2021

UVB-Induced Microvesicle Particle Release in Human Skin in vivo is Diminished Following Oral Vitamin C and E Antioxidant Administration

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UVB-Induced Microvesicle Particle Release in Human Skin *in vivo* is Diminished Following Oral Vitamin C and E Antioxidant Administration

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Clinical Science and Research

Scholarship in Medicine Final Report

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Abstract

An important question in photobiology asks how Ultraviolet B (UVB, 290 – 320 nm) radiation, which mostly absorbs in the outer epidermis of skin, can generate a systemic response such as immunosuppression. Previous *in vitro* and *ex vivo* studies demonstrate UVB-dependent release of bioactive molecule-containing microvesicle particles (MVPs) from keratinocytes. Furthermore, MVP release is diminished upon antioxidant administration. The purpose of this study is to examine UVB-induced MVP release and antioxidant response *in vivo.* In this IRB-approved study, 8 male participants with Fitzpatrick type I or II skin were treated with 1000 J/m^2 UVB irradiation to a 5 by 5 mm area of volar forearm skin. 4 hours later, punch biopsies and erythema measurements were performed. This procedure was repeated 8 days later following a course of oral antioxidants. On average, tissue MVP release increased 1.8-fold (+/- 0.31, P = 0.02) following UVB treatment. Following a course of oral antioxidants, the average UVB-induced tissue MVP release did not differ from the control (0.9-fold +/- 0.13, P = 0.23). There was no significant change in UVB-induced erythema between pre- and post-antioxidant administration. These studies suggest that UVB-MVP are dependent upon reactive oxygen species.

Key Words: (Microvesicle particles, UVB radiation, Platelet-activating factor)

Introduction/Literature Review

This study is designed to investigate whether ultraviolet B radiation (UVB) found in sunlight causes release of microvesicle particles (MVPs) in human skin in vivo, and if antioxidants can inhibit their release. Microvesicle particles are small, 100-micron cellular fragments that bud off keratinocytes and released into the extracellular environment. MVPs, containing bioactive contents such as cytokines and Platelet-activating factor (PAF) agonists, are thought to serve a messenger function within the body and be a mediator of systemic response to UVB radiation of the epidermis [1].

Previous studies have demonstrated that the lipid mediator PAF is produced in human skin upon exposure to UVB light and leads to systemic immunosuppression [2,3,4]. Furthermore, UVB-induced PAF production in human skin is blocked by antioxidants [2,3]. Additional studies show that MVPs are released in human skin upon exposure to both UVB radiation and PAF. While UVB-mediated MVP release is blocked by pretreatment of cell lines with antioxidants vitamin C or N-acetyl cysteine, MVPs are still released upon exposure to the PAF-agonist Carbomyl-PAF (CPAF) [5]. These results suggest that UVB-dependent MVP release is mediated via PAF-agonist production and PAF Receptor (PAFR) activation [5].

Previous studies were performed on either human tissue explants or cell cultures, but not on live humans. Therefore, the purpose of this study is to investigate if UVB radiation induces MVP formation on human subjects *in vivo*, and if MVP release can be blocked by antioxidants. Blocking MVP release may have clinical utility by reducing systemic inflammatory response upon sun exposure and reducing severity of sunburns.

Hypothesis/Specific Aims/Research Questions

Upon exposure to UVB radiation, human skin will produce a marked increase in microvesicle particle formation and release. Additionally, microvesicle particle release due to UVB exposure will be diminished following oral antioxidant administration.

Methods

Context/Protocol

This study is designed to test if localized UVB treatment will increase the microvesicle particle count in the treatment area, and if administering antioxidants will decrease this response. The amount of vitamin C and vitamin E administered was chosen from a previous study [6] in which participants who were given 2000 mg of vitamin C and 1000 mg of vitamin E daily for 8 days had a diminished erythematous response to UVB light. These amounts have also been shown to be safe [6]. Only male participants were recruited to control for fluctuating hormones associated with the menstrual cycle and any affect they may have on MVP release or inflammatory response. While the original goal was to enroll twelve participants, contact restrictions secondary to the COVID-19 pandemic allowed for completion of a statistically-meaningful eight participants. All participants fully completed the study. Appointments and procedures for this study were carried out in the Pharmacology Translational Unit (PTU), located in the Wright State Physicians building.

Inclusion Criteria: Adult male subjects aged 21-45 with Fitzpatrick type I or II skin ("fair" complexion) will be enrolled. A person with Fitzpatrick type I skin always sunburns and never tans, while one with Fitzpatrick type II skin sunburns easily with minimal tanning.

Exclusion Criteria:

1. Unable to understand informed consent
2. Any condition that compromises wound healing
3. Large volar arm tattoos
4. Currently taking medication(s) known to be a photosensitizer, anti-inflammatories, or vitamin C or E
5. Renal compromise or nephrolithiasis
6. History of hypertrophic scarring or keloids
7. Allergy to vitamin C, vitamin E, or lidocaine
8. Use of tanning beds or UVB exposure to volar forearm within the past 3 months
9. Recent (1 week) use of sunscreen to volar forearms

Study Protocol:

Day 0:

1. Volar forearm skin was photographed and a 5 x 5 mm area of skin treated with 1000 J/m^2 of UVB irradiation, approximately 3 minimal erythema doses (MED; ~350 J/m2). A MED is the amount of UVB radiation able to induce mild erythema by 24 hours and equivalent to a minor sunburn.
2. On the back of the same side, four separate 5 x 5 mm areas of skin were treated with 200 J/m^2, 400 J/m^2, 600 J/m^2, and 800 J/m^2 of UVB irradiation. These areas allowed us to measure the effect of antioxidants on dose-dependent UVB-induced erythema.

3. Four hours later, the UVB-treated volar forearm was photographed and erythema was measured with a mexameter (non-invasive instrument that measures redness). A 5 mm punch biopsy was performed on the UVB-irradiated area and another nearby, untreated spot on the same volar forearm. The 5 mm punch biopsies were performed as follows: the area was appropriately sterilized and then anesthetized with 1% lidocaine with 1:100000 epinephrine. One or two sutures were placed following the punch biopsy and participants were scheduled to return 10-14 days later for suture removal. Wound care instruction, antibiotic ointment, and adhesive bandages were provided.

Day 1:

1. The UVB-treated areas on the back were photographed and their erythema measured via mexameter.
2. The participants were dispensed over-the-counter vitamin C (1000 mg capsules) and vitamin E (1000 mg capsules). Participants were instructed to take two vitamin C and one vitamin E capsule daily for 8 days, starting this day. 14 vitamin C capsules and 7 vitamin E capsules were given in a 7-day weekly medicine planner, with the appropriate tablets in each compartment. The participants completed the first dose (Day 1) in the office and were instructed to use the medicine planner the following day. Subjects were instructed to bring the medicine planner back on day 8 so any remaining capsules could be counted and compliance ensured.

Day 8:

1. Any remaining vitamin capsules in the participant's medicine planner were counted

2. The contralateral volar forearm was photographed and subsequently treated with UVB irradiation. Two skin biopsies were removed 4 hours later (same as day 0).
3. On the contralateral back, 4 small areas of skin were treated with increasing amounts of UVB irradiation (same as day 0).

Day 9:

1. The UVB-treated areas on the contralateral back were photographed and their erythema measured via mexameter.

Compensation: Participants were compensated a total of $300 for study completion. Compensation was dispensed as follows:

Day 0: $25 for UVB procedures and $50 for skin biopsies

Day 1: $50 for the mexameter readings

Day 8: $25 for UVB procedures and $50 for skin biopsies

Day 9: $100 for mexameter readings and completion of the study

Data Collection

Two primary types of data were collected:

1. Tissue microvesicle particle count: Following the punch biopsy as described above, the tissue sample was placed in a sterile salt solution containing collagenase and dispase, where it was stored overnight before microvesicle particle counting. The tissue sample lysate was collected and centrifuged at 2,000 *x g* for 20 minutes at 4°C to remove debris. The sample supernatant was further centrifuged at 20,000 *x g* for 10 minutes at 4°C for further tissue and cellular component removal. Microvesicle particles were pelleted from this supernatant

by centrifugation at 20,000 *x g* for 70 minutes at 4°C. Microvesicle particle concentration was determined using a NanoSight NS300, a device that uses dynamic light scattering (DLS) and Brownian motion to characterize nanoparticles.

2. Skin erythema measurement: As described in the protocol above, a mexameter was used to measure erythema of UVB-treated area. Adequate time was given to allow an erythematic response to develop. A nearby area of skin that was not treated with UVB irradiation was read and used as a baseline. Mexameter readings were repeated 5 times at each site and averaged. The mexameter uses light reflectance to provide a numerical value of erythema. Specifically, the mexameter emits a known quantity of light and a receiver detects the amount of light reflected by the skin. The amount of light the skin absorbs is calculated from these two numbers. The mexameter displays erythema as a unitless number between 0 (lowest) and 999 (highest). As hemoglobin is primarily responsible for this redness, the mexameter uses two wavelengths of light (green 568 nm light and red 660 nm light) to measure erythema and minimize noise.

Data Analysis

All data analysis was completed using Microsoft Excel for Macintosh. Statistical significance of MVP release (figure 1) and erythema (figure 2) between control non UVB-treated skin, UVB-treated skin pre-antioxidant administration, and UVB-treated skin post-antioxidant administration was evaluated using a one-tailed paired Student's t-test on fold change data. A paired t-test was used as we are comparing the means of the same group of 8 male participants under varying condition, i.e., pre- and post- antioxidant administration. A one-tailed test was selected as we are only testing a change in tissue MVP release and erythema in one direction. Fold change data, and not raw numerical data, was used because of varying basal starting points of tissue MVP

concentration and erythema. The magnitude of change with respect to the starting point is of more interest than the numerical value of the change itself. These t-tests confirmed the first part of our hypothesis –there is a significant increase in skin MVP release following UVB irradiation. A two-tale z-test evaluated the statistical significance of the average fold change in UVB-induced MVP release and UVB-induced erythema in pre-antioxidant versus post-antioxidant trials. A z-test was chosen because the standard deviations of these two populations were known. Comparing these two populations (pre-antioxidants and post-antioxidants) evaluated the second part of our hypothesis, that MVP release is diminished following antioxidant supplementation. Alpha was set at 0.05 for each statistical test.

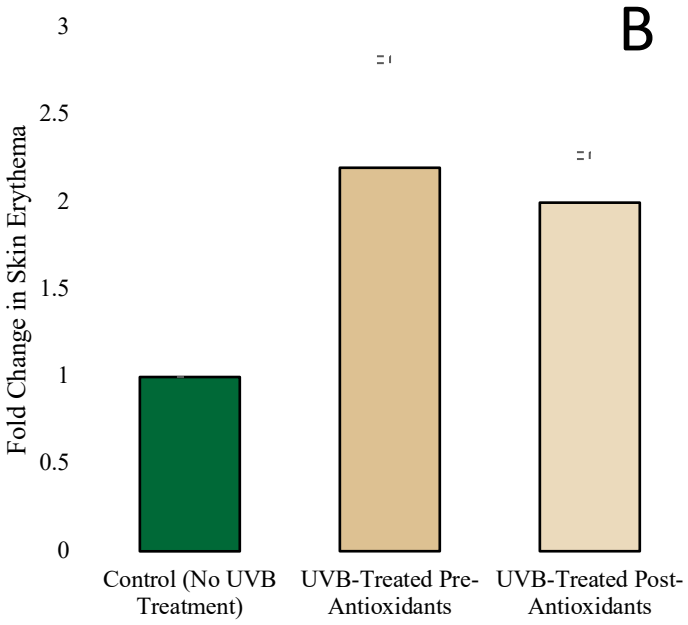
Results

An approximately 2-fold increase in erythema of the volar forearm skin was elicited following 1000 J/m^2 UVB treatment in both the pre-antioxidant and post-antioxidant cohorts (Figures 1A, B). These erythematic responses are statistically significant when compared to control non UVB-treated skin ($P = 0.002$ pre-antioxidants, $P < 0.001$ post-antioxidants, Figure 1A). There is no appreciable difference in UVB-stimulated erythema between the pre- and post-antioxidant trials ($P = 0.49$, Figures 1A, B). Prior to antioxidant administration, the average tissue MVP release increased 1.8-fold ($P = 0.02$) upon exposure to 1000 J/m^2 UVB irradiation (Figures 2A, B). After an 8-day course of antioxidants, the average fold of tissue UVB-induced MVP release was 0.9 and not statistically different than the control non UVB-treated sample ($P = 0.21$, Figures 2A, B). Tissue samples collected after antioxidant therapy demonstrated an appreciable reduction (P 0.0074) in UVB-induced MVP release when compared to similar pre-antioxidant samples (Figures 2A, B).

A

Subject #	Age	Erythema Control (Fold)	Erythema post UVB (Fold)	Erythema Control + Antioxidants (Fold)	Erythema post UVB + Antioxidants (Fold)
	28	165 (1.0)	431 (2.6)	159 (1.0)	433 (2.7)
	26	223 (1.0)	406 (1.8)	216 (1.0)	382 (1.8)
	25	113 (1.0)	430 (3.8)	179 (1.0)	485 (2.7)
	29	187 (1.0)	385 (2.1)	230 (1.0)	378 (1.6)
	24	252 (1.0)	443 (1.8)	246 (1.0)	398 (1.6)
	27	123 (1.0)	305 (2.5)	108 (1.0)	252 (2.3)
	23	247 (1.0)	302 (1.2)	240 (1.0)	367 (1.5)
	29	189 (1.0)	354 (1.9)	223 (1.0)	369 (1.7)
Mean fold change (+/- SEM)		**1.0**	**2.2 (+/- 0.60) P = 0.002**	**1.0**	**2.0 (+/- 0.25) P < 0.001**
z-Test:		**P = 0.49 Δ Fold UVB-induced erythema pre- vs. post-antioxidant administration**			

Figure 1: UVB-irradiation of human skin produces a significant quantifiable erythema response. A 5 mm x 5 mm area of volar forearm skin was treated with 1000 J/m^2 UVB irradiation in 8 male subjects. Erythema response of non UVB-treated control skin and UVB-treated skin was evaluated and recorded 4 hours later. This procedure was then repeated following an 8-day course of vitamin C and E antioxidants. Mexameter data points were averaged from 3 readings. **A)** Erythema measurements are numerically presented as unitless mexameter data and fold change +/- SEM after being normalized to control skin. **B)** Skin erythema in fold change



A

Subject #	Age	Control MVP x 10^{11} / g (Fold)	UVB MVP x 10^{11} / g (Fold)	Control MVP x 10^{11} / g (Fold)	UVB + Antioxidants MVP x 10^{11} / g (Fold)
	28	5.1 (1.0)	8.1 (1.6)	5.6 (1.0)	7.4 (1.3)
	26	10.8 (1.0)	1.6 (0.15)	2.9 (1.0)	1.4 (0.5)
	25	0.95 (1.0)	1.6 (1.7)	3.6 (1.0)	2.4 (0.7)
	29	2.8 (1.0)	9.4 (3.3)	6.7 (1.0)	3.1 (0.5)
	24	6.2 (1.0)	12.0 (1.9)	6.4 (1.0)	7.1 (1.1)
	27	3.3 (1.0)	5.8 (1.8)	5.6 (1.0)	7.2 (1.3)
	23	9.5 (1.0)	23.3 (2.5)	4.8 (1.0)	2.6 (0.5)
	29	0.96 (1.0)	1.5 (1.5)	7.4 (1.0)	8.7 (1.2)
Mean fold change (+/- SEM)		1.0	1.8 (+/- 0.31) P = 0.02	1.0	0.9 (+/- 0.13) P = 0.21
z-Test:		P = 0.0074 Δ Fold UVB-induced MVP release pre- vs. post-antioxidant administration			

B

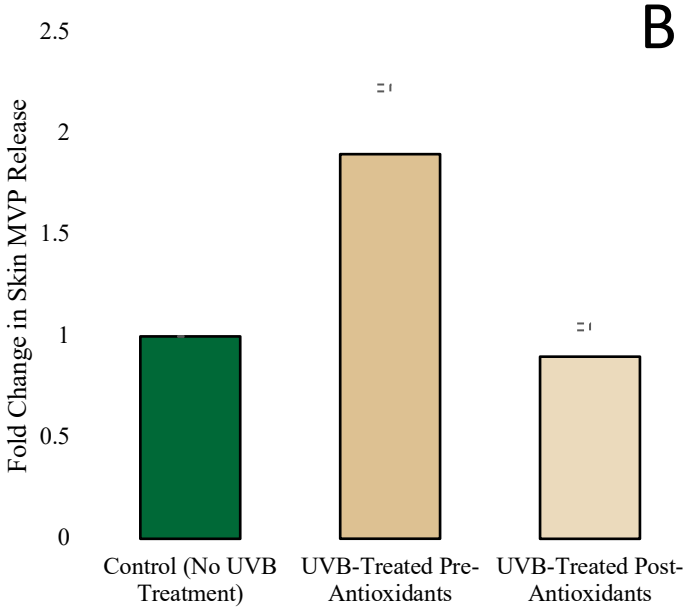


Figure 2: UVB-induced MVP release is diminished after antioxidant supplementation. Eight male subjects were treated with 1000 J/m^2 of UVB irradiation to the volar forearm and 5 mm punch biopsies were collected 4 hours later on both UVB-treated and control (non UVB-treated) skin. This procedure was repeated 8 days later on the contralateral volar forearm following an 8-day course of daily 2000 mg vitamin C and 1000 mg vitamin E antioxidants. **A)** MVP data are numerically presented as both particle levels per gram of sample tissue and fold change +/- SEM

treated skin. **B)** Skin MVP release data in fold change +/- SEM are visualized with a bar graph.

Discussion/Conclusion

Upon exposure to UVB-irradiation, there was a significant increase in skin MVP release *in vivo*. These findings are in agreement with previous *in vitro* and *ex vivo* studies where cellular cultures or human skin explants were used [1]. Administration of oral antioxidants vitamin C and E reduced tissue UVB-induced MVP response to basal (non UVB-treated control) levels, supporting prior *in vitro* and *ex vivo* findings. Seeing how keratinocyte MVP release is stimulated by both CPAF (PAF agonist) and UVB-irradiation, it is postulated that UVB-induced MVP release from keratinocytes is indirectly mediated through activation of PAFR [5]. *In vitro* studies have found UVB-stimulated production of PAF and oxidized glycerophosphocholine PAFR agonists via induction of reactive oxygen species (ROS) [3,7]. PAF-agonist generation through an ROS-generating pathway explains the effectiveness of ROS-neutralizing vitamin C and E antioxidants. Previous data from Eberling-Konig et al. demonstrate an increased minimal erythema dose (MED, dose required to elicit sunburn) and decreased cutaneous blood flow in participants taking antioxidant vitamins. These data suggest a protective effect of antioxidants, raising the amount of UVB required to obtain a sunburn and decreasing sunburn severity. Our data expands these findings, adding diminished systemic immunosuppression to these antioxidants' repertoire. Of interest, we did not observe a noticeable decrease in UVB-induced erythema after vitamin C and E administration. This is likely secondary to the larger, approximately 3 MED of UVB administered and capable of overcoming photoprotective effects.

Future research can evaluate if systemic antioxidant administration significantly affects sunburn duration or identify the therapeutic photoprotective dose. Additionally, establishing the time frame photoprotection occurs after antioxidant administration and how long these effects last after cessation may be of interest. The prominent limitations of this study include its small, but still

significant, sample size (N=8) and the use of a single, moderate dose of UVB irradiation to stimulate MVP release. Sample size was negatively affected by the COVID-19 pandemic and subsequent limitations on university-sponsored clinical research activities. Stimulating MVP release with a single ~ 3 MED of UVB may not best represent a population's typical daily UVB exposure, which is more likely to be brief intermittent exposures. Future studies evaluating dose-dependent UVB-induced MVP release and antioxidant inhibition *in vivo* may further elucidate this relationship.

In brief, we were able to demonstrate and quantify UVB-dependent release of immunosuppressive MVPs from epidermal keratinocytes *in vivo*. UVB-induced MVP release was diminished following systemic antioxidant administration. These data may have future clinical implications in treatment and prevention of sunburn and associated immunosuppression.

References

1. Fahy K., Liu L., Rapp C.M., Borchers C., Bihl J.C., Chen Y., Simman R., Travers J.B. UVB-generated Microvesicle Particles: A Novel Pathway by Which a Skin-specific Stimulus Could Exert Systemic Effects. Photochem Photobiol. 2017 Jul;93(4):937-942.
2. Berry D., Yao Y., Yi Q., Konger R.L., Travers J.B. Ultraviolet B radiation of human skin generates platelet-activating factor receptor agonists. Photochem Photobiol 2010 July;86(4):949-54.
3. Yao Y., Wolverton J.E., Zhang Q., Marathe G.K., Al-Hassani M., Konger R.L., Travers J.B. Ultraviolet B radiation generated platelet-activating factor receptor agonist formation involves EGF-R-mediated reactive oxygen species. J Immunol. 2009 March 1;182(5):2842-8.
4. Zhang Q., Yao Y., Konger R.L., Sinn A.L., Cai S., Pollok K.E., Travers J.B. UVB radiation-mediated inhibition of contact hypersensitivity reactions is dependent on the platelet-activating factor system. J Invest Dermatol. 2008 July;128(7):1780-7.
5. Bihl J.C., Rapp C.M., Chen Y., Travers J.B. UVB Generates Microvesicle Particle Release in Part Due to Platelet-activating Factor Signaling. Photochem Photobiol. 2016 May;92(3):503-6.
6. Eberling-Konig B., Placzek M., Przybilla B. Protective effect against sunburn of combined systemic ascorbic acid (vitamin C) and d-alpha-tocopherol (vitamin E). J Am Acad Dermatol. 1998 May; 38(1):45-8.
7. Marathe G.K., Johnson C., Billings S.D., Southall, M.D., Pei Y., Spandaum D., Murphy R., Zimmerman, G., McIntyre, T., Travers, J. Ultraviolet B radiation generates platelet-activating factor-like phospholipids underlying cutaneous damage. J Biol Chem. 2005, 280 (42): 35448-57.