English translation of the Danish study protocol dated 2017-04-18.
Translated 2019-07-26.

Effect of single dose of imipramine on urethral and anal sphincter in healthy female subjects measured by Urethral Pressure Reflectometry (UPR) and Anal Acustic Reflectometry (AAR)

Signature of primary investigator

I've read all the pages in this protocol. I agree to conduct the study under this protocol. I confirm that the study will be conducted according to the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines and applicable local law. I will make sure that all subinvestigators and other relevant employees have access to copies of this protocol and ICH GCP guidelines so they can work according to them.

Main Investigator and Sponsor

Signature:

Collaborators

Production, labeling, randomization, blinding and packaging of study drugs
Region Hovedstadens Apotek
Marielundvej 25, 2730 Herlev

Data processing and data analysis
Chief physician, dr. with. Niels Klarskov
Department of Gynecology-Obstetrics Department G, Herlev and Gentofte Hospital

Monitor
GCP unit at Copenhagen University
Hospital Bispebjerg Hospital, Building 51, 3rd floor
Bispebjerg Bakke 23, 2400 Copenhagen NV

Time schedule

Expected first visit for first person: Earliest 2017-04-18.
Expected last visit for the last subject: Latest 2017-12-31.

Contents

Abbreviations

5-HT 5-Hydroxytryptamine or serotonine

AAR Anal Acoustic Reflectometry

ADR Adverse Drug Reactions

AE Adverse Events

CNS Central nervous system

CRF Case Report Form

GCP Good Clinical Practice

ICH International Conference on harmonization

ON Onuf's Nucleus or Onuf’s core

RMSE Residual Mean Squared Error

SAE Serious Adverse Events

SD Standard Deviation

SUI Stress Urinary Incontinence

SUSAR Suspected Unexpected Serious Adverse Reaction

TCA Tricyclic antidepressants

TVT Tension free Vaginal Tape

UOP Urethral Opening Pressure

UP Urethral Pressure Profilometry

UPR Urethral Pressure Reflectometry

Summary

Stress urinary incontinence (SUI) and fecal incontinence are common disorders of the general population. Pharmacological treatment can increase sphincter tone for the external urethral sphincter and thus better the symptoms of SUI or increase the tone of the external anal sphincter and thus better symptoms of feecal incontinence. In Denmark, only one registered medicine exists for the treatment of SUI; duloxetine. There is no approved drug for the treatment of fecal incontinence, which acts on the tone of the internal or external anal sphincter. We want to investigate an alternative medical treatment in the form of imipramine; a tricyclic antidepressant (TCA) with known adverse reactions. Previous studies with imipramine have shown varying degrees of efficacy in patients with SUI. Imipramine has never been studied in relation to fecal incontinence. Reflectometry is a simple, safe and valid way to assess the pressure conditions in the urethra (UPR) and the anal canal (AAR). In total, we will examine 16 healthy female subjects in a double-blind, placebo-controlled, crossover study, in which single-dose imipramine 50 mg or placebo is given. The effect on SUI is assessed by the change in Urethral Opening Pressure (UOP) measured by Urethral Pressure Reflectometry (UPR). UOP has previously been shown to be a good objective measure, which correlates with the subjective effects of drugs on SUI. UPR requires fewer subjects compared to urinary diaries or Urethral Pressure Profilometry (UPP) to find statistically significant differences (greater power). In case of change in UOP of more than 10 cm H_2O compared to placebo, the effect is clinically relevant and there will be a basis for further studies in relation to reduction in urine incontinence episodes, side effects and subjective improvement with imipramine use in patients with SUI. This may, in the long term, it may allow medical treatment with imipramine in patients with SUI who do not tolerate duloxetine. The Anal Acustic Reflectometry (AAR) measurements are exploratory. We will examine the pressure conditions in the anal canal after intake of imipramine 50 mg to assess whether imipramine could have a role in the treatment of stool incontinence, and examine the statistical variation of AAR, so that it will be possible to estimate the required number of subjects in phase 1 studies in which AAR can be used to assess the effect of new drugs against fecal incontinence.

Background

Stress urinary incontinence (SUI):
SUI is frequent with a prevalence of 30 % to 50% with the highest prevalence from 25 to 49 years of age (1). SUI is defined symptomatically as involuntary urine leakage during increased abdominal pressure, e.g. by coughing, sneezing, lifting or other physical activity (2). First-line treatment of SUI is conservative in terms of weight loss, smoking cessation, pelvic floor training and intravaginal devices (pessaries, tampons, etc.). In case of failure of conservative treatment, surgical treatment is offered, and the evidence is solid for mid urethral, suprapubic loosely fixed polypropylene slings (TVT, etc.), which can be performed as outpatient interventions in local analgesia (3).

Medical treatment of SUI:
Drug therapy in patients with pure SUI is generally used only, when surgery is contraindicated, or not desired by the patient (3). In Denmark, only one drug, duloxetine, is registered with the indication "stress urinary incontinence in women". However, the effect is small and, compared to placebo, only a few percent of patients have effect on SUI symptoms (11% versus 8%). Also, the number of incontinence episodes is reduced by only approx. 1 pr. day (4). Side effects are frequent, especially side effects from the central nervous system (CNS) such as nausea, sleep disorders, nervousness, headache, decreased libido and anorgasm (4). Treatment effects and side effects can be assessed within a few weeks and, in the absence of efficacy, treatment should be stopped (4).

Fecal Incontinence:
Fecal incontinence occurs in up to 8% of the general population (5-7). Fecal incontinence is often due to decreased function of the internal or external sphincter either due to trauma or degeneration (8).

Medical treatment of fecal incontinence:
A possible treatment may be to increase the pressure in the anal canal pharmacologically (9). Thus, it has been shown that resting pressure measured by anal manometry is increased by local treatment with methoxamine (potent adrenergic alpha agonist) (10). Unfortunately, a clinical effect on anal incontinence in this topical treatment in a clinical placebo controlled trial (11) has not been demonstrated.

Imipramine in general and side effects:
We want to investigate an alternative medical treatment in the form of the TCA imipramine. Imipramine is approved for the treatment of moderate to severe depression and for enuresis nocturna (urination during sleep) (12). Imipramine is an old preparation and has been on the market in Denmark since 1976 (13). The side effects are known, and the most frequently observed are dry mouth, dizziness, sexual disorders, withdrawal symptoms by abrupt discontinuation, myoclonus, confusion and blurred vision (13).

Imipramine - pharmacodynamics:
Imipramine affects the catecholamine metabolism in the CNS. Imipramine is a potent inhibitor of noradrenaline and serotonin (5 - HT) reuptake by the presynaptic, noradrenergic nerve endings. Imipramine is one of the least sedative TCAs and has only moderate antimuscarinic activity (13).

Imipramine - pharmcokinetics:
Imipramine is rapidly and completely absorbed from the gastrointestinal tract after oral administration, and the plasma concentration peak within 1 hour. Imipramine undergoes first pass metabolism in the liver to the primary, active metabolite, desimipramine via the enzyme CYP2C19. Imipramine is eliminated mainly in the urine and the half-life is 6 to 18 hours - that is to say all imipramine is eliminated after

approx. 4 days if liver and kidney function is normal.

Imipramine and SUI:
Imipramine has previously been used for the treatment of SUI. There is, however, no randomized studies that illustrate the efficacy and side effects (3), but two smaller studies have described a positive effect on the number of urinary incontinence episodes, and the pressure profile in the urethra measured by UPP (14,15). The mode of action of treating SUI is unknown (3). Rat studies have shown that immediately following imipramine administration the threshold for the vesicourethral micturition reflex on CNS level is increased, whereby emptying of the bladder is inhibited. Similarly, cat studies have shown that stimulation of 5-HT receptors modifies spinal reflexes, which are involved in bladder control. Onuf's nucleus (ON), located at the sacral part of the medulla spinalis, contains the motor neurons that control the activity of the external urethral sphincter. ON is affected by both serotonergic and noradrenergic nerve cells. Thus, it is pharmacodynamically plausible that an increased concentration of norepinephrine and serotonine in the synaptic cleft by imipramine administration may affect the bladder and the external utrethral sphincter, so that the bladder emptying is inhibited (16).

Imipramine and Stool Incontinence:
The striated external sphincter of the anal canal, like the urethral sphincter, is innervated by ON (17). Theoretically, TCAs could increase the maximum squeeze pressure and, to a lesser degree, the resting pressure. In an older, uncontrolled study, amitriptyline (another TCA) had clinical effect on stool incontinence (18). In this study, in addition to reduced intestinal peristalsis, increased resting and squeezing pressure in the anal canal was also measured. The squeeze pressure increased from 108 cm H_2O to 123 cm H_2O, which, however, was statistically insignificant. A pressure increase of 15 cmH_2O or more is probably clinically relevant. It has been shown that opening pressures at rest and during squeeze measured with AAR correlate with severity of stool incontinence, while the same does not apply to rest and squeeze pressure measured by conventional manometry (19). It is therefore possible, that AAR can provide a more reliable assessment of new pharmaceuticals for the treatment of fecal incontinence. Variation between the predose measurements and the changes in the placebo period can clarify whether AAR is suitable for phase 1 studies, and can be as a basis for sample size calculations for future studies.

Imipramine dose and time for UPR and AAR:
Imipramine will be administered as a single 50 mg dose, which is the recommended starting dose for treatment with imipramine (12). UPR will be performed 1 hour after tablet intake, corresponding to the time for peak plasma concentration, t_{max} (13). AAR will be performed immediately after UPR.

Objective

We will examine, whether imipramine increases the resistance of the external, urethral sphincter, and hence whether imipramine could have a role in the medical treatment of SUI, where there currently exists only on medication on the Danish market with this indication.
Exploratory, we will investigate if imipramine increases the resistance of the anal canal, and thus if imipramine could have a role in the medical treatment of fecal incontinence. We will investigate the variation in the measurements of AAR, and thus whether AAR can be used for future phase 1 studies with pharmacological treatment of fecal incontinence and allow sample size calculations for such studies.

Hypothesis

The 0-hypothesis is that there is no difference in UOP in healthy subjects before and after single-dose placebo and single-dose imipramine 50 mg. The alternative hypothesis is that imipramine single dose increases UOP with a minimum of 10 cm H_2O over placebo.

Explorative hypotheses for AAR measurements:

1) Anal Acustic Reflectometry can be used to test pharmaceuticals with effect on the internal and external anal sphincter.
2) Imipramine has an effect on the anal opening pressure during squeeze.

Purpose

The aim is investigate whether imipramine has a role in the medical treatment of SUI and / or faecal incontinence and to assess whether AAR in the future can be used for phase 1 trials for pharmaceuticals against fecal incontinence.

Design

The study is a placebo-controlled, double-blind, randomized, crossover study. We recruit 16 healthy female subjects and investigate them on two different trial days preceded by a screening day.
The participants are recruited and called for an information day, where written and oral information is given. Subsequently, there is the possibility of reflection time (up to 7 days) before signing written consent. When the written consent is signed, the participants are summoned for the screening day, when the inclusion criteria and the exclusion criteria are reviewed incl. objective examination by doctor and measurement of resting blood pressure. If inclusion criteria are fulfilled and none of the exclusion criteria are met included the participant and assigned a blinded medication kit. On the same day (if consent is given immediately) or the second day (if time is needed before signing the consent), trial day 1 is performed; see under procedures. There is a wash out period of minimum 7 days between trial day 1 and trial day 2. Five days after trial day 2, the participants are called by to identify and follow-up any Adverse Events (AE).
Production of placebo, packaging, blinding, randomization and labeling is handled by Region Hovedstadens Apotek according to GMP. Region Hovedstadens Apotek produces 16 medicine kits consisting of placebo and imipramine. Medicine kits are randomized by Region Hovedstadens Apotek. Each medicine kit is assigned one randomization number from 1 to 16. Of these 16 medicine kits, 8 will contain placebo on trial day 1 and imipramine on trial day 2, and 8 medicine kits will contain imipramine on trial day 1 and placebo on trial day 2. The contents of the medicine kits are blinded to everyone involved until data collection is completed (unless individual subjects are unblinded based on side effects). When trial participants are included they are assigned a randomization number: First test participant gets number 1, next participant gets number 2 etc. This corresponds to the a randomization number on the medicine kit from which the patient receives trial medication. In addition, 4 extra medicine kits are produced which can be used in the event of a dropout.

Inclusion criteria

- Signed informed consent.
- Healthy.
- Woman.
- Non-smoking.

- Age 18 years to 55 years, both inclusive.
- Normal weight, ie. BMI 18.5 to 30.0 kg / m2. Weight minimum 50 kg.
- Consent not to breastfeed during the study or 1 week after the study.
- Negative pregnancy test on trial day 1 and trial day 2.
- Consent not to get pregnant during the study.
- Consent not to participate in other clinical trials concurrently with this study.

Inclusion criteria

- Known hypersensitivity to imipramine.
- history of significant cardiovascular, gastrointestinal, endocrinologic, haematologic, immunological, metabolic or genitourological disease (incl. surgery after pelvic trauma, urethral hypermobility or prolapse of the organs of the pelvis), pulmonary disease, neurological disease, dermatological disease, psychiatric disease, kidney disease, malignant disease and / or other major diseases assessed by the investigator.
- History of or symptoms of urinary incontinence.
- Treatment of infection at least one week before trial day 1, ie. fever or symptoms of viral infection, bacterial infection (including upper respiratory tract infection) or fungal infection (not cutaneous).
- Clinically significant findings assessed on clinical study on screening day.
- Pregnant.
- Pulse <40 or> 100 beats per minute. Mean systolic blood pressure> 140 mmHg or mean diastolic blood pressure> 90 mmHg (average of 3 measurements on screening day). If blood pressure or heart rate differs from this, an additional 3 measurements can be performed.
- Prescription drugs or over-the-counter or natural remedies at least 2 weeks before trial day 1 and trial day 2. Exclusive paracetamol up to 2 g / day and exclusive contraception.
- Smoking 3 months before trial day 1 and trial day 2.
- Alcohol consumption, ie. > 14 drinks per week within 3 months before the trial day 1 and experimental day 2.
- Substance abuse within 3 months before trial day 1 and trial day 2.
- Any condition that allows the investigator not to include the subject.

Recruitment

Healthy volunteers from previous UPR trials in Zelo Phase-1 unit, which have expressed interest in participating in future UPR studies will be informed about the trial and have the opportunity to contact us for inclusion. If there are not recruited enough subjects this way, we will recruit with advertisement on www.forsøgsperson.dk. All recruitment - and the information - must be approved by the Regional Research Ethics Committee.

Reimbursement

Allowance of DKK 125 per hour and DKK 1000 in nuisance bonus for the UPR measurements and AAR measurements on trial day 1 and trial day 2. Thus, compensation of DKK 250 is granted for information day and screening day (approximately 2 hours), DKK 1375 for trial day 1 (3 hours and 1000 DKK nuisance bonus) and 1375 DKK for trial day 2 (3 hours and 1000 DKK nuisance bonus). A total of DKK 3000 in allowance if the entire trial is carried out. The reimbursement must also be authorized by the Regional Research Ethics Committee.

Procedures

Information day: Subjects receive written and oral information about the trial. The subject is entitled to an assessor. Total approx. 30 minutes duration. The subject is offered reflection time, but if the subject is ready to sign written consent, the next part is done immediately.

Screening day: Written consent is obtained. An objective examination is carried out and the medical history and information about the consumption of medicine incl. herbal remedies and supplements are obtained. Objective examination by doctor. Blood pressure x3 is measured after 5 minutes of rest in a supine position. Total approx. 1 hour duration.

Trial day 1: Pregnancy is excluded by pregnancy test via urine sample. The subject is randomized to one of the 16 prefabricated, blinded medicine kits. The subject's bladder is emptied by sterile disposable catherization and then 150 ml of 9% NaCl are instilled the bladder. UPR is performed with 10 measurements, while the subject is relaxing the pelvic floor. Then 5 measurements while the subject is squeezing the pelvic floor. Measurements in the urethra are followed by measurements in the anal canal, where also 10 measurements are made while the subject is relaxed, and 5 measurements while the subject squeeze in the pelvic floor. The subject consumes two tablets according to the randomization and trial day. This will be either two placebo tablets or two tablets of Imipramin DAK 25 mg. The subject relaxes for 1 hour. Again, the bladder is emptied by sterile disposable catherization and then the bladder is filled with 150 ml of 9% NaCl followed by UPR with 10 measurements, while the subject relaxes the pelvic floor, then 5 measurements while the subject squeezes the pelvic floor. The measurements in the urethra are followed by measurements in the anal canal, where also 10 measurements are made while the subject is relaxed, and 5 measurements while the subject is squeezing the pelvic floor. After that, any AE and ADR are registered. Total approx. 3 hours duration.

Trial day 2 : Minimum 1 week after trial day 1 to ensure elimination of the drug. Procedure is identical to trial day 1. Total approx. 3 hours duration.

A total of 4 days of total approx. 8 hours duration.

Methods

UPR

UPR is a validated method for simultaneously measuring the pressure ratio and the cross-sectional area of the urethra. A very thin and very flexible polyurethane bag is introduced into the urethra. Sound waves measure the cross-sectional area of the urethra while pumping air into the polyurethane bag, thereby providing knowledge of the pressure in the urethra. The opening and closing pressure, opening and closing elasticity and the hysteresis can thus be measured by UPR. UPR is more reproducible, with lower variability (and therefore greater statistical power), compared to UPP (20,21). No risk or complication of the procedure has been demonstrated beyond limited discomfort by catheter insertion and a minor risk of cystitis, which, however, is minimized by sterile technique.

AAR

Anal Acoustic Reflectometry (AAR) is a reproducible method of simultaneously measuring pressure and cross-sectional area in the anal canal (22). It has been shown that opening pressure at rest and during squeeze measured with AAR correlates with severity of fecal incontinence, while the same does not apply to rest and squeeze pressure measured by conventional manometry (19). AAR are performed with the same equipment and catheters as used in UPR. No risk or complications of the study have been demonstrated beyond limited discomfort by having catheters inserted into the anal canal.

Statistics

Power calculation:

The primary endpoint is the difference (*delta*) in average baseline UOP and 1-hour UOP. A clinical relevant *delta* between placebo and imipramine is estimated to be 10 cm H_2O. From previous UPR studies of pharmacological intervention vs. placebo there was a residual mean squared error (RMSE) of 5.4 for measurement of UOP (20) - this corresponds to the standard deviation (SD) of the difference in UOP measurements. This gives Cohen's *d* of 10/5.4 ≈ 1.85. We will investigate 16 subjects to minimize the risk of type II errors. For un-paired t-test, Cohen's *d* = 1.85, α = 0.05 and n = 16 , we have strength = 99% to detect a clinically relevant difference of 10 cm H_2O (23).

Power calculation for AAR:

For a sample size of 16 subjects. A clinically relevant difference of 15 cm H_20 in pinch opening pressure, SD of 21 for squeeze opening pressure (22), alpha of 0.05, gives a power of 80%.

Results presentation

Baseline characteristics (age and weight) of trial participants are summarized with mean and SD.
The results from UPR and AAR are described descriptively with mean and SD. Specifically, within-subject SD for AAR is also described to allow future sample size calculations.
The UOP and opening pressure of the anal sphincter for placebo / imipramine are analyzed according to Grizzle's method for cross-over studies (24), that is with a CROS test: One unpaired, two-sided t-test to assess the effect of the intervention. A p value < 0.05 is deemed statistically significant and an average difference greater than 10 cm H_2O for UPR and 15 cm H_2O for AAR are deemed clinically significantly.

Time schedule

Enrollment begins when the necessary permits are available and continue with inclusion until 16 subjects are examined according to this Protocol.
Expected first trial day for first subject: Earliest 18/4 2017.
Expected last test day for last test subject: No later than 31/12 2017.

Place

The experiment will take place in the Zelo phase 1 unit, entrance 11B at Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 Copenhagen NV.

Practical opportunities

The Zelo Phase 1 Unit and the associated Department of Clinical Pharmacology have extensive experience in performing clinical trials. The UPR measurements and the AAR measurements will be performed by a nurse trained and experienced in performing this procedure.

Rights

Sponsor and primary investigator with overall responsibility is doctor Jonatan Kornholt.

Subinvestigator is the chief physician, Dr. med. Jesper Sonne, chief physician dr. med. Niels Klarskov and doctor, PhD David Peick Sonne.
UPR is performed by the nurse Troels Riis.
Data is stored and processed by the chief physician, dr. med. Niels Klarskov in accordance with the approval.
All results - both positive and negative - will be applied for as soon as possible in a scientific journals. We expect to publish two articles; one about UPR and one about AAR.
The author order for article one will be: Jonatan Kornholt, David Peick Sonne, Troels Riis, Jesper Sonne and Niels Klarskov.
The author order for the second article is : Jonatan Kornholt, David Peick Sonne, Troels Riis, Jesper Sonne and Niels Klarskov.

Quality control

Consent will be obtained from the trial subjects that third parties (e.g. inspector from the Scientific Ethics Committee / Data Inspectorate / The Danish Medicines Agency / GCP) can gain access to health information.

Ethics

The project is carried out in accordance with the ICH-GCP. The project is approved by the Regional Research Ethics e Committee, the Data Protection Agency and the Danish Medicines Agency. The study will be monitored by the GCP (Good Clinical Practice Unit). The study will, prior to the inclusion of subjects, register in the Clinical Trials Database.

Information and consent

Subjects are informed both in writing and orally about the purpose and content of the project – in accordance with the recommendations of the Scientific Ethics Committee. The subjects are also given the folder "*Trial subjects' rights in a health science research project* " from the Central Science Ethics Committee.
The first meeting is an information interview with one of the doctors responsible for the trial. Here, the subject will be informed orally and given written information. The person in question has the right to bring along an assistant. The meeting will take place in quiet undisturbed locations with time for questions. The information itself deals with the experiment and its advantages and disadvantages and must be easily understood by the subject.
The subject is informed about the right to refuse information about significant health conditions. The informing doctor is responsible for ensuring that the information is understood. The subject then has the right to a minimum of one-week reflection time.
On the screening day, written consent is obtained from the subject. At the declaration of consent, the investigator of the trial notes that written and oral information is given as stated above.
The subjects are informed that they may withdraw their commitment to participate in the trial at any time without this having any effect on current or subsequent treatment or controls.
The subject is informed if, in the course of the experiment, significant information about his or her health condition arises he/she will be informed of this, unless the subject explicitly states that he does not want this information.

Results

When the results of the study are available, one of the project's doctors will if possible inform the participating subjects about the result. Information on the participating subjects are protected under the *Act on processing of personal data* and the *Law on patients' rights* .

Side effects and risks

Sterile disposable catherization and UPR and AAR measurement results in immediate discrete discomfort during measurement and minimal risk of developing cystitis, which is minimized by sterile technique.

The side effects of imipramine are well known (12) . The known side effects of imipramine are available in the Summary of Product Characteristics 4.8.

The psychiatric and cardiac side effects are considered irrelevant in relation to a single dose. Possible adverse reaction are expected to primarily be anticholinergic (e.g. dry mouth, dizziness, and hypotension) and hypersensitivity reactions such rash and urticaria.

Pregnancy

Imipramine does not appear to be associated with an increased risk of malformations (25–27). There is a risk of withdrawal symptoms associated with delivery. The subjects are screened for pregnancy with pregnancy test (urine) prior to both test days. Only single-dose low dose (50 mg) imipramine is given. It is therefore assessed that there is no risk if pregnancy occurs immediately after the trial day, since the concentration of the drug is reduced to approx. 6- 40% (half-life 6-18 hours) of the dose and because maximum dose is already low, and as the substance has not been shown to be teratogenic in spite of the high usage for many years.

Pros and cons

Disadvantages: Possible side effects of the study drug (though low dose and single dose), low risk of cystitis associated with UPR (minimized by sterile technique), and discomfort associated with UPR and AAR (transient nuisance) .
Advantages: The subjects will have no immediate advantage by participating in this trial. The participants contribute to the gathering of useful new knowledge about the effect of the trial drug on the pressure in the urethra and the anal canal. This can be beneficial for future patients with stress urinary incontinence and fecal incontinence.

Adverse Events

All Adverse Events are registered on the CRF at the following times:

- At the end of trial day 1 and trial day 2.
- At the start of the trial day 2 (AEs since trial day 1).
- Five days (corresponding to > 5 half-lives) after the last test day (contact by phone).

Any SAE (Serious Adverse Events), is immediately (within 24 hours) reported to the sponsor (Jonatan Kornholt) according to the SOP for this. All AEs are followed until they are completed - also beyond the duration of the trial. AE is terminated when the subject is in normal health or stable condition. All AE, Adverse Drug Reactions (ADR), Serious Adverse Reactions (SAR), Serious Adverse Events (SAE) and Suspected Unexpected Serious Adverse Reactions (SUSAR) are handled in accordance with ICH-GCP and applicable legislation (from the Danish Medicines Agency):

"The sponsor must immediately notify the Danish Medicines Agency if, during the trial, unexpected and serious suspected adverse reactions (Suspected Unexpected Serious Adverse Reactions , SUSARs) are observed, cf. 2. No. 1.

The definitions are as follows, cf. the Executive Order on clinical trials:

- *incident: any undesirable event in a patient or subject in a clinical trial after treatment with a drug, without necessarily being related to this treatment and the adverse event*
- *side effect: any harmful and undesirable reaction to a test drug regardless of dose*
- *Unexpected side effect: an adverse reaction whose nature or severity does not correspond to the product information (eg, the Investigators- Brochure for an unapproved test drug or the SPC, in the case of an approved product)*
- *Serious event or serious side effect: An event or side effect, which, regardless of dose, results in death, is life-threatening, involves hospitalization or hospitalization, results in significant or persistent disability or incapacity for work or leads to congenital anomaly or malformation.*

Reference is made to the guideline in the area:
Guideline for reporting adverse reactions arising from clinical trials (Detailed guidance on collection , verification and presentation of adverse event / reaction reports arising from clinical trials on medicinal products for human use (CT 3)). These guidelines can be found on the Commission website under "Volume 10".

Sponsor's obligations

Pursuant to section 89 (1) of the Danish Medicines Act. 2, no. 1, the sponsor must immediately notify the Danish Medicines Agency if there are unexpected and serious suspected adverse reactions during the trial.

The sponsor must ensure that any information on unexpected and serious suspected adverse reactions (SUSAR) that is fatal or life-threatening is recorded and reported to the Drug Administration as soon as possible and within 7 days of the knowledge of such a suspected adverse reaction.

Within 8 days of the report, the sponsor must notify the Danish Medicines Agency of all relevant information about the sponsor's and investigator's follow-up to the report.

All other unexpected and serious suspected adverse reactions must be reported to the Danish Medicines Agency no later than 15 days after the sponsor has been informed of these.

Any report must be accompanied by comments on any implications for the trial.

(...)

All adverse reactions and events are reported at the end of the trial in the final report to the Danish Medicines Agency.

Investigator's obligations

Furthermore, the investigator has the following obligations, cf. the Order for Clinical Trials: Investigator must immediately report any serious incidents to the sponsor, with the exception of the serious events identified in the trial protocol or investigator brochure as events that do not require immediate reporting.

The report must be followed up by a detailed written report, and in both the immediate reporting and in the subsequent report, the investigator must identify the subjects with a personal code number.

Investigator must also report to the sponsor events and / or abnormal analysis results that are stated as critical to the safety of the subjects in the trial protocol.

The alert must be in accordance with the rules and deadlines set out in the Protocol. When reporting deaths, the investigator must provide any additional information requested by the sponsor.

The Sponsor / Sponsor Investigator is required to report any suspected serious or unexpected adverse drug reactions.

In order for a side effect to meet the reporting requirement, the side effect must be serious, ie. resulting in death, life-threatening, hospitalization or prolongation of hospitalization, resulting in significant or persistent disability or incapacity or leading to congenital anomaly or malformation.

In addition, the side effect must be related to the test drug. In other words, it would say that it is supposed to be a causal link between the intake of the drug and the side effect that has occurred.

Both investigator and sponsor (or sponsor investigator) must assess causality.

The sponsor must not, therefore, disclose the investigator's assessment, why reports where the sponsor does not agree with the investigator should still be reported.

The side effect should be unexpected, which is a side effect whose nature or severity does not match the product information (eg Investigators Brochure for an unapproved test drug or the SPC if it is an approved drug).

If the serious side effect is not listed in this product information, it is considered unexpected.

In the case of a blinded trial, the assessment of the adverse reaction must be made before blinding. From blinded trial, all SUSAR then unblinded before reporting to the authorities.

Follow-up reports (follow up) should only be submitted if applicable. What information is relevant or not should be based on a medical and scientific assessment. "

Reference document
The reference document for imipramine is the Summary of Product Characteristics for Imipramine "DAK".

Annual report
As the trial lasts for less than 1 year, no annual report is submitted, but at the end of the trial, the final report is submitted in accordance with applicable legislation. From the Danish Medicines Agency:

"After the end of the trial, the sponsor must, within 90 days of the end of the trial, inform the Danish Medicines Agency that the trial has been completed by Schedule for completion of clinical trials . (...)

As soon as possible and no later than 1 year thereafter, the test result must be entered in EudraCT. The data will then be published on www.clinicaltrialsregister.eu. This replaces that the trial result must be submitted to us [the Danish Medicines Agency]. You can see more here how to enter the results in EudraCT . As data becomes publicly available, there will in future not be a requirement to submit a publication to us. (...) "

Medical charts

Only healthy subjects are recruited for the trial, and therefore no records will be created or will be recorded during the study. CRF is documented instead.

Unblinding

Participants' study medication is always unblinded if SUSAR occurs. If other adverse events occur, the investigator or treating doctor will decide if unblinding is needed. Unblinding is done by the investigator (or subinvestigator): They open the envelope with the number corresponding to the subject's randomization number (stored envelopes are locked in the phase-1 unit where the experiment takes place). The envelopes contain information about the given test drug; whether placebo is given day 1 or day 2 and if there is given imipramine day 1 or day 2. Sponsor is informed of unblinding by person or telephone.

Drop out

In case of drop out, the reason must be registered on the CRF. If the cause is AE, this is followed until AE is complete - beyond the duration of the trial. AE is complete when the patient is in normal health again or the condition is stable.
As a rule, there is not unblinding on drop out, unless the cause for drop out is one of the situations specified in the section "Unblinding". All subject that drop out are offered a telephone call 5 days after intake of trial medication to detect any AE.
Data from drop out is not analyzed, as the analysis is performed as balanced crossover study (see statistics section). We recruit and therefore include trial subjects until a total of 16 participants have completed the trial, ie. both trial day 1 and trial day 2. When recruiting subjects after the first 16, calls are made to Region Hovedstadens Apotek in relation to which medicine kit the next subject must consume; to preserve the balanced design and to maintain blinding.

Data

On screening day, personal data, incl. CPR number, inclusion and exclusion criteria, medical history and any findings by objective examination on Case Report Form (CRF) made for this purpose. On trial day 1 and trial day 2, the date, results of the pregnancy test, subject ID, time for the intake of tablets, and time for UPR - and AAR measurements, and any AE. Data from the UPR measurement is electronic and transferred to encrypted USB key and from there to the data logged folder according to the approval of the Data Protection Agency.

Data storage

Data from UPR and AAR up are kept in anonymous form on PC, to which only the data-processing personal have access. All files are placed in a locked cabinet, to which only the data controller has access. Data is stored for 5 years, after which data is deleted. Envelopes with data on blinded medicine are delivered from Region Hovedstadens Apotek together with the medicine kits and stored together with Trial Master File and CRF locked in office at Zelo Phase-1 Unit.

Data controllers & data processors

Primary data manager and data processor: Chief physician, dr. med. Niels Klarskov, Department of Gynecological-Obstetrics, Herlev and Gentofte Hospital.
Secondary data processor: Doctor Jonatan Kornholt, Department of Clinical Pharmacology, Bispebjerg Hospital.

Financing

The trial is funded by the Department of Clinical Pharmacology's research funds.

Budget

	Price
Subject reimbursements	3,000 DKK * 16 subjects = 48,000 DKK
Manufacture and packaging of medicine	11,000 DKK in starting fee 300 DKK * 20 medicine kit = 6,000 DKK Total 17,000 DKK
Application to The Danish Medicines Agency	7,734 DKK
Total	72,734 DKK

Administrative conditions

The study is performed according to current legislation and ICH-GCP. The study must be approved by the Danish Medicines Agency, the Regional Science Ethics Committee and the Data Protection Agency. The study is monitored by the GCP.

The study is carried out in collaboration between the Department of Clinical Pharmacology (Bispebjerg Hospital), the Zelo Phase 1 unit (Bispebjerg Hospital) and the Gynecological-Obstetrics Department G (Herlev and Gentofte Hospital).
Contact person Clinical Pharmacological Department (Bispebjerg Hospital): Doctor Jonatan Kornholt.
Contact person Zelo Phase 1 unit (Bispebjerg Hospital): Chief physician , dr. med. Jesper Sonne.
Contact person Gynækologisk-Obstetisk Afdeling G (Herlev and Gentofte Hospital): Chief physician, dr. med. Niels Klarskov.

Insurance

Injuries to healthy subjects are covered by both the Patient Statement and the Labor Market Business Insurance. All areas of the Danish health service and all authorized healthcare professionals are covered by a publicly funded compensation scheme. The scheme covers if the patient is injured in connection with treatment in a public hospital, a private hospital, with his or her doctor, with a specialist doctor or with other privately-practiced authorized healthcare professionals. “Patients” also include people who participate in health science trials. The insurance also covers drug damage. It is the duty of the investigator to inform the subject about the insurance available if a drug injury occurs in a subject.

Monitoring

The experiment is monitored by the GCP unit at Copenhagen University Hospital. The trial participants consent that the GCP unit and the Danish Medicines Agency, as required, can monitor and make audits on all data incl. source data.

References

1. Minassian VA, Drutz HP, Al-Badr A. Urinary incontinence as a worldwide problem. Int J Gynecol Obstet. 2003;82(3):327–38.

2. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: Report from the standardisation sub-committee of the international continence society. Am J Obstet Gynecol. 2002;187(1):116–26.

3. Dansk Selskab for Gynækologi og Obstetrik (DSOG). Medicinsk behandling af urininkontinens hos kvinder [Internet]. 2010 [cited 2016 Nov 22]. Available from: http://www.dsog.dk/gynkologi/

4. pro.medicin. Stressinkontinens hos kvinder [Internet]. 2016 [cited 2016 Nov 22]. Available from: http://pro.medicin.dk/Sygdomme/Sygdom/318326

5. Melville JL, Fan M-Y, Newton K, Fenner D. Fecal incontinence in US women: A population-based study. Am J Obstet Gynecol. 2005;193(6):2071–6.

6. Perry S, Shaw C, Mcgrother C, Matthews RJ, Assassa RP, Dallosso H, et al. Prevalence of faecal incontinence in adults aged 40 years or more living in the community and the Leicestershire MRC Incontinence Study Team.

7. Whitehead WE, Borrud L, Goode PS, Meikle S, Mueller ER, Tuteja A, et al. Fecal incontinence in US adults: epidemiology and risk factors. Gastroenterology. 2009 Aug;137(2):512–7, 517-2.

8. Hayden DM, Weiss EG. Fecal Incontinence: Etiology, Evaluation, and Treatment.

9. Kumar L, Emmanuel A. Internal anal sphincter: Clinical perspective. Surgeon. 2016 Nov 20;

10. Simpson JAD, Bush D, Gruss HJ, Jacobs A, Pediconi C, Scholefield JH. A randomised, controlled, crossover study to investigate the safety and response of 1R,2S-methoxamine hydrochloride (NRL001) on anal function in healthy volunteers. Color Dis. 2014 Mar;16(s1):5–15.

11. Siproudhis L, Graf W, Emmanuel A, Walker D, Shing RNK, Pediconi C, et al. Libertas: a phase II placebo-controlled study of NRL001 in patients with faecal incontinence showed an unexpected and sustained placebo response. Int J Colorectal Dis. 2016 Jun 13;31(6):1205–16.

12. pro.medicin. Imipramin DAK [Internet]. 2016 [cited 2016 Nov 22]. Available from: https://pro.medicin.dk/Medicin/Praeparater/474

13. www.produktresume.dk. Imipramin DAK - Produktresume [Internet]. 2015 [cited 2016 Nov 22]. Available from: http://www.produktresume.dk

14. Lin H-H, Sheu B-C, Lo M-C, Huang S-C. Comparison of treatment outcomes of imipramine for female genuine stress incontinence. BJOG An Int J Obstet Gynaecol. 1999 Oct;106(10):1089–92.

15. Gilja I, Radej M, Kovacic M, Parazajder J. Conservative treatment of female stress incontinence with imipramine. J Urol. 1984 Nov;132(5):909–11.

16. Hunsballe JM, Djurhuus JC. Clinical options for imipramine in the management of urinary incontinence. Urol Res. 2001 Apr 27;29(2):118–25.

17. Thor KB, De Groat WC. Neural control of the female urethral and anal rhabdosphincters and pelvic floor muscles. Am J Physiol Regul Integr Comp Physiol. 2010;299:416–38.

18. Santoro GA, Eitan BZ, Pryde A, Bartolo DC. Open study of low-dose amitriptyline in the treatment of patients with idiopathic fecal incontinence. Dis Colon Rectum. 2000 Dec;43(12):1676–81.

19. Hornung BR, Mitchell PJ, Carlson GL, Klarskov N, Lose G, Kiff ES. Comparative study of anal acoustic reflectometry and anal manometry in the assessment of faecal incontinence. Br J Surg. 2012 Dec;99(12):1718–24.

20. Klarskov N, Scholfield D, Soma K, Darekar A, Mills I, Lose G. Measurement of Urethral Closure Function in Women With Stress Urinary Incontinence. J Urol. 2009;181(6):2628–33.

21. Klarskov N, Darekar A, Scholfield D, Whelan L, Lose G. Effect of fesoterodine on urethral closure function in women with stress urinary incontinence assessed by urethral pressure reflectometry. Int Urogynecol J. 2014 Jun 21;25(6):755–60.

22. Mitchell PJ, Klarskov N, Telford KJ, Hosker GL, Lose G, Kiff ES. Anal Acoustic Reflectometry: A New Reproducible Technique Providing Physiological Assessment of Anal Sphincter Function. Dis Colon Rectum. 2011 Sep;54(9):1122–8.

23. Champely S. pwr: Basic Functions for Power Analysis. 2016.

24. Grizzle JE. The Two-Period Change-Over Design and Its Use in Clinical Trials. Biometrics. 1965 Jun;21(2):467.

25. Briggs GG, Freeman R. Drugs in Pregnancy and Lactation. In: 10th ed. Philadelphia : Wolters Kluwer Health; 2015. p. 727.

26. Janusinfo. Läkemedel och fosterpåverkan [Internet]. 2016 [cited 2017 Mar 6]. Available from: http://www.janusinfo.se/Beslutsstod/Lakemedel-och-fosterpaverkan/?docid=1308

27. Dansk Psykiatrisk Selskab, Dansk Selskab for Obstetrik og Gynækologi, Dansk Pædiatrisk Selskab, Dansk Selskab for Klinisk Farmakologi. Anvendelse af psykofarmaka ved graviditet og amning - kliniske retningslinjer. 2014.