The long term effects of coal tar treatment on the microbiome and DNA methylome in atopic dermatitis skin.

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We hypothesize that the long lasting therapeutic effect of coal tar could be mediated by effects on the skin microbiome and/or effects on cellular memory by epigenetic changes. In atopic dermatitis patients, coal tar therapy could modify the skin...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Epidermal and dermal conditions
Study type	Observational invasive

Summary

ID

NL-OMON40800

Source
ToetsingOnline

Brief title
Long term effects of coal tar on eczema skin.

Condition

- Epidermal and dermal conditions

Synonym
Atopic dermatitis, eczema

Research involving
Human

Sponsors and support

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

Intervention

Keyword: coal tar, DNA methylation, microbiota, skin

Outcome measures

Primary outcome

The outcome of the study A and B is a nearly complete skin microbiome of each of the samples. Analysis of the taxonomic units will allow us to correlate the presence or absence of certain taxa with coal tar treatment of normal and eczema skin. This study is expected to generate hypotheses that can be tested subsequently in larger groups using a more focused and less expensive approach. The outcome of study C is a methylome analysis of a limited number of patients before and after coal tar treatment. Only when gross changes are observed, a controlled follow-up study will be initiated.

Secondary outcome

Not applicable.

Study description

Background summary

Atopic dermatitis: clinical features and therapy
Humans have evolved facing a continuous exposure to infectious agents, and it is likely that our genetic make-up has been shaped by the selective pressure from external microorganisms and our commensal flora of the skin and gut. Recent data on two major inflammatory skin diseases, psoriasis and atopic dermatitis (*eczema*) have indicated that the chemical and physical barrier of the skin, which protects us against infection, plays a crucial role in the development of these diseases [1-3]. Atopic dermatitis is a disease characterized by inflammation and itch, affecting up to 15% of children in the Western society. Skin of these patients has a poor antimicrobial defense as demonstrated by several labs including our own. Skin of atopic dermatitis patients is often infected and nearly always colonized by Staphylococcus

aureus. Recently, it was found that mutations in filaggrin, a gene involved in skin barrier function, explains a large part of the heritability of this disease.
Patients with atopic dermatitis are often treated with topical corticosteroids, systemic immunosuppressants or UV light therapy. Most of these treatments show short remission times, or even rebound effects. In our day care practice patients are often treated with coal tar, an ancient topical therapy of (until recently) unknown mechanism. Recently, our group found coal tar to activate the aryl hydrocarbon receptor (AhR), thereby unraveling (a part of) the working mechanism. Activation of AhR by polycyclic hydrocarbons, which are present in large amounts in coal tar, appears to (a) enhance epidermal differentiation leading to restored epidermal barrier function thereby attenuating allergen exposure, and (b) dampens the inflammatory cues of the T helper (Th)2 response [4]. Treatment of patients with coal tar shows a favorable remission time in comparison to other topical therapies, like corticosteroid application. A scientific explanation for this has not been supplied thus far.

Metagenomics
Metagenomics is the study of genetic material recovered directly from environmental samples or individuals. While traditional microbiology and microbial genome sequencing and genomics rely on clonal cultures, early environmental gene sequencing cloned specific genes (often the 16S rRNA gene) to produce a profile of diversity in a natural sample. Such work revealed that the vast majority of microbial biodiversity has been missed by cultivation-based methods. These studies have used "shotgun" Sanger sequencing or massively parallel pyrosequencing to get largely unbiased samples of all genes from all the members of the sampled communities (so-called microbiomes). Recent investigations have provided an inventory of the skin and gut microbiome by current next generation sequencing (NGS) technologies allowing an unbiased identification of virtually all skin-inhabiting bacteria [5, 6]. Since a correlation between skin microbiome and atopic dermatitis has been reported [7], restoring the normal skin microbiome could hold a therapeutic effect in itself.

Epigenetics
Epigenetics is the study of heritable changes in gene activity that are not caused by changes in the DNA sequence; it also can be used to describe the study of stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. The term also refers to the changes themselves: functionally relevant changes to the genome that do not involve a change in the nucleotide sequence. Examples of mechanisms that produce such changes are DNA methylation and histone modification, each of which alters how genes are expressed without altering the underlying DNA sequence. Epigentic changes are likely to be involved in cellular differentiation of normal epidermal morphogenesis. Recent studies have indicated that diseases (such as psoriasis) can induce epigenetic changes in skin tissue that may contribute to maintenance of disease [8]. Also, recent pilot studies have shown distinct

tissue-specific patterns of DNA methylation associated with atopic dermatitis [9].

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2. Hollox, E.J., et al., Psoriasis is associated with increased beta-defensin genomic copy number. Nat Genet, 2008. 40(1): p. 23-5.
3. de Cid, R., et al., Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. Nat Genet, 2009. 41(2): p. 211-5.
4. van den Bogaard, E.H., et al., Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. J Clin Invest, 2013. 123(2): p. 917-27.
5. Gao, Z., et al., Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. PLoS One, 2008. 3(7): p. e2719.
6. Grice, E.A., et al., Topographical and temporal diversity of the human skin microbiome. Science, 2009. 324(5931): p. 1190-2.
7. Kong, H.H., et al., Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res, 2012. 22(5): p. 850-9.
8. Roberson, E.D., et al., A subset of methylated CpG sites differentiate psoriatic from normal skin. J Invest Dermatol, 2012. 132(3 Pt 1): p. 583-92.
9. Rodriguez, E., et al., An Integrated Epigenetic and Transcriptomic Analysis Reveals Distinct Tissue-Specific Patterns of DNA Methylation Associated with Atopic Dermatitis. J Invest Dermatol, 2014.

Study objective

We hypothesize that the long lasting therapeutic effect of coal tar could be mediated by effects on the skin microbiome and/or effects on cellular memory by epigenetic changes. In atopic dermatitis patients, coal tar therapy could modify the skin microbiome in such a way that colonization by S.aureus is reduced and that a *normal* skin microbiome is established (largely consisting of S.epidermidis and P.acnes, no detectable S.aureus). In addition, coal tar therapy could induce epigenetic changes in epidermal (stem) cells or lymphocytes, that would render the skin resistant to disease for a prolonged period, thereby explaining the remission time.

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Our study aims to address these issues in:
(A) a short term study to address the effect of coal tar on the skin microbiome of healthy volunteers
(B) a long term study of the microbiome of atopic dermatitis patients that are treated with regular coal tar therapy in a day care setting
(C) a pilot study to investigate if coal tar application on lesional atopic dermatitis skin causes gross epigenetic changes in the skin cells

Study design

A. EFFECT OF COAL TAR ON THE MICROBIOME OF HEALTHY VOLUNTEERS
Our goal is to study the effect of coal tar on the skin microbiome in a group of healthy volunteers (n=10). Three areas (4 x 4 cm, divided in 4 quadrants) on the lower back will be treated as follows:

1. control without treatment
2. lanette/vaseline followed by zinc-oxide paste(base ointment)
3. coal tar solution (LCD) 10% in lanette/vaseline followed by coal tar (pix lithantracis) 5% in zinc oxide paste

Treatment will be stopped at day 7. Skin scrape samples (non-invasive) will be taken before, during and after treatment at day 0, 2, 7 and 14, from the 4 quadrants respectively (to avoid repeated sampling from the same area). Microbial DNA will be extracted from these skin scrape samples and analyzed. All healthy volunteers will be reimbursed for their travel costs, based on public transportation, and receive ¤50 for participation in the study.

Methodological considerations
This is an explorative study on the microbiome of a small group of healthy individuals. The microbiome analysis will generate large amounts of experimental data (>10.000 datapoints per sample, comparable to microarray studies), creating the problem of family wise errors. For this reason the analysis will not primarily focus on the quantitative comparison of individual taxa but rather on the shifts of the microbial communities within samples (at higher taxonomical aggregation levels, from genus to phylum).

Analysis of the skin microbiome
Microbial DNA from skin samples will be extracted and amplified using the universal 16S rRNA primers and subjected to deep sequencing technology. Because of the rapidly evolution of sequencing techniques, the exact method of deep sequencing is to be determined. Bioinformatics analysis will be performed in collaboration with NIZO (The Dutch Dairy Institute, Ede) and CMBI (Centre for Molecular BioInformatics, Nijmegen). We have previously performed such studies with success (see CMO 1243; this has resulted in a publication by Zeeuwen et al 2012, (Genome Biol. 2012 Nov 15;13(11):R101)

B. EFFECT OF COAL TAR ON SKIN MICROBIOME OF AD PATIENTS
We aim to analyze the skin microbiome of adult patients with atopic dermatitis before, during and after treatment with coal tar (n=10). In addition we will analyze the effect of long term coal tar treatment on the skin microbiome. This is a regular AD treatment in the day care centre of our department. Patients are enrolled for coal tar treatment via the out-patient department and will be asked to participate in the following study protocol. Patients that are already on coal tar treatment are excluded. Patients receiving therapy with indifferent

ointments or corticosteroids are eligible. Two areas of lesional AD skin, the right and left inner elbow (ca 5 x 8 cm) are selected for the experiment. These two areas will be divided in 4 quadrants for sampling at the first 4 timepoints.

Area #1: lanette/vaseline followed by zinc-oxide paste(control treatment, base ointment)
Area #2: coal tar solution (LCD) 10% in lanette/vaseline followed by coal tar (pix lithantracis) 5% in zinc-oxide paste

Five patients will have coal tar on the right arm and base ointment on the left, and 5 patients vice versa. Except for the arm with the base ointment, the patients receive the standard regular skin care. Patients will receive the regular instruction by the nurse practictioner how to apply the ointments and bandages at home. Patients will visit the day care clinic, 4 times during the first 2 weeks. Thereafter, the visit frequency is reduced, as assessed by the dermatologist. Third week: the experimental treatment is stopped and patients are only treated by their regular coal tar therapy, at a frequency determined by the medical staff.
Skin scrapes are taken at baseline (t=0) and at the 3 following visits to the day care clinic (end of week 2). When patients have finished the therapy at the day care clinic (on average between 4 and 12 weeks treatment), a skin scrape will be taken from the experimental area #2 (= exit measurement). The patients will be asked to continue participation in our study by bi-monthly visits and skin sampling of experimental area #2. Patients are asked to contact us when they feel that their eczema exacerbates. We will then take skin scrapes again from area #2.
Patients that participate in our study will be reimbursed for unscheduled travel costs based on public transportation. Apart from travel costs, patients that volunteer to participate will receive ¤50 when the exit measurement is done. Patients that participate in the follow-up study will receive an additional ¤50.

Methodological considerations
The design is constrained by various practical, ethical and financial reasons. We have opted for the simplest protocol possible, in order not to interfere with daily practice at the day care centre, and to minimize the burden of the patient. Nevertheless we feel that we will extract sufficient useful information from these investigations.
The microbiome analysis will generate large amounts of experimental data (>10.000 datapoints per sample, comparable to microarray studies), creating the problem of family wise errors. For this reason the analysis will not primarily focus on the quantitative comparison of individual taxa but rather on the shifts of the microbial communities between samples (at higher taxonomical aggregation levels, from genus to phylum).

Analysis of the skin microbiome
Microbial DNA from skin samples will be extracted and amplified using the

universal 16S rRNA primers and subjected to deep sequencing technology. Because of the rapidly evolution of sequencing techniques, the exact method of deep sequencing is to be determined. Bioinformatics analysis will be performed in collaboration with NIZO (The Dutch Dairy Institute, Ede) and CMBI (Centre for Molecular BioInformatics, Nijmegen). We have previously performed such studies with success (see CMO 1243; this has resulted in a publication by Zeeuwen et al (Genome Biol. 2012 Nov 15;13(11):R101).

C. EFFECT OF COAL TAR ON SKIN DNA METHYLOME
In a pilot study (3 patients) we want to investigate if gross epigenetic changes can be observed in atopic dermatitis skin, following coal tar treatment. When such changes are observed, a larger study with appropriate controls will be designed. This will then include larger sample numbers and other therapeutic modalities. In view of the high costs associated with this kind of research, we think it is justifiable to perform an uncontrolled pilot study first to see if there is any effect.
The study will be performed in 3 AD patients that will be treated with coal tar solution (LCD) 10% in lanette/vaseline followed by coal tar (pix lithantracis) 5% in zinc-oxide paste. We will take three 4-mm full thickness skin biopsies from lesional skin, before and after topical treatment for 7 days (6 biopsies in total).
Patients will be recruited via our list of volunteers, our day care centre or out-patient department. Volunteers will receive a ¤15,- fee per biopsy and additional travel costs will be reimbursed, based on public transportation.
Biopsies will be processed for DNA extraction and subsequent methylome analysis.

Analysis of the skin DNA methylome
DNA methylation status from skin biopsies will be analyzed in collaboration with the Department of Molecular Biology (Radboud Institute of Molecular Life Sciences, RIMLS, Nijmegen). Because of the rapid evolution of methylome analysis techniques, the exact method of analysis will be chosen in a later state. Bioinformatics analysis will be performed in collaboration with the CMBI (Centre for Molecular BioInformatics, Nijmegen).

Study burden and risks

Collection of skin scrapes is not invasive and poses a limited burden on the participants. Full thickness skin biopsies usually heal well, with a minor scar. Individuals that have a tendency to develop hypertrophic scars or keloids are excluded. None of the participants have direct benefits of the proposed studies.
For patients that participate in study B there is the following burden: normally they would be completely treated with coal tar, but here one arm will be treated with base ointment without coal tar for 2 weeks. From week 2 onwards they will receive the regular coal tar therapy.

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

Patients with:
Atopic dermatitis

Exclusion criteria

pregnancy in first trimester

Study design

Design

Study type:	Observational 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):	22-06-2015
Enrollment:	23
Type:	Actual

Ethics review

Approved WMO Date:	08-12-2014
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)
Approved WMO Date:	16-06-2015
Application type:	Amendment
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)

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	NL48646.091.14