.

Headache diary is also used to record the migraine attacks during the 1-month prospective run-in (baseline) period before randomization into the study arms.

In the IHS Guidelines, there are no specific recommendations about the design of such a headache diary, other than it should be an **easy-to-use**, either a paper-and-pencil form or an electronic diary that captures all predefined assessment measures (efficacy, tolerability and safety).[72]

These diaries are submitted to the study monitor on monthly basis, so as to confirm the compliance of each subject with the study protocol as well as to record the efficacy endpoints, tolerability and safety of the test preparations.

4.3.Study endpoints

The single most important aim of this study is to establish whether Acetium® capsule is an effective measure in decreasing the frequency (or totally aborting) the headache episodes among patients suffering from migraines, as strongly suggested by the spontaneous testimonials of several individual patients.[68] The null hypothesis of the study implicates that Acetium® capsules are no better than placebo in migraine prophylaxis during the intervention period of 3 months.

4.3.1.Primary endpoints

Rejection or not of the null hypothesis is based on comparison of the two strata (Acetium® and placebo) against **two primary study endpoints** (efficacy measures): 1) **Number of migraine attacks** (NMA) per evaluation interval (1 month), and 2) **Number of migraine days** (NMD) per one month. The frequency of headache attacks or headache days, either during the entire treatment period (3 months) or during the last treatment interval (1 month) is compared with the baseline frequencies, to disclose the differences in efficacy measures between the two arms.

4.3.2.Secondary endpoints

In addition to these primary efficacy endpoints (NMA, NMD), the IHS Guidelines recommend using potential **secondary efficacy endpoints** in analysing the results.[72] Such potentially useful secondary endpoints include: i) Intensity of headache (4-tier nominal scale); ii) Attack duration in hours (potentially biased by treatment); iii) Drug consumption for symptomatic or acute treatment (NMDs treated with abortive agents and the number of drug administrations for acute therapy); iv) Patients'

preferences and satisfaction (a secondary global outcomes); v) Responder rate (proportion of study subjects with >50% improvement in NMA or NMD, as compared to baseline values).

4.4.Statistical analysis

All statistical analyses will be performed using the SPSS 25.0.0.1 for Windows (IBM, NY, USA) and STATA/SE 15.1 software (STATA Corp., Texas, USA). The descriptive statistics will be conducted according to routine procedures. Frequency tables will be analysed using the $\chi 2$-test, with the likelihood ratio (LR) or Fisher's exact test for categorical variables. Differences in the means of continuous variables (e.g. attack frequency) are analysed using non-parametric (Mann-Whitney or Kruskal-Wallis) test for two- and multiple independent samples, respectively.

4.4.1.Conventional techniques

Standard statistics are used to compare the efficacy of the two study arms on migraine attack frequencies. The effects of test preparation and placebo can be analysed separately in non-parametric paired samples t-test (Wilcoxon signed ranks test) to compare the pairs of the baseline- and follow-up attack frequencies in each arm. Another way is to calculate the efficacy measures (reduction of the baseline attack frequencies by treatment) and compare these effects between the two arms. Similarly, the statistics used to compare the two arms with regard to the secondary endpoints is straightforward. For categorical outcomes, conventional regression models can be used, where the results are expressed as crude OR (odds ratio), and their 95% confidence intervals (95% CI).

4.4.2. Life-table techniques

More sophisticated approaches can be used to analyse these longitudinal data, pending on the obtained results. If, e.g. complete and sustained abortions of the attacks are recorded, different life-table techniques can be used to compare the two study arms, using univariate survival (Kaplan-Meier) analysis, where the attack disappearance dates represent the event of interest, and the stratum-specific (test vs. placebo) estimates are calculated using the log-rank (Mantel-Cox) statistics. The same approach can be used to calculate the difference in the duration of attack abortion (i.e., remission time, RT) between the two study arms. The independent effect of Acetium® (adjusted for potential confounders) can be analysed using the multivariate Cox proportional hazards regression

models, where all recorded baseline characteristics of migraine (Questionnaire) can be entered as covariates, including the eventual stratification variable (baseline attack frequency).

4.4.3.Generalised linear models (GEE, panel Poison)

In addition, using the permanent attack disappearance (abortion) as the event, the effect of Acetium versus placebo can be modelled also using the regression techniques based on count variables, i.e., Poisson regression. In this approach, attack disappearances are expressed as **events per person time (months) at risk**, and the two arms can be compared using the **incidence rate ratio** (IRR) statistics. When applied to panel type of data recorded at each FU visit (panel Poisson), all covariates subject to random intra-subject variation (by FU visits) can be adequately controlled, in this longitudinal setting. A similar type of approach based on panel data, i.e., generalized estimating equation (GEE) modelling, can be used to estimate the effect of Acetium/Placebo on persistence (=sustainability) of attack remission (AR), using the AR (yes/no) recorded at each FU visit as the dependent variable, and adjusted for potential confounders in multivariate GEE. A wide variety of such potential confounders should be examined, including e.g. age, gender, migraine type, migraine onset, migraine severity, attack frequency, triggers like alcohol and cigarette smoking, previous interventions, current medication, etc.

4.4.4.Modelling of migraine outcomes by competing-risks regression

Migraine prevention trial is more complex than merely showing a reduction in attack frequency vs. no such reduction, as dichotomous (yes/no) outcome. Indeed, if carefully modeled, it can be anticipated that there are several possible outcomes to be observed during the Acetium intervention, which can be treated as **competing events.** These include the following: **i) no effect** (=NMA and/or NMD remain unchanged as compared with the baseline), **ii) abortion** of NMA/NMD (=disappearance of all headache episodes since the onset of intervention**), iii) relapse** (=disappearance of NMA/NMD for a period of time but subsequent reappearance of the attacks), and **iv) reduction** of attacks (=both NMA and NMD are reduced at study endpoint, but not entirely aborted).

If the i) the longitudinal data be utilized in full, ii) intra-subject variation of the repeated measurements at FU visits be taken into account, and iii) the multiple-outcome dependent variable (no effect, abortion, relapse, reduction) be treated in a single statistical model, we need to apply a

special technique, known as **competing-risks regression**,[73,74] This elegant technique can be used to model the impact of Acetium intervention on the competing risks outcomes of this trial, adjusted for other covariates as potential confounders. The major advantage to conventional (Cox) models is that while usual censoring (exclusion) from the study in Cox prevents you from observing the event of interest, a competing event prevents the occurrence of the event of interest. In simple terms, competing-risks regression generates hazard for the events of interest, while simultaneously keeping the subjects who experience competing events still "at risk" so that they can be adequately counted and have no chance of failing.

4.5.Power analysis

Due to the fact that several optional tools are available for statistical analysis of these data, also the power of the study can (and needs to) be analysed differently, following the algorithms specified for each of these statistical techniques. In the simplest approach (for attack frequency or the effect size, i.e., reduction by treatment), the power can be calculated using the two-sample mean test, comparing mean attack rates (or reduction) in the Acetium® and placebo arms. The study (n=100 per study arm) is adequately powered (Type II error 0.80, type I error 0.05) to detect a true difference in attack frequency between **4 attacks/month** in the placebo and **2.4 attacks/month** in the Acetium arm, i.e., the difference in effect size of **1.6 attacks**. Within this range, the study power is sensitive to any decrease in this difference and also critically dependent on the SD in the two arms. Given that the study subjects are selected among patients with 2-8 monthly attacks, there figures seem reasonable estimates for the basis of these power calculations.

Using univariate survival (Kaplan-Meier) analysis for AD (attack disappearance), with log-rank test for comparing the study arms, this sample size (n=200) is adequately powered to detect the true difference between the arms with HR=0.668, in other words a 33.2% difference in AD between Acetium and placebo arms. This power, however, is sensitive to censoring. For the analysis by Cox proportional hazards regression, the study power estimates are the same, and also here, the power is sensitive to censoring. Using the Poisson regression for count outcomes (AD events/person time at risk), the cohort of 200 subjects is also adequately powered (α=0.05; β=0.80) down to IRR 1.3 between the Acetium and placebo study arms.

5.STUDY EXECUTION AND TIME-TABLE

For execution of the study, the company decided to build up a multi-centre consortium with specialist neurologists working in six medical centres in Finland, supervised by two internationally recognized experts in the field of vascular headaches (prof. X.Y. and Z.X.). The execution of the study is monitored following the principles of good clinical practice (GCP) by a certified GCP monitor. For this purpose, the company recently hired a project coordinator, who (in addition to study monitoring) will also assist the investigators in all practical steps during the study execution.

The study protocol will be subjected for approval by the institutional review board (IRB). Meanwhile the final protocol is under evaluation for ethical approval by IRB, preparatory measures will be taken by informing about the planned study and asking their co-operation in encouraging the interested migraine patients to contact the study coordinator. As determined from the preliminary enthusiasm in this trial, the required cohort of volunteers can be enrolled within a reasonably short time. Given that each study subject shall complete the 3-month trial period, preceded by 1-month run-in time and 1-month post-trial surveillance, we expect that the study can be completed during a period of 6-12 months, pending on the cohort size of course.

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ANNEX 1. **MIGRAINE HISTORY RECORD**

Date of Interview:	Day:	Month:	Year:	
Name:				
Date of Birth:	Day:	Month:	Year:	AGE:
Gender:	Female:		Male:	
Profession:				
Chronic co-morbidities	Yes:		No:	
If yes, list the most important ones:				
Migraine diagnosis made:	Year:		Age at onset:	
The exact diagnosis (ICD-10 code):	G43.0: Migraine without aura		G43.1: Migraine with aura	
	G43.2: Status migrainosus		G43.3: Complicated migraine	
	G43.8: Other migraine		G43.9: Mgraine, unspecified	
Symptoms of migraine (typical attack):	Prodrome (Y/N):		Aura (Y/N):	
	Pain (Y/N):		Postdrome (Y/N):	
Prodrome:	Frequency (%):	Timing (h bef):	Main symptom:	
Aura:	Visual:	Sensory:	Motor:	
	Frequency (%)	Duration (m):	Main symptom:	
Pain:	Unilateral:	Bilateral:	Severity (Mi/Mo/S):	
	Duration (h):	Aggravated by:	Ass. symptom:	
Postdrome:	Duration:		Main symptoms:	
Causes and Triggers:	Genetic background (Y/N, details):			
Physiological issues (describe)				
Dietary aspects (describe)				
Environmental factors (describe)				
Other suspected triggers (list)				
Frequency of attacks (current):	No./Day:	No./Week:	No./Month:	More rarely:
Trend since the migraine diagnosis	Constant:	Increased:	Decreased:	
	Frequency of attacks during the past 3 months (IMPORTANT)			
	No./Day:	No./Week:	No./Month:	No attacks:
Preventive measures ever tested:	YES:		NO:	
	Surgery:		Acupuncture:	
	Behavioral therapy:		Gluten-free diet:	
	Herbal/nutritional supplements:		Manual therapy:	
Medical devices tested:	Neurostimulation:		TMS:	
	Biofeedback:		Hyperbaric Oxygen:	
Preventive medical treatment:	YES:		NO:	
	Beta blockers:		Anti-convulsants:	
	Anti-depressants:		Other:	
	List your current preventive medicines:			
	1	2	3	
Current treatment of acute attacks:	List the medicines:			
	1	2	3	
	4	5	6	
Your self-estimation of your migraine:	Under good control:		Not in satisfactory control:	
	Improved during the years:		Aggravated during the years:	
	Debilitates my daily life:		I can live with it:	
SOME OF YOUR LIFE-STYLE PRACTICES				
Alcohol consumption:				
Regularity and type	No:	Social:	Daily:	Excessive:
Type of alcohol typically used	Beer:	Wine:	Liquors:	Spirits:	Other:
Weekly use	Estimated dosages per week:			
Alcohol intake triggers your migraine?	NO:		YES:	
Did you stop drinking due to migraine?	NO:		YES:	

Smoking history:			
	Never:	Past:	Current:
Age when started smoking			
Regular smoker ever since	Yes:	No:	
If not, describe			
Cigarettes per day (currently)			
The same number, for how long (yrs)?			
Trend in the daily numbers of cigarettes	Constant:	Increasing:	Decreasing:
Other forms of tobacco	Cigars:	Pipe:	Smokeless:
If any of the above, list the amounts			
Smoking triggers your migraine?	NO:	YES:	
Did you stop smoking due to migraine?	NO:	YES:	
SPACE FOR FREE COMMENTS:			

ANNEX 2. **HEADACHE DIARY FOR MIGRAINE PATIENTS**

MONTH:	**SUBJECT CODE:**

Day	**Attack Y/N**	**Time of Onset (h/m)**	**Time at Stop (h/m)**	**Intensity* of headache** (0-3)	**Acute Medicine (=AM) (Y/N)**	**How many pills of AM?**	**Your satisfaction on the test drug#** (1-5)	**Test drug taken; how many?**	**Side effects ascribed to the test drug:** (see footnote for coding)		
									ES (1-3)	**ESR** (1,0)	**D** (h)
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
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31											

RESEARCH PERSONNEL FILLS OUT:

OVERALL EVALUATION OF THE MIGRAINE MONTH

Days	No. attacks	Total Duration	Average Intensity	AM Used (Y/N)	No. AM Used	Average Score	No. Test Drugs	No. of Side Effects	Total D

*__Before taking symptomatic medicine__; (0=no headache; 1=mild; 2=moderate, 3=severe); **#Satisfaction**: (1=very dissatisfied; 2=somewhat dissatisfied; 3=neither satisfied nor dissatisfied; 4=somewhat satisfied; 5=very satisfied); **SIDE EFFECTS**: 1. Event severity **(=ES)**: (1=mild, 2=moderate, 3=severe); 2. Event seriousness (=**ESR**): (1=serious, 0=non-serious); 3. Duration **(=D)**: (hours).