Pilot study on the pharmokinetics of p-phenylenediamine and its metabolites after application to the skin by means of Raman spectroscopy.

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By means of this in vivo study, we try to non-invasively get insights into the pharmacokinetics of PPD applied to the human skin. By studying penetration rate, penetration depth, allocation to different skin departments (stratum corneum, epidermis,...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Other condition
Study type	Observational non invasive

Summary

ID

NL-OMON36061

Source
ToetsingOnline

Brief title
Pilot study on pharmacokinetics of PPD by Raman spectroscopy.

Condition

- Other condition
- Epidermal and dermal conditions

Synonym
allergic contact dermatitis, eczema

Health condition

Farmacokinetiek van de stof p-fenyleendiamine na applicatie op de huid.

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Groningen
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: farmacokinetics, p-phenylenediamine (PPD), Raman spectroscopy, skin penetration

Outcome measures

Primary outcome

Experiment 1

1a: Determination of PPD penetration as a function of applicationtime and

clearance.

1b: Determination of PPD penetration in the epidermis and dermis as a function

of applicationtime and clearance.

Experiment 2:

Determination of the formation of PPD-metabolites MAPPD, DAPPD and BB after

topical application of PPD.

Secondary outcome

n.a.

Study description

Background summary

With an increasing number of women, but also men and children dyeing their

hair, the prevalence of sensitization and subsequent allergic contact dermatitis (ACD) to p-phenylenediamine (PPD) is rising. [1,2] Although the exact prevalence is not known, large research cohorts estimate the sensitization to PPD in the general population at 0.1 to 2.3%. [3] A less frequent, but other way to become sensitized is the application of a temporary henna tattoo, contact with black rubber materials or even the use of mascara where PPD has been added. [2,4,5] Sensitization is also seen within the framework of cross reactivity with azo-dyes in clothing. [5-7] A contact allergic dermatitis to PPD can be very severe, with erythema, oedema, blistering and crusts. In severe cases this can lead to hospitalization.
ACD is a delayed type hypersensitivity disorder to low molecular weight chemicals. Despite the long recognition of ACD to PPD, the immunological pathway has not been completely elucidated. [8,9]To date, there is no human, in vivo data on the pharmacokinetics (distribution, metabolism, excretion) of PPD after application to the skin. In vitro experiments showed that in aqueous solution or in contact to the skin, PPD is susceptible to sequential oxidation and self-conjugation, resulting in formation of instable intermediates like benzoquinonediimine, benzoquinone and the stable end product Bandrowski*s Base (BB). [17-19] Some of these substances are considered possible haptens. [18] However, there is much debate on which hapten is responsible in the sensitization to PPD. In addition, several studies have shown that (N-acetyltransferase 1) NAT1-enzymes in the human skin are able to acetylate PPD to mono-acetyl PPD (MAPPD) and di-acetyl PPD (DAPPD). This acetylation is considered a detoxification step, because MAPPD and DAPPD have a reduced to absent potential to sensitize. [20]
There are several methods to get insight in the dermal absorption and kinetics of a chemical. The most relevant method is a human in vivo absorption measurement, as used in experimental exposure studies with volunteers. Logically, these are often invasive experiments which are regulated tightly and are possible to a limited extent. In addition to the developed in vitro studies, a number of less invasive methods like plasma- and excretion-measurements, micro dialysis and *tape stripping* exist. [10]However, each of these methods has its specific limitations (i.e. destructiveness, lack of accuracy and/or spatial resolution). An entirely different in vivo method, spectroscopy, offers several advantages: it is fast, non-invasive and is able to provide *real-time* in vivo data on the penetration of a chemical through the skin. Unlike Infrared Spectroscopy (IS) which only reaches the outer layer of the stratum corneum, the Confocal Raman Microspectroscopy (CRM) is suitable to obtain information on the molecular skin structure to the depth of several hundred micrometers below the skin. [11]
By means of this in vivo, non-invasive study on the pharmacokinetics of PPD after application to the skin, we try to understand and contribute to research into the aetiology of contact sensitization to PPD. In order to obtain a reference spectrum, the kinetics of PPD will be examined in healthy subjects first. In subsequent studies we might examine PPD sensitized subjects in order to study possible differences. Therefore, a better understanding of the kinetics of PPD, may in the future lead to definition of subjects with an

increased risk of sensitization to PPD and hence, to reduction of sensitization. CRM is an elegant, non-invasive tool that provides detailed information on molecular structure and composition of the skin. [21] In addition, it enables the monitoring of skin molecular changes after topical application of drugs. [21]
In CRM the sample is illuminated by monochromatic laser light. This light interacts with the molecules in the sample, which then are able to vibrate internally or rotate around different axes.
Interaction between the molecules and the incident light leads to scattering of this light. Most of the scattered light is found at frequencies of this incident light (called elastical- or Rayleigh scattering). A small fraction however, is found at wavelengths longer than that of the incident light and is called inelastical- or Raman scattering. This scattered light exhibits frequency shifts with respect to the incident light, which are associated with molecular vibrations within the molecule. Each frequency shift, which can be recorded and translated by an optical dispersive system into a so called Raman spectrum, is dependent on the atom masses, chemical bonds and molecular structure and interactions and is therefore highly molecule specific. Therefore, a Raman spectrum represents a *fingerprint* by which the molecule can be identified. [22]
By comparing *blank* skin (without application of PPD) with spectra derived from skin after topical application, PPD can be detected in the skin. The depth, at which PPD is located, can be determined with so-called water profiles. These profiles correlate the depth of the skin with the water gradient, naturally present in skin. [22]

Study objective

By means of this in vivo study, we try to non-invasively get insights into the pharmacokinetics of PPD applied to the human skin. By studying penetration rate, penetration depth, allocation to different skin departments (stratum corneum, epidermis, dermis) and metabolism (converting of PPD into the trimer Bandrowski*s Base and/or the detoxification products mono-acetyl PPD and di-acetyl PPD) we try to understand the immunologic pathway of PPD.

If this pilot-study provides us insights into the pharmacokinetics of PPD in the skin of human volunteers, in the future we might be able to detect possible pharmacokinetic differences between healthy volunteers and sensitized subjects. Eventually, this may lead to definition of subjects with increased risk or reduction or avoidance of sensitization

Furthermore, several concepts exist on the mechanism of T-cell activation by PPD. The hapten-protein concept suggests that PPD represents a pro-hapten which will be modified after penetration through the skin, in order to subsequently being presented by antigen presenting cells to naïve T-cells.[17,23] Another concept states that PPD is able to directly - by means of a reversible non-covalent binding to MHC-class II molecule without further modification -

stimulate T-cells. [18,23] This PPD-protein complex seems to represent an additional antigenic signal for T-cells of allergic patients. Advanced techniques in Raman microscopy may allow visualizing binding of PPD to residential skin proteins. This is a possible follow-up experiment for the future.

Study design

Experiment 1: PPD penetration and clearance
a. Stratum corneum (SC) penetration as a function of application and clearance time
b. Deep penetration (epidermis (ED)/dermis) for 2 application times and 2 clearance times

Product : 1% PPD petrolatum
Application : covered, preferably Finn-chamber
Site : inner forearm (we can define 3 areas per arm and use left and right)
Volunteers : at least 2

Required : measurement of SC thickness (water profile)
measurement of PPD profile (fingerprint profile) across entire SC thickness

Experiment 1a:

Measurement times:
Each spot requires both a water profile (SC thickness) and a PPD profile.
Because of biological variation, take 10 repeat measurements per area.
Water profiles are measured (required) -4 to 40 micrometer in 4 micrometer steps at 1s/frame (appr. 24s / profile).
Fingerprint profiles can be measured 0 to 24 micrometer in 2 micrometer steps at 6 s/frame (appr. 91s / profile). Alternative settings for range and step size are possible (e.g. larger steps to save time at the cost of spatial resolution).
Total time for 10 repeats (including overhead times): 26 min.

Time scheduling
Based on a total time of little under 30 min per area, a time schedule is prepared to best fill all the desired time points, at highest efficiency.
(Based on an explorative experiment, it is not likely that we will see much PPD after 6 hrs or later. If necessary, we can verify one if PPD is still visible 6 hrs after 30 min application. If it is, we can then expand.)

Measurements
Application time T = 0 T = 1 hr T = 2 hrs T= 4 hrs T= 6 hrs T = 12 hrs T = 24 hrs
5 min. X X X X
30 min. X X X X

2 hrs. X X X X X X X
48 hrs. X

With the application and time points given above, a full series can be measured in 3 days for 2 volunteers. This should be done 3x to obtain a more reasonable number of 6 volunteers.

Experiment 1b:
Based on an exploratory experiment, a small amount of PPD is expected in the viable epidermis after 2 hrs or longer application. In order to determine the depth of penetration beyond the SC, the required signal collection time is considerably longer than in exp. 1a. This should be explored first, which can be done in about half a day.

Explorative experiment:
Time: 0.5 day
Volunteers: 2 volunteers)
After 48 hrs application (deepest penetration; moreover, this is the applicationtime used in diagnostic patch testing)
- measuring water profiles to determine SC thickness
- measuring deep fingerprint profiles (0-40 micrometer and deeper in 4 micrometer steps), with long exposure time of 20s per point. This results in ~3.5 minutes per profile.
- Repeat only a few times. Based on the outcome a detailed experiment can be designed.

Experiment 2: formation of MAPPD, DAPPD, BB
- Raman spectra of MAPPD, DAPPD and BB must be established first.
- These experiment requires high quality spectra (= long exposure times) in order to identify and quantify the downstream products.
- Exploratory experiments are required to determine if downstream products can be detected at all, and how long we should measure in order to quantify the amounts.

Study burden and risks

The burden for the subjects in this pilot study will consist of:

Voorafgaand aan deelname: anamnestisch uitsluiten van allergie voor PPD, actieve huidziekte onderarmen en mogelijk zwangerschap, d.m.v. vragenlijst meegestuurd met informatiebrief. Tevens anamnese tijdens een eerste afspraak (30 min.) en inspectie van de onderarmen naar evt. actieve huidziekte en uitleg zelf bereiden en aanbrengen PPD pleister.

- Prior to participation: excluding sensitization to PPD and pregnancy by

taking history, excluding active skin disease by questionnairre attached to the information letter. In addition taking a history during a first visit (30 min.) together with physical examination of the lower arms and instruction of PPD patch test preparation and application.
- Three consecutive measuring days, according to a schedule as optimal as possible. Day 1 :6.5 hrs, day 2: 5.5 hrs and day 3: 4.5hrs (including breaks).
- Measurements will take place at the Erasmsu Medical Centre in Rotterdam
- True burden exists of: application of several patchtest (plaster containing 1%PPD in petrolatum) during 5min, 30min, 2 hrs and 48hrs. After removal of the patchtest, the skin will be wiped clean by a tissue, where after the CRM measurements can start.
- For one measurement (application 2hrs, measurement T=12), the patch test has to be applied and removed at home, by the subjects themselves. They will be carefully instructed.
- During (especially the 48hrs) patch test, the patient will be asked to keep the lower-arm dry
- During the measurements, the subject places his/her arm on measurement window of glass, placed above the objective of the Raman spectrometer, which can be seen as an inverted microscope, while resting their hand and elbow on specially equipped armrests. During the 10 repeated measurements of approximately 90sec, the subjects will be asked to keep their arm as still as possible. Between the measurements the subjects will be able to move their arm and relax. The measurements are pain- and harmless.
- For exploratory experiment 1b measurements will be conducted on the researcher and spectroscopist after 48hrs application. Timeframe: 0.5 day.
- For exploratory experiment 2 attempts will be made to detect the formed PPD-metabolites in the skin of the researcher, if spectra of these metabolites will be obtained successfully. Timeframe: 0.5 day

The risks:
- Mild irritation on the spot of the patchtest. To minimize these complaints, hypoallergenic patches will be used. Possible irritation/itch will disappear spontaneously after removal of the patch and can be compared to itch while wearing a normal patch.
- Extremely small chance (<= 0,3 %, in 10-years review) on active sensitization to PPD.
Based on the history we assume that participating subjects don*t have an allergy to PPD.
This means that they have never been exposed to PPD and subsequent never could have been sensititized or that they have been exposed (very likely f.e. through dye in clothing), but are tolerant to PPD and thus do not develop allergic contact dermatitis.

Raman spectroscopie is a unique method to non-invasively study pharmacokinetics of PPD in vivo.[21] Knowledge regarding penetration, distribution and metabolism is necessary to unravel the to date unknown mechanism of sensitization to PPD. When more knowledge on the pathway of sensitization in

present, we may react to the field of prevention as well as the field of treatment. For the future, this may contribute to decreasing the prevalence of allergic contact dermatitis to PPD in the general population. In terms of these interests, we consider the negligible risks acceptable. Furthermore, application of the 1%PPD patchtest, needed for this experiment, is being used as a general diagnostic tool on a considerable amount of subjects of the dermatology department.[12] PPD has been included in the European Standard Series (a series with several, common contact allergens) which has been selected after extensive consideration and repeated evaluation by the European Society of Contact Dermatitis. [13] An allergen is being tested in a concentration that maximizes the number of sensitized subjects being detected, while the risk of active sensitization is as low as possible.[14] Scientific studies show that patch testing with PPD on subjects which have not been sensitized prior to the test, or which only showed one positive reaction, do not cause active sensitization and can be can be performed with minimal risk. [14,15] Furthermore, the dose to which subjects will be exposed (in regular patch tests as well in this pilot study) is much lower than the dose in f.e. hair dyeing. [16]

The objective of these experiments is to study the pharmacokinetics of PPD in the skin. Using six healthy volunteers, allows us to get a clear image. For the future, this may help to distract a protocol (optimal application times and follow-up measurements) for experiments on other healthy volunteers and/or sensitized patients, which then will be less time consuming.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

adulthood
legal competence

Exclusion criteria

History of allergic contact dermatitis to PPD
Active eczema or skin disease on the lower-arm (volar side).
Legally incompetence
Pregnancy

Study design

Design

Study type:	Observational non invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Treatment

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	24-11-2011
Enrollment:	6
Type:	Actual

Ethics review

Approved WMO

Date:	17-08-2011
Application type:	First submission
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL36149.042.11