Study Protocol

Project title:

Determination of DNA repair products in urine after UV irradiation of the skin

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Determination of DNA repair products in urine after UV irradiation of the skin

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1. **Title**

Determination of DNA repair products in urine after UV irradiation of the skin.

2. **Purpose**

Problem definition

DNA damage is formed in the skin by sun exposure [1]. The most common DNA damage is called thymine dimers (Figure 1). Many of these injuries are repaired by "Nucleotide excision repair" (NER) and excreted through the urine [2]. The importance of this repair mechanism is seen in patients with defects in their NER, who have a very high risk of developing skin cancer. We want to be able to quantify the amount of thymine dimers in the urine after ultraviolet (UV) irradiation of the skin. With the establishment of such a test system, it will eventually be possible to test potential photoprotective substances or potential photocarcinogenic substances based on damage measured in the urine after a known UV dose. We would like to investigate when the highest secretion of this type of DNA damage occurs using 2 different irradiation regimens. It has previously been possible to quantify thymine dimers in the urine using an isotope method, but that method is no longer available and it is desirable to avoid isotopes [3]. We have developed a new method based on mass spectrometry (UHPLC-MS).

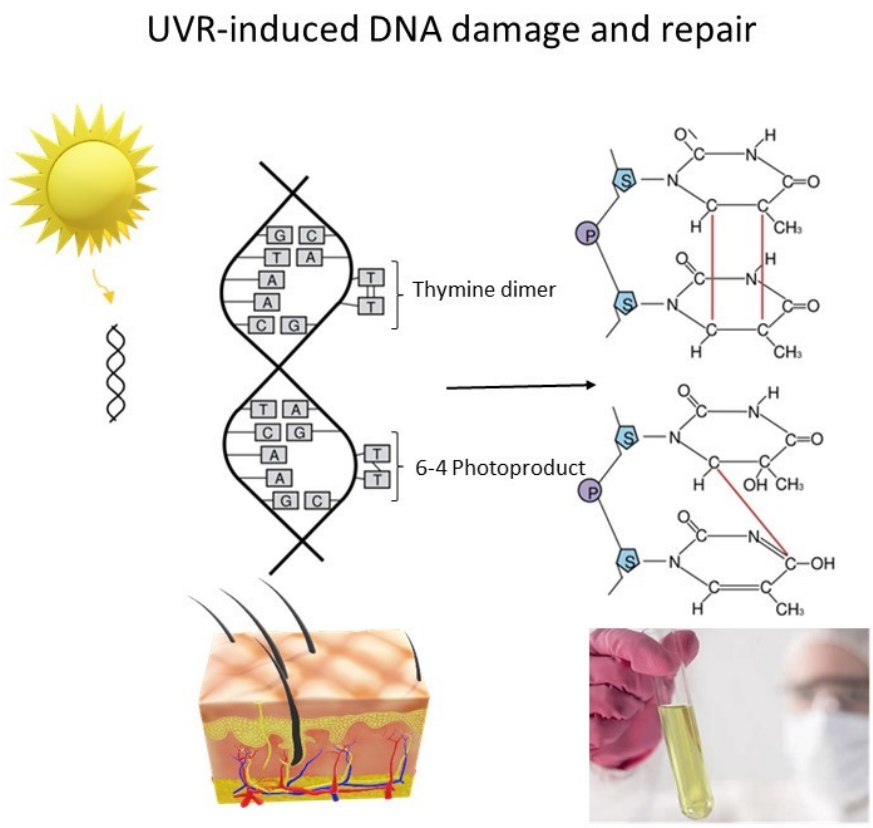

Figure 1. DNA damage of the thymine dimer type occurs by UV irradiation of the skin. The damage will often be repaired and excreted in the urine.

Hypothesis

Our hypothesis is that maximum excretion of thymine dimers in the urine occurs 1-4 days after UV irradiation of the skin.

Endpoints and rationales

The primary endpoint is quantification of thymine dimers in the urine before irradiation as well as every day for up to 7 days after UVR irradiation with two different irradiation regimens in healthy subjects. The 2-3 days where the concentration of excreted thymine dimers is greatest will be the days to collect urine in future studies.

The secondary endpoints are changes in redness and pigment, measured non-invasively using surface reflectance measurement.

The 2 irradiation regimens will be compared so that the greatest amount of thymine dimers can be determined with the least change in pigment and redness.

Background

Ultraviolet (UV) radiation causes DNA damage in the skin that can eventually lead to skin cancer [1]. Thymin dimer is a DNA damage that is only formed when UV rays hit DNA in the skin cells [1]. Nucleotide excision repair (NER) repairs and removes thymine dimers in the individual cell, which are then excreted in the urine [2]. Thymine dimers in the urine are a biomarker for the total DNA repair[3]. In the past, a method for quantifying thymine dimers in urine using isotope labeling[3] has existed. The method was very sensitive but had a long analysis time and in addition it is an advantage from a work environment to avoid working with radioactive isotopes[3].

We have succeeded in developing a new method of analysis using Ultra High-pressure Liquid Chromatography Mass Spectrometry (UHPLC-MS). This method has high sensitivity and high selectivity while having a fast analysis time. In addition, this equipment is capable of analyzing multiple DNA modifications. Furthermore, there is high sensitivity and high selectivity. This study is a proof-of-concept study in which we would like to investigate when the most optimal time for urine collection is after two different irradiation regimens. The perspective is to be able to use the method to find new potential photo-protective as well as photocarcinogenic substances.

3. Method

Study design for healthy subjects

The subjects (n = 16-20) are recruited by a notice on Bispebjerg's hospital website. Based on this, subjects are divided into 2 groups of 8-10 people. Group 1 is irradiated 3 times with 1 standard erythema dose (SED). 1 SED corresponds to approx. 10 min sun around kl. 13 on a good Danish summer day. Group 2 is irradiated once with 3 SED, which corresponds to approx. 30 min around kl. 13 on a good Danish summer day. The irradiation is carried out on day 1 for group 2 and days 1, 2 and 3 for group 1.

Subjects are irradiated in a full body UV cabin (Waldmann, Willing-Schwenningen, Germany) with 26 F85 / 100W UV6 tubes (290-350 nm, broad spectrum). The irradiation time is set on a timer clock, so that the UV cabin switches off after the desired irradiation time. The irradiation time is 16 seconds for a 1 SED and 43 seconds for a 3 SED. The UV cabin is measured using calibrated equipment, which is also used to measure our UV cabins in the clinic, which are used for patients. The subjects stand up in the cabin and have a screen on so that their eyes and face are not exposed to radiation. When lighting, subjects should only wear underwear, which for men are underpants / boxer shorts, while for women it is bras and panties. The experiment is performed between October and March, to avoid that the subjects do not simultaneously receive UV radiation from the sun and thus can form DNA damage.

Subjects must collect morning urine in dispensed containers and must store it in their own freezer until the final visit.

Morning urine (2x 50 ml) is collected before irradiation, called day 1 and even until day 8 after the last irradiation, ie. day 10 for group 1 and day 8 for group 2. 2 x 50 ml x 10 in total 1000 ml of urine are collected for group 1 and 2 x 50 ml x 8 a total of 800 ml of urine for group 2. Before and after each illumination are measured pigment and redness on the subjects as well as on final visits. Pigment and redness measurement are performed on the back, chest and buttocks.

4. **Statistical considerations**

Previous studies have observed an increase of 1.7 nmol with a scattering (SD) of 1.3 nmol 4, power of 0.80, as well as significance level of 0.05 in a paired test, then 7 people must complete the study when we set power to 0.80 and have a significance level of 0.05 and uses the paired test (Power and Sample Size Calculation, PS, Vanderbilt University; version 3.1.2). As the study lasts up to 10 days, dropouts can be expected and therefore 8-10 are included in each of the two groups, ie. a total of 16-20 healthy subjects.

5. **Subjects**

A total of 16-20 subjects are recruited to participate in the study. Men and women aged 18 or over who meet the criteria for inclusion and exclusion will be included. The subjects are recruited through Bispebjerg Hospital's intranet. Subjects will be invited to an initial screening visit where they will receive oral information about the study by trial supervisor Catharina Lerche. The meeting takes place in a separate room to ensure a safe and quiet environment. Subjects will be informed that they can bring an assistant if they wish. Written informed consent will be ensured, and takes place at least 24 hours after the screening visit.

Inclusion criteria

Over the age of 18

Written informed consent from the subject.

Exclusion criteria

Immunosuppressed individuals

Sun holiday / ski holiday / solarium 4 weeks before trial start

Skin disease

Medicines that cause photosensitivity or affect DNA repair

Pregnancy

6. **Risks, side effects and disadvantages in the short and long term**

There is a slight risk of short-term redness after lighting, as well as after staying in the sun. The UV doses (3 SED) have been chosen so that almost all Danes will avoid redness. The total UV dose corresponds to a ½ hour stay in the sun in the middle of the day during the summer in Denmark. All subjects will be informed both orally and in writing about the risks and possible side effects.

Extraction of new biological material or collection of biological material from already existing biobank

No biobank. The urine samples are analyzed immediately after collection. Excess biological material is destroyed immediately after analysis.

7. **Quality control**

   The Helsinki II Declaration will be respected as well as the standards of good clinical research. Respect for privacy as well as for physical and mental integrity in patients is maintained. The study will be registered at the Knowledge Center for Data Reporting, the Capital Region, in the electronic system Pactius.

8. **Data protection and storage**

   The general data protection regulation will be respected. Research using data from the capital region is considered public research. Use and distribution of data collected in this study will be discussed with patients during the consent process. The project is reported to the Knowledge Center for Data Reporting, the Capital Region of Denmark in the electronic system Pactius. A list of the subjects' names, study ID and date of birth is prepared. All collected data will be anonymised and protected by Danish legislation regarding the handling of personal data and the Health Act. Data are registered and stored for 5 years after completion of the study at the Department of Dermatology, Bispebjerg Hospital.

9. **Economy**

   It is Pharmacist Catharina Lerche, Professor Hans Christian Wulf and Engineer Peter Philipsen who have taken the initiative for the experiment and they are all paid by the skin department, Bispebjerg hospital. There are no contributions from companies or external funds to this experiment.

10. **Information for subjects**

    The principle investigator, Catharina M. Lerche, is responsible for providing clear oral and written information about goals, designs and risks of the study, as stated in "Information and consent to participate in health science research projects" by the Ministry of Health and the Elderly. In undisturbed surroundings, the participant will be made aware of their right to have one / or more attendants present, that participation is voluntary and that withdrawal is possible at any time during the study. Subjects are given sufficient reflection time (at least 24 hours). Subjects will be asked to sign a consent form.

11. **Publication of results**

    Positive, negative and conclusive results will be published. The aim is to publish and present the results in a peer-reviewed international dermatology journal and / or at dermatological conferences. The intellectual property rights to the results belong to Bispebjerg Hospital. Publications will comply with the Vancouver Guidelines.

12. **Ethics**

    Personal information and urine samples are processed in accordance with the Personal Data Act and the Health Act. The project is also implemented with minimal health and safety risks for the participants. It is expected that the potential risks of adverse effects in this study are very small. The potential for evidence-based future gains and the future perspectives that this study may provide should be weighed against trial participation. Danish laws on patients' rights and compensation are followed.

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