Effect of Topical Sulforaphane on Skin Fragility Seen in Skin Aging and With Ultraviolet Exposure

NCT03126539

June 16, 2017

Johns Hopkins Medicine - eForm A

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1. Abstract

The cross sectional study investigating whether topical application of sulforaphane (broccoli sprout extract) results in the induction of both keratin 16 and keratin 17 in the basal layer of the epidermis is currently IRB approved (IRB00061421).

The primary purpose of our study is to compare the expression of keratins before and after application of sulforaphane (SF) and determine whether these findings alter skin fragility seen in both intrinsic and extrinsic skin aging as well as in ultraviolet (UV) light exposure.

This investigation will be done in collaboration with researchers from the Department of Biochemistry and Molecular Biology and Bloomberg School of Public Health at Johns Hopkins University. The study population recruited by Johns Hopkins Department of Dermatology will include up to 50 individuals over the age of 18 with healthy skin. Each study participant will have four on-site study visits and will be asked to contribute photographs, undergo non invasive elasticity measurements, use topical sulforaphane for 7 days and contribute up to four biopsy specimens for laboratory study. We will also evaluate any differences in keratins' expression in skin exposed to acute UV light, separately and after pretreatment with sulforaphane.

2. Objectives

- The primary objective of this study is to compare the induction of both keratin 16 and keratin 17 in the basal layer of the epidermis in both photoprotected and photodamaged skin treated with sulforaphane to determine whether SF can improve skin fragility seen in these conditions. This would be visualized by established indirect immunofluorescence assays on skin tissue sections prepared from biopsy material, and would be confirmed and quantified by PCR-based analyses of RNA samples.
- The secondary objective is to investigate distinct keratin expression changes in human skin after acute UV light exposure, separately and in combination with application of topical sulforaphane.

3. Background

Isothiocyanate sulforaphane (SF), derived from broccoli sprouts, has been known to induce an antioxidant response through the Keap1-Nrf2-antioxidant response element pathway.[1] With its chemoprotective effects as the rationale, it has previously been studied for tolerance and safety in humans and has been shown to have virtually no adverse effects.[2-4] This includes a study in which sulforaphane-containing broccoli sprout extracts was formulated in acetone and topically applied to the skin.[2] Additionally, a recent study has shown that oral sulforaphane was safely tolerated in doses of 50-150 μmol daily in children with autism.[5]

SF selectively induces K17 and K16 in skin keratinocytes. Local activation of two distinct signaling pathways, hedgehog and Keap1/Nrf2/ARE, rescued skin blistering in an epidermolysis

bullosa simplex mouse model, correlating SF with the reprogramming of keratin synthesis in epidermis and improving skin fragility.[6]

Sulforaphane might also help in maintaining the collagen levels during photoaging via the inhibition of the AP-1 activation and of the MMP expression. A previous study reported that SF had preventive effects on UV-induced MMP expression, inhibiting the MMP-1 and MMP-3 expression by blocking the NF-jB pathway.[7]

Aging of human skin may result from both the passage of time (intrinsic aging) and via exposure to external factors (extrinsic aging) such as sunlight (photoaging).[8] One hallmark feature of skin aging, regardless of type, is skin thinning or fragility. This is partially due to the breakdown in dermal collagen. An increase in MMP-1 after UV exposure is observed in skin. MMP-1 is an interstitial collagenase able to hydrolyze type I collagen, the major component of the dermis, and plays a crucial role in the disorganization and progressive degeneration of dermal extracellular matrix.[8]

While much is known regarding the dermal changes that occur with skin aging[8], there has been little research in the role that keratins and epidermis play in skin aging and the associated skin fragility. It is also not know whether UV exposure has an effect on epidermal keratins. By assessing the epidermal structure of the skin from both photoprotected and photoexposed sites from individuals before and after the application of sulforaphanes, and before and after acute UV light exposure, we hope to understand whether differences exist in the expression of keratins between photoprotected and photoexposed areas and whether sulforaphane can change these differences.

4. Study Procedures

a) Study design, including the sequence and timing of study procedures

Recruitment: Individuals will be recruited from patient populations seen at the general dermatology clinics of Johns Hopkins Department of Dermatology or from patient populations participating in Johns Hopkins Cutaneous Translational Research Program (CTReP) research studies. Interested study participants will be evaluated after their routine clinical care visits. Participants will also be recruited from other patient populations in the Johns Hopkins Health System through the use of flyers. Study procedures will be conducted at the Johns Hopkins CTReP office located at the Johns Hopkins Outpatient Center. Recruitment may include Johns Hopkins University employees or students but these groups will not be specifically targeted for participation. Interested individuals will be interviewed to see whether they meet the basic eligibility criteria to participate in the study. Once eligibility has been demonstrated, the potential subjects will be instructed to make their first study appointment. We will recruit up to 50 participants over the age of 18 with healthy skin and they will be split into 2 groups: Group A) 20 volunteers that will apply sulforaphane in jojoba oil for up to 7 consecutive nights and have biopsies before and after on both photoprotected and photoexposed areas and jojoba oil only only to photoexposed areas for up to 7 consecutive nights with biopsy after 7 days Group B) 20 volunteers that will have 2 test areas irradiated with UV light and biopsies before and 24 hours after UV light irradiation; one of the UV treated areas will be pre-treated with sulforaphane for up to 7 consecutive nights and the other UV treated areas will be pre-treated with jojoba oil.

Group A

Visit 1(day 0):

During the first scheduled appointment, the research protocol will be discussed with the subject and documentation will be obtained of the subject's consent to participate in the study and the subject's agreement to be contacted about future research studies. Photographs and clinical assessment will be taken.

Once informed consent is obtained, investigators will identify two suitable areas on the photoexposed forearm and one suitable area of the photoprotected upper inner arm for study. Each participant will receive up to 14ml of broccoli sprout extract (BSE) in jojoba oil, containing 7000nmol of sulforaphane. 500nmol of sulforaphane will be applied each night on each suitable area for up to 7 consecutive nights. Additionally, each participant will receive up to 14 ml of jojoba oil only to be applied each night on one of the photoexposed site. All areas will be occluded with saran wrap every night. A demonstration and verbal instructions for how and where to apply study drug will be provided to subjects.

During this visit, investigators will perform clinical assessments, elasticity measurements, and up to two 6 mm punch, one from each area.

Visit 2 (up to day 7):

During this visit, investigators will perform clinical assessment, elasticity measurements and up to three 6 mm punch, one from each area.

Visit 3 (day 10-14):

During this visit investigators will remove sutures from biopsies taken on visit 1.

Visit 4 (day 17-21):

During this visit investigators will remove sutures from biopsies taken on visit 2.

Group B

Visit 1:

During the first scheduled appointment, the research protocol will be discussed with the subject and documentation will be obtained of the subject's consent to participate in the study and the subject's agreement to be contacted about future research studies. Photographs and clinical assessment will be taken.

Once informed consent is obtained, investigators will identify 2 suitable test areas on the photoprotected upper inner arm for study. Test area dimensions will be about 17 x 17 mm in diameter, the spot size produced by the department's UV device. Each participant will receive up to 7 ml of broccoli sprout extract (BSE) in jojoba oil, containing 3500nmol of sulforaphane and up to 7 ml of jojoba oil only 500 nmol of sulforaphane will be applied each night to Test Area #1 for up to 7 consecutive nights. Additionally, jojoba oil only will be applied each night to Test Area #2 for up to 7 consecutive nights. Areas will be occluded with saran wrap every night. A demonstration and verbal instructions for how and where to apply study drug will be provided to subjects. Clinical assessment and elasticity measurements will be performed during this visit as well.

Visit 2:
UV Light Irradiation: Within 1 to 7 days of BSE and jojoba oil only application, investigators will deliver a fixed dose of UV light to Test Area #1 and Test Area #2. Subjects will be asked to return on-site up to 3 days after UV exposure for biopsy.

Visit 3:
During this visit, investigators will perform clinical assessment, elasticity measurements and three 6 mm punch, one from each Test Area and one from normal, unirradiated skin, also on the upper inner arm, to serve as a control.

Visit 4:
Subjects will return on-site 10-14 days after biopsy to have sutures removed.

Photography: Standardized digital photographs will be obtained by study staff using a digital camera and software under standard photographic conditions at each study visit. Participants' eyes will be blacked out to de-identify facial photos. Photograph files will be coded to remove personal identifiers and stored on a secure hard drive in CTReP.

Clinical assessment: Clinical assessments will be performed to 1) record baseline skin findings 2) to identify up to 4 suitable areas for biopsy, 3) give information/demonstration about sulforaphane application and 4) to identify occurrence of any adverse events.

Cutometer: Cutometer readings will be taken from photoprotected and photoexposed areas, before and after sulforaphane and after jojoba oil only application on Group A and from normal skin, Test Area #1 and #2 after UV irradiation on Group B. The cutometer, frequently used in cosmetic dermatology research, applies precise suction to a small area of the skin (6mm diameter). Skin is drawn into the aperture of the probe via negative/vacuum pressure and after a defined time, released again. The resistance of the skin to the negative pressure (firmness) and its ability to return into its original position (elasticity) are displayed as curves (penetration depth in mm/time) in real time during the measurement. This measurement principle provides information about the elastic and mechanical properties of skin surface and enables the study team to objectively quantify skin aging. The procedure is non-invasive and does not cause any discomfort.

Ballistometer: Ballistometer readings will be taken from photoprotected and photoexposed areas, before and after sulforaphane or jojoba oil only application in Group A and from normal skin, Test Area #1 and #2 after UV irradiation on Group B. The ballistometer allows us to measure skin firmness and elasticity by dropping a small probe onto the skin. After the collision of the probe with the skin's surface, we measure the rebound height of the probe. The number of rebounds and amplitude of the rebounds provide information about the rate of energy dampening and bounce profile of the skin's surface. Again, this is a non-invasive procedure and does not cause any discomfort.

UV light irradiation: We will use a Lumera UVB light phototherapy device which allows for targeted delivery of controlled doses of UVB radiation (emission spectrum 290-320 nm).

Skin biopsies: up to five skin punch biopsies (up to 6 mm in diameter) from Group A will be taken in order to conduct the laboratory work. Two from the untreated control areas,two after up to 7 days applying sulforaphane, and one after up to 7 days applying jojoba oil only to a photoexposed site. Up to three skin punch biopsies (up to 6 mm in diameter) from Group B will be taken in order to conduct to the laboratory work. One from control area, one from UV irradiated area after topical jojoba oil only and one from UV irradiated area after topical sulforaphane. All areas will be biopsied using standard punch biopsy tools and following standard clinical protocols, including cleansing the skin with an alcohol wipe and injecting local anesthesia with lidocaine and epinephrine. No more than five total skin biopsies will be obtained from a volunteer over the course of the study. After removal of the tissue sample, one or two sutures are placed to close the circular opening. Sutures are removed and a scar is formed, but typically heals well without complications and blends well with the surrounding skin. Routine post-biopsy care instructions will be provided and volunteers will be instructed to return in 10 to 14 days to have sutures removed and for assessment of healing.

Laboratory studies: Tissue work-up may include but not limited to cryosectioning and immunofluorescence staining and protein or RNA extraction for molecular assays (RT-PCR and/or qPCR for select mRNAs and western blotting for select protein antigens). The levels of keratin 1, keratin 5, keratin 10, keratin 14, keratin 16, keratin 17, and Nrf2 will be studied with both immunofluorescence and RNA PCR. Dr Pierre Coulombe laboratory from the Department of Biochemistry and Molecular Biology and Bloomberg School of Public Health at Johns Hopkins University has successfully applied this strategy.

b. Study duration and number of study visits required of research participants.

The study consists of up to 4 on-site study visits within a 3 week period, including the suture removal visit. We will allow 4 years to complete the study.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.
NOT APPLICABLE

d. Justification of why participants will not receive routine care or will have current therapy stopped.
NOT APPLICABLE

e. Justification for inclusion of a placebo or non-treatment group.
NOT APPLICABLE

f. Definition of treatment failure or participant removal criteria.
Any clinical findings determined by the Investigator to be important and/or unusual will be referred to as an adverse event (AE). Study participants are asked to contact clinic staff

immediately if they experience a reaction to the topical sulforaphane at any time during the study. Expected reactions may be documented in a problem events log. The investigator will use his discretion to remove participants form the study, and all problem events will be reported to IRB.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

NOT APPLICABLE

5. Inclusion/Exclusion Criteria

Inclusion:

1. Participants must be over the age of 18 years old with healthy skin;
2. Must be healthy enough to undergo skin biopsy, UV light irradiation, and other study procedures in the opinion of the investigator;
3. Must be willing to comply with the requirements of the protocol;
4. Must have the ability to understand and communicate with the investigator;
5. Participant must provide informed consent.

Exclusion:

1. Subjects who are unable to provide informed consent;
2. Subject with significant medical history or current skin diseases that the investigator feels is not safe for study participation;
3. Subjects who have been treated with systemic retinoids or steroids within the past month prior to entry to the study;
4. Subjects who have been treated with topical steroids, retinoids or other topical drugs used within 2 weeks prior to entry to the study;
5. Recently treated or current skin diseases that would affect clinical evaluation and biopsy;
6. Subjects with a known allergy to broccoli.
7. Presence or suspicion of bleeding disorder or diathesis which would complicate biopsy.
8. Subjects with a history of excessive scar or keloid formation in the past 10 years.
9. Pregnant or nursing subjects (self-reported).
10. Subjects with known allergy to anesthetics used.
11. Patients with history of investigational drug use in the 30 days prior to entry into the study.

6. Drugs/ Substances/ Devices

Jojoba oil was chosen based on its known lipophilic nature and ability to permeate deeply into skin [9]. In a prior study performed in SKH-1 hairless mice (which feature thicker epidermis, more akin to human than furry mouse strains), topical sulforaphane induced K16 and K17 best when administered in jojoba oil. Dermabase, another widely used vehicle, was also tested in this study; however, as Dermabase was found to induce K16/K17 alone even without sulforaphane

added, it was deemed not to be an appropriate vehicle to measure the effects of sulforaphane-containing broccoli sprout extract. [10]

The equivalent of 500nmol of sulforaphane (delivered in the form of broccoli sprout extract) was chosen as the dosage to be used as it has been previously been shown to be tolerable to human skin up to a dose of 681nmol when it caused a transient 3mm erythematous macule.[10] By using jojoba oil coupled with occlusion under saran wrap, we hope to demonstrate penetration of broccoli sprout extract's effect to the basal layer of the epidermis. Broccoli sprout extract is a natural substance that has been orally ingested for thousands of years. It has been used topically and orally with no adverse events.[9-11]

Lidocaine with epinephrine will be used as a local anesthetic to the biopsy sites prior to the biopsy procedure. The drug used in the study will be the same drug provided for the entire Johns Hopkins Department of Dermatology. There will not be a separate batch of this drug for the sole purposes of this study as this study is not a clinical trial and use of the drug is unlikely to interfere with study results.

Skin elasticity measurements will be obtained using a cutometer (Cutometer® MPA 580) and a ballistometer (Dia-stron Limited Dermal Torsional Ballistometer). The only stimulus to the participant via cutometer is a light vacuum suction of the skin via a 6mm diameter probe. The resistance of the skin to be sucked up by negative pressure (firmness) and its ability to return into its original position (elasticity) are displayed as curves. The accompanying software allows us to calculate different parameters like the ability of the skin to return to its original state and the tiring effects of the skin after repeated suction. The ballistometer probe lightly taps the skin and bounces repeatedly on the test site before coming to rest. An optical sensor monitors the position of the probe and the positional data is transmitted to the PC software. It does not cause any discomfort to the subject, but offers measurements of skin firmness and dynamic resilience of skin. Both devices are non invasive and have been approved by the Department of Clinical Engineering.

The UVB light radiation will be administered using the Lumera Targeted Phototherapy system (Daavlin, Bryan OH, USA). Buttock skin will be irradiated using a hand-held fiber optic cable with an adjustable aperture size. The JHU Department of Dermatology has already purchased this device and it has been approved by the Department of Clinical Engineering. The device has an emission spectrum in the ultraviolet B range of 290-320 nm. This device is FDA approved for the treatment of several dermatologic diseases, such as psoriasis, seborrheic and atopic dermatitis, and vitiligo, and can be safely used on all skin types (I-VI). The system consists of a light source, a spot handpiece, and a light guide that connects the handpiece to the light source. The light source has a timer and an output level control to adjust the intensity of the ultraviolet light. The device will be calibrated using a manufacturer-provided UV meter prior to every use to ensure accurate doses of ultraviolet light.

7. Study Statistics

We are collaborating with researchers from the Department of Biochemistry and Molecular Biology and Bloomberg School of Public Health at Johns Hopkins University. The skin biopsies will be analyzed by immunofluorescence staining and molecular analyses for keratin 1, keratin 5, keratin 10, keratin 14, keratin 16, keratin 17, and Nrf2 activation.

8. Risks

It is possible that there will be skin irritation of the skin. Risks include irritant dermatitis and/or contact dermatitis, itching sensations from the sulforaphane. Risk events, problems, and deviations will be immediately reported by the PI to the IRB.

Risks include potential bleeding and a slight risk of infection from the punch biopsies. As with any cut in the skin, a scar will develop, although its appearance will likely fade over time. We will screen carefully for keloids in our exclusion criteria, but in rare individuals with no prior keloid history, keloids or excessive scarring may develop at the biopsy site. As with any biopsy, there is a risk of pain or discomfort from the procedure.

There are no anticipated risks or discomforts associated with gross mechanical measurements of elasticity.

There is a risk of sunburn from the UV treatment, although the risk is minimal given the small area affected and limited numbers of treatments. There is a risk of ultraviolet injury to the eyes. Both the operator and subjects will wear UV blocking protective eyewear provided by the device manufacturer. The provided eyewear meets the ANSI Z87.1-2003 standard and blocks 99.9% of UV in wavelengths 180-380 nm and has wrap-around side shields. In addition, the treatment area is the buttock, so even inadvertent eye exposure is highly unlikely.

In terms of confidentiality, there are minimal risks as all study participant information will be de-identified. Since this is an exploratory study, no confidential or protected information would be taken outside of the standard. There is a slight financial risk to the participants in the rare event that the above complications occur requiring additional medical care.

9. Benefits

There will be no direct benefit to the participant as a result of this study. Instead, the potential benefit will be to society at large. We hope to better understand the differences on keratins' expression on phototexposed and photoprotected skin, after acute UV light exposure and also if sulforaphane can play a role on this expression and on skin elasticity.

10. Payment and Remuneration

Study participants who undergo all study procedures, including correct application of sulforaphane, non-invasive measurements, UV light irradiation (if in Group B) and biopsies (up to 5for Group A and up to 3 for Group B) will receive a total of $100 at completion of the study.

11. Costs

Study costs will be borne by the Johns Hopkins Department of Dermatology and Dr. Coulombe's lab. The sulforaphane-jajoba oil product will be provided by Dr. Coulombe's lab.

12. References

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