

RESEARCH PROTOCOL

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n.v.t.

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics (in Dutch: officiële productinformatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party

that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.

SUSAR Suspected Unexpected Serious Adverse Reaction

Wbp Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgegevens)

WMO Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

SUMMARY

Rationale: Painful polyneuropathy is a common condition that is difficult to diagnose and treat and has a negative impact on quality of life. Painful polyneuropathy is caused by degeneration of thin nerve fibers. With the current techniques, the diagnosis of thin fiber neuropathy can not be reliably established. Flavoprotein imaging is an optical method with which, among other things, pain transmission in the spinal cord can be measured. Because the epidermis or epidermis also contains a high density of pain nerve fibers, our hypothesis is that flavoprotein imaging is suitable for diagnosing thin fiber neuropathy.

Objective: In order to study whether painful neuropathy can be diagnosed with flavoprotein imaging, first of all a pilot study is needed, with which we want to investigate whether a reproducible and validated flavoprotein signal can be obtained at the middle finger tip of healthy subjects.

Study design:

- studying whether there is a time relationship between administering an electrical stimulus by means of finger electrodes and an optical (flavoprotein) signal on the fingertip of test subjects
- studying whether there is a linear relationship between the strength of the applied electrical signal and the size of the optical (flavoprotein) signal
- studying whether the signal is specific to the flavoprotein wavelength, by changing the filter settings of the camera
- studying whether the optical (flavoprotein) signal can be blocked by administering lidocaine / prilocaine cream on the fingertip
- studying whether the optical (flavoprotein) signal can be blocked by administering a capsaicin 8% patch on the fingertip

Study population: 10 healthy volunteers

Intervention (if applicable): the intervention with lidocaine / prilocaine cream and with capsaicin 8% patches is not the aim of the study, but serves as a negative control.

Main study parameters/endpoints:

- magnitude and time course of the flavoprotein response
- linearity of the electrical stimulation with the flavoprotein response
- block of the flavoprotein response by lidocaine / prilocaine crene and capsaicin patches

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

- time tax 45 minutes per person

-Discomfort: short-term painful electrical stimulation, about 3 hours of deafness of the index finger tip, about 3 days of hypersensitivity to pressure and temperature of the middle finger tip

-risk: none

1. INTRODUCTION AND RATIONALE

Painful polyneuropathy is a form of neuropathic pain that occurs when thin A δ or C nerve fibers become damaged. The exact incidence and prevalence of painful polyneuropathy is not known, but it is estimated that painful neuropathy occurs in 5% of the general population (1). Painful polyneuropathy has a negative influence on quality of life (2, 3) and is difficult to treat. The prevalence of untreatable neuropathic pain is estimated at 1.5% of the general population (4).

Painful polyneuropathy often presents itself as painful, burning or tingling feet. Painful, burning or tingling feet, however, can also be caused by narrowing of small arteries, venous insufficiency, osteoarthritis or rheumatoid arthritis of the foot joints, skin diseases such as a fungal infection or hives, or a marrow fracture, in addition to polyneuropathy. For the treatment of painful feet it is important to determine exactly what the underlying cause is. A problem here is that the aforementioned nerve fibers responsible for a painful polyneuropathy are difficult to investigate in neurological research or with additional examinations (5). With additional investigations are meant here:

-New guidance research / electromyography: this method mainly examines thick or A β sensitive nerve fibers and these are generally not at all or only slightly affected by a painful polyneuropathy

-Quantitative Sensory Testing: this allows sensitivity to temperature and strong mechanical stimuli to be investigated. This research is precise, but subjective. This means that the result is influenced by the concentration and pain experience of the test subject. In addition, the research is very labor-intensive.

Skin biopsies: in skin biopsies of the painful area, endings of epidermal nociceptors can be visualized and quantified by means of immunohistochemistry with the marker substance PGP9.5. Although this method is not 100% sensitive, it is often used as a gold standard for the diagnosis of painful, thin-fiber neuropathy. A disadvantage is that this method is not routinely available in hospitals and that it is very labor-intensive. A result can take 1-2 months.

-Videothermography / pulsed transit time / laser doppler. This method makes use of the fact that thin nerve fibers also regulate peripheral blood flow in addition to pain. This is an indirect measure of painful, thin-fiber neuropathy, of which the suitability for diagnosing painful, thin-fiber neuropathy is currently being investigated.

Because of the above, there is a need for a reliable, rapid and non-invasive method with which the function of thin nerve fibers can be measured. Autofluorescent flavoprotein imaging is a promising new technique for this. Autofluorescent flavoprotein imaging (AFI) is an optical technique that makes use of the property that certain endogenous mitochondrial

proteins, called under blue light, emit a green fluorescent signal in oxidized, or activated, state. Because nerve cells and their endings are very active, for generating the action potentials that are required for signal transmission, a lot of energy is required, AFI can be used to measure the degree of activation of such a nerve cell (6). Our research group has already demonstrated in experimental animals that this method is extremely suitable for studying nociceptive transmission in the spinal cord (Figure 1) (7). As in the spinal cord, there is also a high density of nociceptor endings in the epidermis (figure 2). These endings contain a high density mitochondria (figure 3) (8). Because there is a strong reduction in the number of nociceptor ends in the epidermis (Figure 4) (9) in neuropathy, it can be expected that the AFI signal after a nociceptive stimulus in the epidermis of people with a painful polyneuropathy is also greatly reduced. Thus, AFI could be used in the objectification of a painful, thin-fiber neuropathy.

This research application concerns a pilot study, with which we want to examine in healthy volunteers whether a reproducible and reliable AFI signal can be obtained after nociceptive stimulation in the epidermis.

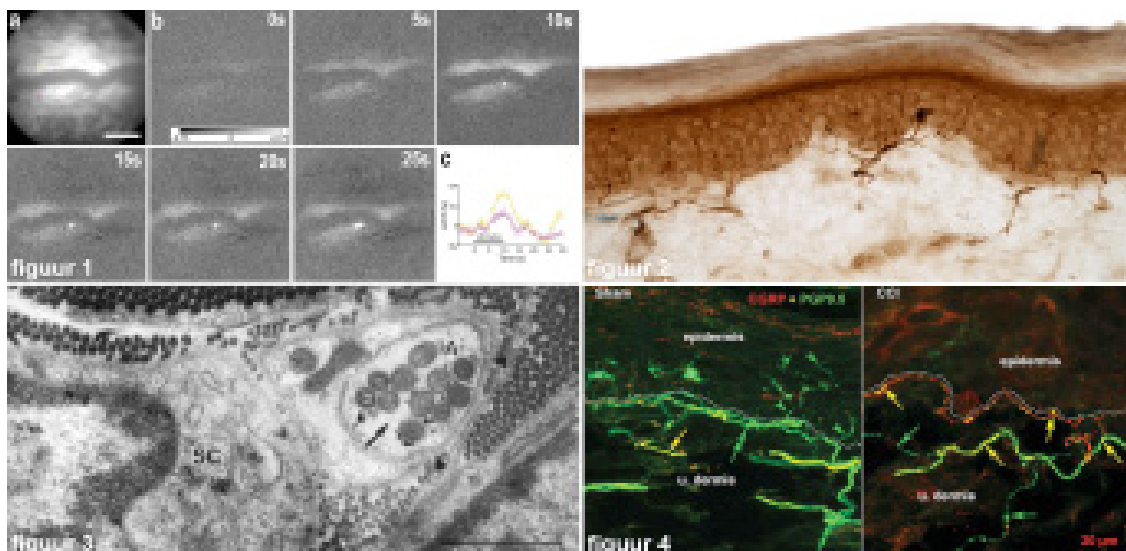


Figure 1: autofluorescent flavoprotein imaging of the spinal cord of the rat, after nociceptive electrical stimulation of the left n. sciatic, for 0-10 seconds. Clearly visible is an increase in the AFI signal especially on the left side (= upper side in the figure), which decreases again after stopping the stimulus.

Figure 2: high density of nociceptor endings in the epidermis, demonstrated by immunohistochemical staining with PGP9.5. The endings have a somewhat "variceus" aspect. In these thickenings there is a high concentration of mitochondria (see figure 3).

Figure 3: electron microscopic recording of an axon terminal (A) and Schwann cell (SC) of a nociceptor termination. The arrow shows 11 mitochondria in this ending.

Figure 4: sharp decrease in the number of epidermal nociceptor endings in an animal model for neuropathy. sham = control animal, CCI = animal with chronic constriction injury (model

for neuropathy), u.dermis is upper dermis. The arrowheads show PGP9.5 labeled epidermal nociceptor endings, which are completely absent in the epidermis in the animal with neuropathy.

OBJECTIVES

Primary Objective:

To study whether painful neuropathy can be measured with flavoprotein imaging, first of all a pilot study is needed, with which we want to investigate whether a reproducible and validated flavoprotein signal can be obtained at the second fingertip of healthy subjects.

STUDY DESIGN

- studying whether there is a time relationship between administering an electrical stimulus by means of finger electrodes and an optical (flavoprotein) signal on the fingertip of test subjects
- studying whether there is a linear relationship between the strength of the applied electrical signal and the size of the optical (flavoprotein) signal
- studying whether the signal is specific to the flavoprotein wavelength, by changing the filter settings of the camera
- studying whether the optical (flavoprotein) signal can be blocked by administering lidocaine / prilocaine cream on the fingertip
- studying whether the optical (flavoprotein) signal can be blocked by administering a capsaicin 8% patch on the fingertip.

2. STUDY POPULATION

2.1 Population (base)

2.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

-10 healthy volunteers

2.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

-younger than 18 years

-ready existing neuropathy

-allergy to local anaesthetics

2.4 Sample size calculation

AFI of the fingertip is a completely new application, there are no data on the basis of which a sample-size calculation can be done. It is a pilot study. The results of this pilot will serve to calculate a sample size for a follow-up study.

3. TREATMENT OF SUBJECTS

3.1 Investigational product/treatment

3.2 the intervention with lidocaine / prilocaine cream and with capsaicin 8% patches is not the aim of the study, but serves as a negative control.

3.3 Use of co-intervention (if applicable)

n.a.

3.4 Escape medication (if applicable)

n.a.

4. INVESTIGATIONAL PRODUCT

4.1 Name and description of investigational product(s)

4.2 Summary of findings from non-clinical studies

n.a.

4.3 Summary of findings from clinical studies

n.a.

4.4 Summary of known and potential risks and benefits

n.a.

4.5 Description and justification of route of administration and dosage

n.a.

4.6 Dosages, dosage modifications and method of administration

n.a.

4.7 Preparation and labelling of Investigational Medicinal Product

n.a.

4.8 Drug accountability

n.a.

5. NON-INVESTIGATIONAL PRODUCT

5.1 Name and description of non-investigational product(s)

-lidocaine/prilocaine creme (EMLA)

-capsaicine 8% patch (Qutenza)

5.2 Summary of findings from non-clinical studies

-EMLA: zie de SPC, <http://db.cbg-meb.nl/IB-teksten/h11015.pdf>

-Qutenza: zie de SPC, <http://www.astellas.nl/sites/default/files/SmPC%20Qutenza.pdf>

5.3 Summary of findings from clinical studies

-EMLA: zie de SPC, <http://db.cbg-meb.nl/IB-teksten/h11015.pdf>

-Qutenza: zie de SPC, <http://www.astellas.nl/sites/default/files/SmPC%20Qutenza.pdf>

5.4 Summary of known and potential risks and benefits

-EMLA 5% (from SmPC) Common (>1/100): Transient local reactions at the application site such as paleness, erythema (redness) and oedema. An initial and usually mild sensation of burning, itching or warmth at the application site. Uncommon (1/1000 to 1/100): An initial mild burning, itching sensation or warmth at the application site; local paresthesia at the application site, e.g. tingling sensation; and skin irritation at the application site. Rare (<1/1000): Methaemoglobinaemia (especially at longer application time in children with atopic dermatitis or mollusca contagiosa). Corneal irritation after accidental eye exposure.

-Qutenza 8% patch: On application a sensation of prickling and/or burning will occur for approximately 180 minutes, together with an area of erythema of the skin. In case of severe pain, a cold pack can be applied to the site of application to reduce pain, and oral analgesics, such as paracetamol, can also be provided. Hypersensitivity to pressure and heat can occur for up to two days. As with any medication, rare side effects cannot be excluded beforehand.

5.5 Description and justification of route of administration and dosage

-the route and dosage of lidocaine/prilocaine and the capsaicin patch is being used according to the SmPCs of lidocaine/prilocaine and the capsaicin patch.

-studies using cutaneous capsaicin patch 8% have demonstrated that this regimen is suitable to cause epidermal denervation in healthy subjects (10).

5.6 Dosages, dosage modifications and method of administration

-the route and dosage of lidocaine/prilocaine and the capsaicin patch is being used according to the SmPCs of lidocaine/prilocaine and the capsaicin patch.

-a small patch of 8% capsaicin will be applied to the middle finger for 60 minutes.

5.7 Preparation and labelling of Non Investigational Medicinal Product

-lidocaine/prilocaine cream will be present at the outpatient clinic of the Dept. of Neurology and supplied from regular pharmacy stock

-capsaicin 8% patches will be delivered to Erasmus MC by the manufacturer in their original packaging, cut to size on- site and used within 2 hours of opening the package.

5.8 Drug accountability

All treatments for the study will be dispensed from the Department of Clinical Pharmacy of Erasmus MC. Drug accountability will be done in accordance with the pertaining SOPs of the pharmacy. Unused lidocaine/prilocaine crème will be returned to the pharmacy at the end of the clinical phase. Waste capsaicin patches will be destroyed along with other medical waste at Erasmus MC.

METHODS

5.9 Study parameters/endpoints

5.9.1 Main study parameter/endpoint

- magnitude and time course of the flavoprotein response
- linearity of the electrical stimulation with the flavoprotein response
- block of the flavoprotein response by lidocaine / prilocaine cream and 8% capsaicin patches

5.9.2 Secondary study parameters/endpoints (if applicable)

n.a.

5.9.3 Other study parameters (if applicable)

n.a.

5.10 Randomisation, blinding and treatment allocation

n.a.

5.11 Study procedures

1) Each subject will undergo a series of electrical stimuli (5Hz, for 10 seconds) with AFI measurement. Each stimulus strength (0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mA) will be administered twice. The stimulus is interrupted when the pain intensity exceeds 7. Thereafter, a (non-painful) 2000Hz, 1.0mA stimulus will be administered as a (negative) control for 10 seconds. The stimuli are administered by a Neurometer, a validated before (11). The middle finger is illuminated with a white light source, from which blue light with a wavelength of 480nm is filtered. This light excites oxidized flavoproteins. The signal is picked up by a digital camera that is connected to an upright microsoop. The stimulation and measurements take 15 minutes.

2) At the end of the above measurements, the middle finger tip is smeared with lidocaine / prilocaine cream. An hour later the test person returns for the same measurements described above (15 minutes).

3) At the end of the measurements described under 2) an 8% capsaicine patch is applied to the middle finger tip. After an hour the researcher comes along and the plaster is removed. A week later the test person returns to undergo the measurements as described under 1) again (15 minutes).

5.12 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

5.12.1 Specific criteria for withdrawal (if applicable)

n.a.

5.13 Replacement of individual subjects after withdrawal

If test subjects lose weight, other test subjects will be approached, so that there are at least 10 participants in the (pilot) study

5.14 Follow-up of subjects withdrawn from treatment

subjects will be followed-up after withdrawal if medically required.

5.15 Premature termination of the study

n.a.

6. SAFETY REPORTING

6.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

6.2 AEs, SAEs and SUSARs

6.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

6.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse events.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

6.2.3 Suspected unexpected serious adverse reactions (SUSARs)

n.a.

6.3 Annual safety report

n.a.

6.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

6.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

n.a.

7. STATISTICAL ANALYSIS

7.1 Primary study parameter(s)

-correlation coefficients between stimulus strength and AFI signal amplitude will be calculated. The statistical significance of the correlation will be calculated using linear regression analysis.

-repeated-measures ANOVAs will be performed between baseline recordings and recordings 1 hour after application of lidocaine/prilocaine cream and between baseline recordings and recordings 1 week after application of a capsaicine patch. Finally, a paired t-test will be used to compare 1.0mA, 5Hz and 1.0mA, 2000Hz electrical stimulation.

7.2 Secondary study parameter(s)

n.a.

7.3 Other study parameters

n.a.

7.4 Interim analysis (if applicable)

n.a.

8. ETHICAL CONSIDERATIONS

8.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO)

8.2 Recruitment and consent

8.3 Subjects are recruited by colleagues, not subordinates, to approach the principal investigator orally. After this, a patient information form will be provided. Test subjects have a week to think about participation.

8.4 Objection by minors or incapacitated subjects (if applicable)

n.a.

8.5 Benefits and risks assessment, group relatedness

8.6 The importance, namely the development of a new diagnostic tool for painful neuropathy, in our view weighs against the minimal discomfort (in terms of time-load / risk) that goes with it.

8.7 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 9 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;

3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

8.8 Incentives (if applicable)

The subjects do not receive financial compensation for participating in the study.

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 Handling and storage of data and documents

The subjects are coded with a number from 1-10, in the order in which they participate in the study.

9.2 Monitoring and Quality Assurance

n.a.

9.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

9.4 Annual progress report

Not applicable, since the planned study period is 4 months

9.5 End of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature

termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

9.6 Public disclosure and publication policy

This pilot study will be registered at clinicaltrials.gov. The results of this study will be published, if applicable. The authorship guidelines of the Vancouver Protocol (<http://www.icmje.org/>) will be followed regarding co-authorship.

10. STRUCTURED RISK ANALYSIS

10.1 Potential issues of concern

a. Level of knowledge about mechanism or action

-for lidocaine / prilocaine cream and capsaicin 8% patches see section 5.2

With regard to the safety of the neurometer: the electrical stimulus is generated by a low voltage source, or a battery. For safety reasons, the Neurometer is constructed in such a way that it does not generate a stimulus when it is connected to the mains.

b. Previous exposure of human beings with the test product (s) and / or products with a similar biological mechanism

-for lidocaine / prilocaine cream and capsaicin 8% patches see section 5.2

- as far as we know, there have never been safety incidents with a Neurometer

c. Can the primary or secondary mechanism be induced in animals and / or in ex-vivo human cell material?

Yes, but the purpose of the above pilot study is to see if the AFI method is applicable to people. This method has already been applied to rats (7).

d. Selectivity of the mechanism to target tissue in animals and / or human beings

No, see above

e. Analysis of potential effect

Because lidocaine / prilocaine cream and capsaicin 8% patches are both already registered drugs, although not registered for use in healthy volunteers, but in patients prior to surgery or in patients with neuropathic pain, these drugs are safe at the dose used and there are no serious side effects to be expected.

f. Pharmacokinetic considerations

for this we refer to section 5.2 (SPC forms)

g. Study population

10 healthy volunteers

h. Interaction with other products

none, because both lidocaine / prilocaine cream and the capsaicin 8% patches only work locally on the skin

i. Predictability of effect

does not apply

j. Can effects be managed?

Lidocaine / prilocaine cream is a negative control experiment in the pilot study, but also serves to prevent the acute burning pain after administration of the capsaicin patch.

10.2 Synthesis

There is a minimal risk of moderately severe burning pain in the fingertip after application of the capsaicin 8% patch, despite lidocaine / prilocaine ointment an hour before. In that case an ice pack or paracetamol can be prescribed, because the pain always changes after 180 minutes.

11. REFERENCES

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