Study Protocol

OPIOID INDUCED GAIT ATAXIA

Danish title: *Måling af opioid-induceret gangataksi*
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Study registration

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1) Specification of when the collected biological material will be destroyed. 2) Addition of the used questionnaires as appendices. 3) Specification of which pamphlets that are provided to potential participants in relation to oral and written information. 4) A Danish translation of the Act on Processing of Personal Data. 5) A statement that any further financial support of the study will be disclosed to the participants and that the Health Research Ethics Committee will be informed of this.
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Roles and responsibilities

MH conceived of the study. HB is the grant holders. RC provides statistical expertise in clinical study design and RC is conducting the primary statistical analysis. All authors contributed to refinement of the study protocol and approved the current version.

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1 BACKGROUND

Musculoskeletal pain is extremely common and the leading cause of physical disability. Opioids are potent analgesics that may be used to treat musculoskeletal pain, such as knee osteoarthritis (1). Several opioid analgesics are available and are increasingly used for the treatment of pain because, in contrast to NSAIDs, opioids do not produce gastrointestinal bleeding or renal problems. This makes opioids an attractive option.

Disequilibrium and gait disturbance are the most common causes of falls, which are becoming a serious social problem for the increasing geriatric population. Important side effects to opioid usage are dizziness and gait ataxia (2) which may cause loss of limb coordination and unsteady gait that predispose to falls. In the elderly, fall related fractures are considered a serious risk factor for increased disability and approximately 20% die within the first 3-6 months after a hip fracture (3). Because balance deficits are not uncommon among musculoskeletal pain patients (3;4), gait ataxia and increased risk of falling is a particular safety issue in knee osteoarthritis. However, no studies have assessed opioid induced gait ataxia in humans.

While the analgesic effects of opioids are well known, empirical clinical evidence suggest that there are differences in the dizziness and gait ataxia profiles of the different opioid types. However, this has not been investigated scientifically under controlled conditions, in part due to lack of sensitive methods to discriminate between levels of ataxia. Three-dimensional biomechanical movement analysis is a sensitive method to assess gait ataxia, as excess variability in gait parameters, however it is unknown if this method can discriminate between the gait ataxia induced by different opioid types.

This study is designed as an experimental, randomized, double-blind, cross-over study of the differences in pharmacologically induced gait ataxia between two different opioid formulations and placebo in healthy volunteers and patients with knee osteoarthritis.

1.1 Investigational Agents

This study includes 2 investigational agents, *Tapentadol* and *Tramadolhydrochlorid*, and 1 inert placebo.

Tapentadol is a centrally acting analgesic acting as a μ-opioid receptor agonist and as a norepinephrine reuptake inhibitor (5). The analgesic effect of Tapentadol is direct without conversion to a pharmacologically active metabolite.

Tramadolhydrochlorid is a centrally acting analgesic acting as a μ-opioid receptor agonist, induction of serotonin release, and inhibition of norepinephrine reuptake (6;7). The analgesic effect of Tramadolhydrochlorid is almost exclusively mediated through the pharmacologically active metabolite O-desmethyltramadol, a potent μ-opioid receptor agonist.

1.2 Overview of relevant data

There are no studies evaluating the effects of opioid induced gait ataxia.

The vast majority of knowledge is from clinical studies of safety, in which frequencies of side effects are reported. In a recent meta-analysis on efficacy and safety of opioids in osteoarthritis self-reported dizziness were among the most common side effects (2).

One study has shown a significant relationship between cognitive and motor function decrements and plasma opioid concentration (8). Another study has shown that there seems to be an inhibitory effect of opioids on motor neurons (9).

1.3 Risk/Benefits

The analgesic effects of opioids are well known, and opioids are among the world's oldest known drugs for pain relief.
The most common side effects in patients taking opioids for pain relief include nausea, constipation, dizziness, somnolence, and vomiting. Infrequent adverse reactions in patients taking opioids for pain relief include: dose-related respiratory depression, confusion, hallucinations, delirium, urticaria, hypothermia, bradycardia/tachycardia, orthostatic hypotension, dizziness, headache, urinary retention, ureteric or biliary spasm, muscle rigidity, myoclonus (with high doses), and flushing. Risk of opioid dependencies with one opioid dosage (Tapentadol and Tramadolhydrochorid) is estimated not to exist.

1.4 Dose Rationale

This study includes 2 investigational agents; *Tapentadol* and *Tramadolhydrochlorid,* and 1 inert placebo. Both active agents are given as extended release formulations as tablets.
Tapentadol is administered in a 50 mg formulation (Palexia Depot, Grünenthal).
Tramadolhydrochlorid is administered in a 100 mg formulation (Mandolgin Retard, Sandoz).
These dosages are chosen because they are considered equianalgesic.
All participants are given both investigational agents and the inert placebo. One tablet is administered per study visit. The order of tablets is decided through randomization. The study visits are separated by at least 7 days.

1.5 Population

When investigating the potential differences in dizziness and gait ataxia between different opioid types in a population that has pain it is difficult to discern the effects of pain relief from central nervous system side effects, because pain relief in itself may affect gait variability in a way that may mask gait ataxia. Therefore, a healthy control group without pain is included because dizziness and gait ataxia in this population with confidence can be attributed to side effects on motor function in the central nervous system (gait ataxia). This information can be used to distinguish between increased gait variability caused by pain relief from centrally derived gait ataxia in the patient population.

2 STUDY OBJECTIVES

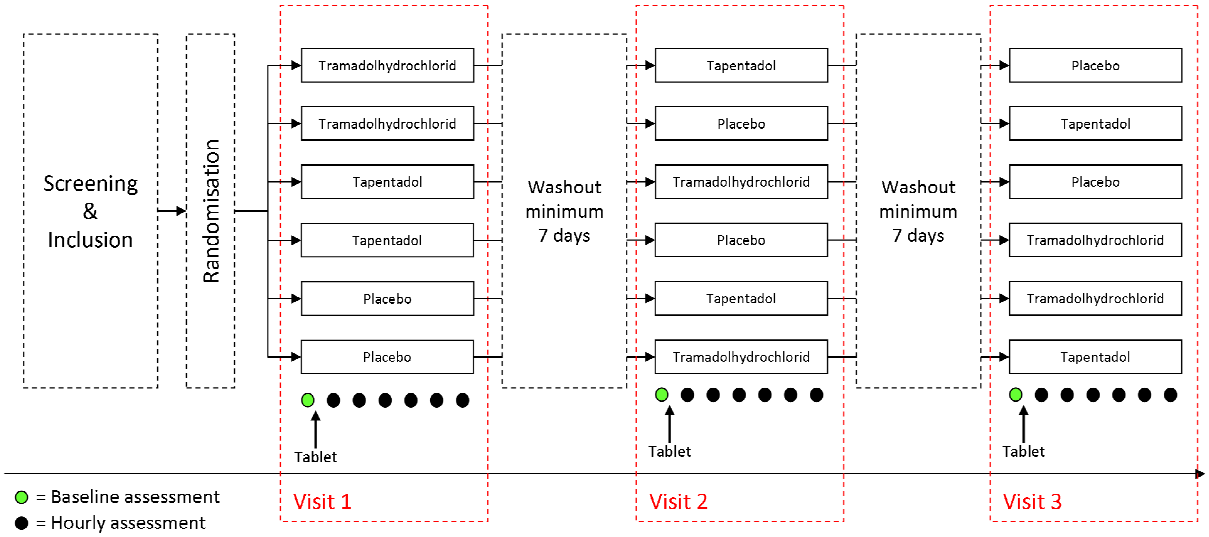
The purpose of this study is to investigate if differences in gait ataxia induced by two different single-dose opioid formulations and inert placebo can be detected by 3D gait analysis in healthy volunteers and knee osteoarthritis patients.

3 STUDY DESIGN

The study is designed as an experimental single center, double-blind treatment, cross-over study with placebo, Palexia Depot, and Mandolgin Retard, with a minimum of 7days wash-out periods. All participants are required to attend to 3 full day examinations at The Clinical Motor Function Laboratory at The Parker Institute, Department of Rheumatology, Copenhagen University Hospital - Frederiksberg.

3.1 Study Design/Type

The study is designed as an experimental single center, double-blind treatment, cross-over study with inert *placebo*, *Tapentadol* (Palexia Depot), and *Tramadolhydrochlorid* (Mandolgin Retard), with a minimum of 7days wash-out periods.
At day 1 (Visit 1), baseline measurements are carried out before tablet administration. Immediately after baseline measurements one tablet is administered, and hourly measurements are performed for 6 hours. Subsequently all participants enter a minimum 7 day washout period, after which they return to the facility to repeat the procedures at Visit 2 (day 8+) and Visit 3 (day 15+). The order of treatments will be randomized (1:1:1). The design is illustrated below:



3.2 Primary Study Endpoints/Secondary Endpoints

Primary outcome:

- Change from the post-washout baseline in gait variability over time

Secondary outcomes:

- Change from the post-washout baseline in autonomous nerve system assessments over time
- Change from the post-washout baseline in plasma concentrations of opioid metabolites over time
- Change from the post-washout baseline in participant assessment of dizziness, nausea, and other adverse effects over time
- Change from the post-washout baseline in knee pain (patients only) using 100 mm Visual Analogue Scale (VAS) over time

3.3 Randomization

The biostatistician is responsible for preparation of the randomized group allocation list before initiation of the study. Two randomization lists are developed; one for the OA patients and one for the healthy volunteers. The same randomization technique is used for both lists:

Each participant is randomized to experimental group ABC, ACB, BCA, BAC, CBA, or CAB. ABC, ACB, BCA, BAC, CBA, or CAB denotes the order of the investigational products and placebo.
Generation of the code defining the actual denotation of A, B, and C is pending, but to illustrate the allocation generation, A could denote *Tramadolhydrochlorid,* B could denote *Tapentadol*, and C could denote *Placebo*.
In such allocation code, ABC denotes *Tramadolhydrochlorid* at the first visit, *Tapentadol* at the second visit, and *Placebo* at the third visit. ACB denotes *Tramadolhydrochlorid* at the first visit, *Placebo* at the second visit, and *Tapentadol* at the third visit. BCA denotes *Tapentadol* at the first visit, *Placebo* at the second visit, and *Tramadolhydrochlorid* at the third visit. BAC denotes *Tapentadol* at the first visit, *Tramadolhydrochlorid* at the second visit, and *Placebo* at the third visit. CBA denotes *Placebo* at the first visit, *Tapentadol* at the second visit, and *Tramadolhydrochlorid* at the third visit. CAB denotes *Placebo* at the first visit, *Tramadolhydrochlorid* at the second visit, and *Tapentadol* at the third visit.

The randomization will be 1:1:1 meaning that 4 participants (2 healthy volunteers and 2 patients) are randomly assigned to each order of agents. To account for drop-outs during the study, the randomization list will have 20 randomizations for both OA patients and healthy controls (yielding a total of 40 randomizations).
A coded randomization list will be available to the clinical staff administering the investigational products.
After obtaining signed informed consent form the participant, baseline measurements will be completed, and the clinical staff will administer the experimental product according to the coded randomization list.
Participants, scientific, clinical technical and assistive staff and other persons directly involved in clinical and preclinical examinations, measurements, and data analyses will be blinded to the group allocation.
Information that potentially can unblind study staff and/or participants will not be shared and will be stored under lock and with limited access, until the database of the study is locked.

3.4 Maintenance of the randomization

Prior to the study initiation, two folders (one for the healthy volunteers and one for the knee OA patients) each containing 20 envelopes are created. Each envelope will contain a piece of paper with the randomization code written on it representing the order of tablet administration but in a coded form (e.g. “123”, “132”, “231”, “213”,“312”, and “321”, corresponding to the order of products A, B, and C (see 3.3)). The order of the envelope content matches the randomization list. It is ensured that the envelopes are closed and opaque. The envelopes are numbered consecutively from 1-20 and placed in the folder according to the number (no. 1 in front – no. 20 in the back). The envelope numbers match the randomization numbers. The folders are stored in a locked locker in the clinical motor unction laboratory. Duplicates of the randomization list and envelopes are stored under lock in the research administration of the Parker Institute.
When a participant has completed the baseline assessments at Day 1, the participant is randomized. An envelope is drawn from the folder, starting from the front envelope. On the

front of the envelope, the participant's name, date of birth, screening number, and present date is written.

The investigator can immediately and any time request unblinding due to participant safety. Unblinding by the investigator is done by contacting the research administration of the Parker Institute and requesting a given participant's envelope to be opened in order to reveal the order of agent administration. Once unblinded, a participant cannot continue in the study.

The entire group allocation can be unblinded in the case of suspected unexpected adverse events related to the study.

3.5 Study Treatment

Tapentadol is administered as a single dose in a 50 mg formulation (Palexia Depot, Grünenthal). *Tramadolhydrochlorid slow release* is administered as a single dose in a 100 mg formulation (Mandolgin Retard, Sandoz). *Inert placebo* is administered as inert calcium tablets.
The tablets will be encapsulated to mask the tablets. The encapsulation will be performed at *Sygehusapotek Fyn, Odense Universitetshospital* according to GMP (Good Manufacturing Practice).
The tablets will be packed in triplets with one of each investigational product and one placebo. Each tablet will be packed in a small duma. On each duma, the number "1", "2" or "3" will be printed, to define the order of tablet administration. There will be an equal amount of each investigational product labeled "1", "2", and "3". A triplet of small dumas including one of each investigational products and one placebo will be packed together in a sealed labeled plastic container.
The content of each plastic container corresponds to the randomization list such that the plastic container allocated for a participant randomized to "123" will have product A in the duma labeled "1" to be administered at the first visit, product B in the duma labeled "2" to be administered at the second visit, and product C in the duma labeled "3" to be administered at the third visit. A participant randomized to "132" will have product A in the duma labeled "1" to be administered at the first visit, product C in the duma labeled "2" to be administered at the second visit, and product B in the duma labeled "3" to be administered at the third visit. A participant randomized to "231" will have product B in the duma labeled "1" to be administered at the first visit, product C in the duma labeled "2" to be administered at the second visit, and product A in the duma labeled "3" to be administered at the third visit. A participant randomized to "213" will have product B in the duma labeled "1" to be administered at the first visit, product A in the duma labeled "2" to be administered at the second visit, and product C in the duma labeled "3" to be administered at the third visit. A participant randomized to "312" will have product C in the duma labeled "1" to be administered at the first visit, product A in the duma labeled "2" to be administered at the second visit, and product B in the duma labeled "3" to be administered at the third visit. A participant randomized to "321" will have product C in the duma labeled "1" to be administered at the first visit, product B in the duma labeled "2" to

be administered at the second visit, and product A in the duma labeled "3" to be administered at the third visit.

3.6 Duration

The study is designed as an experimental cross-over study with a minimum 7 days wash-out period. Thus, the participants are expected to participate in the study for at least 15 days, with three days of clinical assessments (Visit 1, 2 & 3).

3.7 Discontinuation

3.7.1 Causes for Individual Subject Discontinuation

Participants may discontinue the study at any time by withdrawal of informed consent. Sponsor and investigator may discontinue a participant. The reason for discontinuation will be recorded on the CRF. Criteria for individual subject discontinuation by the sponsor or investigator include, but are not limited to:

- Any medical condition, including an Adverse Event, that, in the opinion of the Investigator, may jeopardize the subject's safety if he or she continues in the study
- Subject noncompliance with study schedule or procedures

3.7.2 Study discontinuation

Sponsor has the right to terminate this study at any time. Reasons may include the following:

- The incidence or severity of events that compromise the safety of the participants or pose a health risk
- Unsatisfactory subject enrollment

3.8 Product Accountability

The investigator and study personnel will be responsible for accounting for investigational agents (distribution, storage, and records) and following the guidelines for good clinical practice and national regulations.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Recruitment

Healthy volunteers are recruited via advertisements in local newspapers. Patients with knee osteoarthritis are recruited from the Osteoarthritis outpatients clinic at Copenhagen University Hospital Frederiksberg.

4.2 Inclusion Criteria

Healthy subjects:

- In general good health, in the opinion of the Investigator, based on medical history and physical examination.
- Ability to comprehend and a willingness to provide written informed consent
- Age between 50 and 75 years
- No opioid usage 3 months prior to the study
- No musculoskeletal pain requiring medical attention during the previous 3 months
- Willing and able to complete study visits and procedures

- Willing to hold activity, exercise level, and concurrent treatments/therapies generally consistent during the study
- A body mass index (BMI) of ≤30

Patients with knee osteoarthritis

- Diagnosis of knee OA
- Ability to comprehend and a willingness to provide written informed consent
- Age between 50 and 75 years
- No opioid usage 3 months prior to the study
- Knee pain of at least 40 mm on a 0-100 mm visual analog scale (without analgesics) representing the average pain intensity during the previous week
- Willing to discontinue all pain medications 24 hours before and during examination visits
- Willing and able to complete study visits and procedures
- Willing to hold activity, exercise level, and concurrent treatments/therapies generally consistent during the study
- In general good health, in the opinion of the Investigator, based on medical history and physical examination.
- A body mass index (BMI) of ≤30

4.3 Exclusion Criteria

The same exclusion criteria apply for both healthy subjects and patients with knee osteoarthritis:

- Clinical signs of gait ataxia assessed by clinical neurological examination
- Independent or unsteady walking (i.e. dependence on walking device or stumbling gait)
- Counter indications to either of the investigational products (see Summaries of product characteristics; Appendix A and B), including but not restricted to:
    - Allergy towards one or more of the investigational products or their excipient(s).
    - Significant respiratory depression
    - Current or serious asthma
    - Hypercapnia
    - Suspected or diagnosed paralytic ileus
    - Acute intoxication by alcohol, hypnotica, centrally acting analgesics, psychopharmaca or other pharmaceuticals.
    - Renal dysfuntion
    - Hepatic dysfunction
    - Diseases of the biliary tract
    - Acute pancreatitis
    - Usage of monoamine oxidase inhibitors within the last 14 days
    - Galactose intolerance
    - Lactase deficiency
    - Glucose/galactose malabsorption
    - Epilepsy
- Previous usage of opioids without pain reliving effect.

- Patients who have a documented history of an allergic reaction or a clinically significant intolerance to opioids
- Malignant pain
- Excessive joint laxity in the lower extremities indicative of functional ligamentous deficiency.
- Dependency of walking aid (stick, cane, roller etc.).
- Positive Clock Drawing Test
- Abuse of alcohol, medicine and narcotics within past 5 years.
- History of symptoms of autoimmune disorders
- Diabetes
- Pregnancy or breast feeding
- History, diagnosis, or signs and symptoms of clinically significant neurological disease
- History, diagnosis, signs or symptoms of any clinically significant psychiatric disorder

4.4 Subject Withdrawal and Replacement

In case of events during the clinical visits and measurements that preclude complete records, subjects are invited to re-enter the study at a later time. In such case the participant will be re-randomized.

4.5 Concomitant medication and therapies

Between visits Acetaminophen/Paracetamol (500 mg) to a maximum of 8 tablets per day is allowed. Participants must not take this medication for at least 24 hours before any assessment visit.
Concomitant non-pharmacological therapies (e.g. physiotherapy, acupuncture etc.) are allowed, but not for at least 24 hours before any assessment visit.
Concomitant pharmacological therapies for other conditions than musculoskeletal pain are allowed.

4.6 Monitoring for subject compliance

The administration of the investigational product is performed in the laboratory overlooked by the clinical staff.

5 OUTCOME ASSESSMENTS

5.1 Gait ataxia (primary outcome):

Gait ataxia is assessed using biomechanical gait analyses. 3-D kinematic gait data is obtained using reflective markers, arranged in the Plug-in-Gait configuration and a 6-camera Motion Analysis System sampling at 100 Hz. Ground reaction forces and moments are obtained with two 6-channel force platforms operating at 1500 Hz. The participants walk a distance of 10m 25 times at self selected comfortable speed, while kinematic and kinetic data is recorded. 25 series of gait cycles will be recorded.
From the gait analyses gait ataxia is quantified as step-to-step variability. Ataxia is defined as increased (or excess) variability during normal walking.

The analyses of gait ataxia are done at The Parker Institute, Copenhagen University Hospital at Frederiksberg.

5.2 Autonomous nerve system (secondary outcome):

The autonomic nervous control of heart rate and blood pressure will be analyzed through the use of quantitative test of maximal capacity as well as of ambient activity. Blood pressure and interbeat intervals will be recorded continuously on a beat by beat basis by CNAP-monitor (photoplethysmography) and by one standard precordial lead in the ECG. The maximal capacity will be quantified from the Valsalva manoeuvre primarily using the blood pressure response during phase 2 as a measure of sympathetic activity. Further quantification will be made by deep breathing test analyzing heart rate changes as a measure of parasympathetic function. The ambient activity will be measured from spontaneous fluctuations in heart rate through the use of both time and frequency analysis as well as by deterministic methods.
The analyses of autonomous nerve system are done at Syncope Center, Copenhagen University Hospital at Frederiksberg.

5.3 Plasma concentrations of opioid metabolites (secondary outcomes):

Blood samples are drawn from the cubital region every hour for 7 hours (7 samples) during each study visit. 10 ml is drawn at each time point (70 ml each visit, total 210 ml throughout the study). For storage of the samples a research bio-bank is established. The purpose of the bio-bank is to store the biological material safely and controlled until batch analyses can be performed. Once analyses have been completed, the material will be destroyed. The material is stored for a maximum 5 years and is subsequently destroyed – irrespective of whether analyses have been completed or not.
The analyses of plasma concentrations of active ingredients and metabolites are done at The Faculty of Pharma, University of Copenhagen.

5.4 Patient reported dizziness (secondary outcome; appendix F):

Current self reported dizziness is measured every hour for 7 hours (7 samples) during each study visit. Dizziness is measured using a Visual Analog Scale (VAS) ranging from 0–100 (0=no dizziness; 100=worst imaginable dizziness) with 101 point resolution on a validated electronic touch screen.

5.5 Knee pain (secondary outcome; appendix G):

Current knee pain is measured every hour for 7 hours (7 samples) during each study visit. The VAS for measuring pain intensity is an acceptable measure used in many chronic pain trials. This study will use a VAS ranging from 0–100 (0=no pain; 100=worst imaginable pain) with 101 point resolution on a validated electronic touch screen.

6 SAFETY

It is not expected that participation in the study will lead to significant risks or complications for the participants. The study drugs are approved in Denmark and are often used as pharmacological pain therapy. Risk of opioid dependencies with one opioid dosage (*Tapentadol* and *Tramadolhydrochorid*) is estimated not to exist.

All assessments are performed by qualified staff with relevant education and training.
Transportation to and from the research facility to the participants' home will be arranged, because the investigational agents are associated with impaired ability to drive a car (cf Summaries of Product Characteristics). Also, the participants are required to have an escort on their way from the research facility and not to be alone during the afternoon, evening and night following an assessment visit.
It is not expected that safety issues will occur that will lead to suspension or termination of the study.

6.1 Adverse Events (AE)

An AE is any untoward medical occurrence (e.g., sign, symptom, disease, syndrome, intercurrent illness) that occurs in a study subject following exposure to the study drugs, regardless of the suspected cause. Only subjects who have been exposed to a study drug can experience AEs. Untoward experiences occurring prior to first study drug administration (day 1) are part of past medical history.
Because this is an experimental study with only one exposure to each study drug no adverse events other than the outcomes mentioned in this protocol (gait ataxia and self reported dizziness) will be recorded.
The study drugs have been thoroughly tested for safety and adverse events and it is assumed that the adverse events profiles of the study drugs are well-described.
Only event categorized as serious adverse reactions (SAR), serious adverse events (SAE) or unexpected serious suspected adverse reactions (SUSAR) will be recorded.
According to Good Clinical Practice guidelines the following definitions are applied:

SAE (serious adverse event): A SAE is any untoward medical occurrence that at any dose

- Results in death,
- Is life-threatening
- Requires inpatient hospitalization
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect, or
- Requires intervention to prevent permanent impairment or damage

SAR (serious adverse reaction):

- The event must be a SAE.
- There must be a certain degree of probability that the event is an adverse reaction to the administered drug.

SUSAR (suspected unexpected serious adverse reaction):

- The event must be a SAE.
- There must be a certain degree of probability that the event is an adverse reaction to the administered drug.
- The adverse reaction must be unexpected, i.e. not foreseen in the Summaries of Product Characteristics.

6.2 Adverse Event Reporting

The occurrence of any SUSAR leads to immediate reporting (as fast as possible – not later than 7 days (fatal or life-threatening SUSARs) and not later than 15 days (other SUSARs) after the sponsors has been informed) to the Danish Health and Medicines Authority using the appropriate electronic reporting system. SUSARs are also reported annually and at study termination to the Danish Health and Medicines Authority and the Health Research Ethics Committee.

	Danish Health and Medicines Authority		Health Research Ethics Committee	
SUSAR	**Immediately**	**Yes***	Immediately	No
	At study termination	**Yes**	**At study termination**	**Yes**
SAR	Immediately	No	Immediately	No
	At study termination	**Yes**	**At study termination**	**Yes**
SAE	Immediately	No	Immediately	No
	At study termination	**Yes**	**At study termination**	**Yes**

* As fast as possible – not later than 7 days (fatal or life-threatening SUSARs) and not later than 15 days (other SUSARs) after the sponsors has been informed.

6.3 Contraception

Female subjects of child-bearing potential and spouses or partners of male subjects of child-bearing potential must use contraception throughout study participation. Appropriate contraceptive methods include hormonal contraceptives (oral, injected, implanted or transdermal), tubal ligation, intrauterine device, vasectomy, or double barrier methods. Abstinence is an acceptable form of birth control, though appropriate contraception must be used if the subject becomes sexually active. Sterile male partner or use of double barrier (condom in combination with diaphragm) is acceptable if usage is ensured. At the information interview potential participants are informed that usage of either of the investigational products is not recommended during pregnancy. Sterile or infertile females are not required to use contraception. To be considered sterile or infertile the female must generally be surgically sterilized (vasectomy/bilateral tubectomy, hysterectomy or bilateral ovarectomy) or be postmenopausal, defined as absence of menstruation at least 12 months before study enrolment.
Whether a potential female participant is fertile and whether she is sexually active is assessed by a medical doctor based on an interview at the screening visit. It is emphasized to the participant that in case the fertility status or sexual activity changes from being not present to present during the study, the participant must inform the investigator of this, in order to make appropriate consideration about contraceptives.

7 STATISTICAL PLAN

7.1 Statistical Methods

All data analyses will be carried out according to this prespecified analysis plan. Analyses are done applying SAS software (v. 9.2; SAS Institute Inc., Cary, NC, USA). The repeated measures of changes from the wash-out baseline assessment (hour 0 at each visit) are analyzed using a Repeated-Measures Analysis of Covariance (via PROC MIXED): a factor for

drug (Tapentadol, Tramadolhydrochlorid, and Placebo), a factor for time (hour; 6 levels), group (healthy controls and patients) drug-by-time, drug-by-group, group-by-time, and drug-by-time-by-group interactions, with the daily baseline values as covariates. Participants will be handled as a random factor.
In case of non-normal distribution, data transformation may be necessary.
Estimated least-squares mean effects over each individual Time point (Hours 1-6) will be reported for each drug and group. All estimated means will be presented with standard errors and 95% confidence intervals. For the differences between drugs, significance tests (p-values) will be shown.
Exploratory analyses are done with demographic variables and the secondary outcomes (plasma concentration of opioid metabolites, autonomous nerve system, pain and self-reported dizziness) as covariates, to assess the effects of these on the primary outcome.

7.2 Sample size considerations
No previous studies exist on the effects of opioids on gait ataxia, so it is not possible to make sample size estimations based on previous data from similar studies. However, one study investigating changes in gait variability using the same methodology have shown significant increases in gait variability during dual task walking (walking and talking) on a sample of elderly (age above 70 years) with intact cognition. Gait variability increased by 0.05 (arbitrary unit) during dual task walking (P=0.03). Extrapolating these results to the present study, a sample size of 24 will make it possible to detect a mean difference of 0.03 arbitrary units (at the P=0.05 level) with a power of 80.4%
Because it is assumed that the gait ataxia induced by opioids will be significantly more pronounced than what is induced by dual task walking (walking while talking), and due to practical considerations a total of 24 participants (12 healthy volunteers and 12 patients) that complete the protocol (defining the per-protocol population) will be included in the analyses.

7.3 Subject Population(s) for Analysis
The primary analyses will be based on the per-protocol (PP) population, defined as subjects that have completed the entire study.

7.4 Significance
Tests of statistical significant difference between the two investigational products for all endpoint analyses will use a significance level of 0.05.

7.5 Termination Criteria
The criterion for termination of the study is when 12 healthy volunteers and 12 patients have completed the protocol.

8 ETHICAL CONSIDERATIONS
This study will be conducted according to Danish and international standards of Good Clinical Practice for all studies. Applicable government regulations and national Committee on Health Research Ethics research policies and procedures will also be followed.

This protocol and any amendments will be submitted to the Committee on Health Research Ethics of the Capitol Region of Denmark for formal approval to conduct the study.
Before any study related procedures are commenced potential participants are informed orally and in writing about the purpose, course and potential risks and benefits of participation in the study. The pamphlet *'Forsøgspersoners Rettigheder i et Sundhedsvidenskabeligt Forskningsprojekt*' is handed out together with the written information.
Potential participants are informed of their rights to withdraw from the study at any time without this impacting on their future assessments and/or treatment at Frederiksberg Hospital, or by any members of the study staff. After the information is distributed, read and understood, the informed consent is given voluntarily by the participant, by signing a consent form before participation can take place.

8.1 Oral information
When a potential participant contacts the study, an appointment is made for delivery of oral information. A detailed description of the procedures for the information interview is attached this protocol as Appendix C.

8.2 Written information
All potential participants for this study will be provided written information describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The written information will be given to the potential participant at the first contact with the study. The written information is attached this protocol as Appendix D.

8.3 Informed consent
Consent to participation in the study is given on the basis of the written and oral information.
An informed consent form has been prepared. The form must be signed and dated by the participants prior to participation in the study. A copy of the form is provided to the participants. The investigator and his designated delegates can receive the signed consent form. Prior to consent, it must be ensured that a potential participant has been given sufficient time to consider his or her participation. Informed consent form is attached this protocol as Appendix E.

8.4 Ethical aspects of the planned interventions
It is not expected that participation in the study will lead to significant risks or complications for the participants. However, some of the known side effects of opioids - including gait ataxia that is the focus of this study – are expected to occur (cf. the Summary of Product Characteristics).
The participants are exposed to a minimum of opioids during the study, and the study purpose and design ensures the participants' safety. Risk of opioid dependencies with one opioid dosage (Tapentadol and Tramadolhydrochorid) is estimated not to exist.

It is not expected that individual participants will gain direct benefits from study participation – except for pain relief in the knee OA patients during two of the three study visits (Visit 1, 2 or 3).
The difference both in affinity and in the internal cell response is related to the analgesic and adverse effects of the individual opioids, in terms of alleviation of pain and side effects, respectively, in the individual patient. The ability to discern gait ataxia related to different opioids will be of significant importance in the description of the safety profiles of the opioids, which in turn may impact clinical decision related to prescription of opioids to patients with chronic pain. Gait ataxia might be used as a surrogate measurement for opioid side effects, for example in respect to influence on patients cognitive impairment. Any discomforts and disadvantages that the participants may experience are considered to outweigh the scientific gain and perspectives this study will yield.

8.5 Ethical aspects of the planned outcome assessments

Gait analyses, Autonomous Nerve System assessments and questionnaires
The methods are all non-invasive and are not associated with any predictable risks. The methods are considered without any ethical problems.
Blood samples
The risk associated with the procedure is very minimal, but includes development of hematoma around the skin perforation site, and in extremely rare cases infection at the same site. The risks are so minimal, that it usually does not require separate information of the participants. No other predictable risks are expected to be associated with blood sample collection. 10 ml is drawn at each time point (70 ml each visit, total 210 ml throughout the study). The methods are considered without any ethical problems.

9 COMPLIANCE WITH LAWS AND REGULATIONS

The Parker Institute will conduct this clinical research study under related legislation and regulations, including the Act on Processing of Personal Data (Danish: lov om behandling af personoplysninger). The Parker Institute will comply with International Conference on Harmonisation (ICH) E6 Good Clinical Practice (GCP): Consolidated Guidance, and other national laws and regulations, as applicable.

10 STUDY CONDUCT

This study will be conducted in compliance with the protocol approved by the Committee on Health Research Ethics of The Capitol Region of Denmark. The Danish Medicines Agency has deemed this study as a “tool trial” and is thus not subject to a notification duty. No deviation from the protocol will be implemented without the prior review and approval of the Committee on Health Research Ethics and The Danish Medicines Agency except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the Committee on Health Research Ethics and The Danish Medicines Agency as soon as possible.

10.1 Notification to the Danish data protection agency

Because the study is carried out at a hospital department, the project is regarded as "public" in accordance with the Data Protection Agency guidance. Therefore the

notification of the study to the Data Protection Agency is handled by the public authority which the hospital department belongs, in this case the Capital Region of Copenhagen. Thus, the study is notified to "Enheden for Informationssikkerhed" under the Capital Region of Copenhagen's IT department, which is responsible for the further notification to the Data Protection Agency.

10.2 Subject confidentiality

Subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.
With the subject's permission, medical information may be shared with his or her personal physician or with other medical personnel responsible for the subject's welfare.
If the data from this study are published, the presentation format will not include names, recognizable photos, personal information or other data which compromises the anonymity of participating subjects.

10.3 Retention of records

Danish regulations require that the records and documents pertaining to this study must be retained by the Investigator for 5 years after the study.
Records to be retained include, but are not limited to CRFs, consent forms, source documentation, laboratory test results, medication inventory records, and regulatory documents.

10.4 Quality assurance

All data will be entered into a study database for analysis and reporting. Any data captured electronically (e.g., biomechanical gait analysis data) will be stored electronically in a separate database according to standard procedures at The Parker Institute. Upon completion of data entry, the databases will be checked to ensure acceptable accuracy and completeness. System backups and record retention for the study data will be consistent with The Parker Institute standard procedures.
Individuals involved in study evaluations will be trained to perform the efficacy evaluations and activity measurements described in the protocol.

10.5 Finance and insurance

This study is initiated by the sponsor, physiotherapist Marius Henriksen PhD and his executive professor Henning Bliddal that is the investigator of this study.
The project is supported financially by

- Mundipharma Research Limited (unrestricted grant of DKK 550.000/ £ 60.000)
- The Oak Foundation (grant for operations costs of The Parker Institute. The amount dedicated for this study is not specified)

, which be disclosed on the written information material.
The sponsor, investigator, and biostatistician have all received study grants, travel grants to participate in scientific meetings, and speaking fees from the financial sponsor (Mundipharma). The investigator, sponsor and biostatistician own no shares, share options, and are not employed by the financial sponsor (Mundipharma). The financial sponsor (Mundipharma) will have no role in the acquisition, analysis, and interpretation of

the data or in the preparation of and decision to publish of the results. Funding of the study is a continuous process. Any future grants provided for this study will be disclosed in the written information material (including grant provider and amount), and the Health Research Ethics of The Capitol Region of Denmark will be notified (including grant provider and amount).

The participants are insured by the Danish Patient Insurance Association.

10.6 Publication plan

According to international law, all results will be published. We will seek to publish the results as scientific articles in international journals with a peer review policy. Publication of the results will be done after the study is terminated and data is analyzed. All outcomes will be published, i.e. both positive, negative, and inconclusive results. The main result will be submitted by the sponsor, with representatives from the participating centers as co-authors. Any spin-off articles (regarding ancillary analyses) will typically be dealt with by the specialists in their field. Other authors are included according to the ICMJE recommendations.

11 LIST OF APPENDICES

Appendix A: Summary of product characteristics; Palexia
Appendix B: Summary of product characteristics; Mandolgin
Appendix C: Procedures regarding oral information (in Danish)
Appendix D: Written information material (in Danish)
Appendix E: Informed Consent Form (in Danish)
Appendix F: Visual Analogue Scale for participant reported dizziness (in Danish)
Appendix G: Visual Analogue Scale for participant reported knee pain (in Danish)

12 REFERENCES

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