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Circadian Rhythm and UV-Induced Skin Damage: An In Vivo Study

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ABSTRACT

Exposure of the skin to ultraviolet (UV) irradiation causes many detrimental effects through mechanisms related to oxidative stress and DNA damage. Excessive oxidative stress can cause apoptosis and cellular dysfunction of epidermal cells leading to cellular senescence and connective tissue degradation. Direct and indirect damage to DNA predisposes the skin to cancer formation. Chronic UV exposure also leads to skin aging manifested as wrinkling, loss of skin tone, and decreased resilience. Fortunately, human skin has several natural mechanisms for combating UV-induced damage. The mechanisms operate on a diurnal rhythm, a cycle that repeats approximately every 24 hours. It is known that the circadian rhythm is involved in many skin physiologic processes, including water regulation and epidermal stem cell function. This study evaluated whether UV damage and the skin's natural mechanisms of inflammation and repair are also affected by circadian rhythm. We looked at UV-induced erythema on seven human subjects irradiated with simulated solar radiation in the morning (at 08:00 h) versus in the afternoon (at 16:00 h). Our data suggest that the same dose of UV radiation induces significantly more inflammation in the morning than in the afternoon. Changes in protein expression relevant to DNA damage, such as xeroderma pigmentosum, complementation group A (XPA), and cyclobutane pyrimidine dimers (CPD) from skin biopsies correlated with our clinical results. Both XPA and CPD levels were higher after the morning UV exposure compared with the afternoon exposure.

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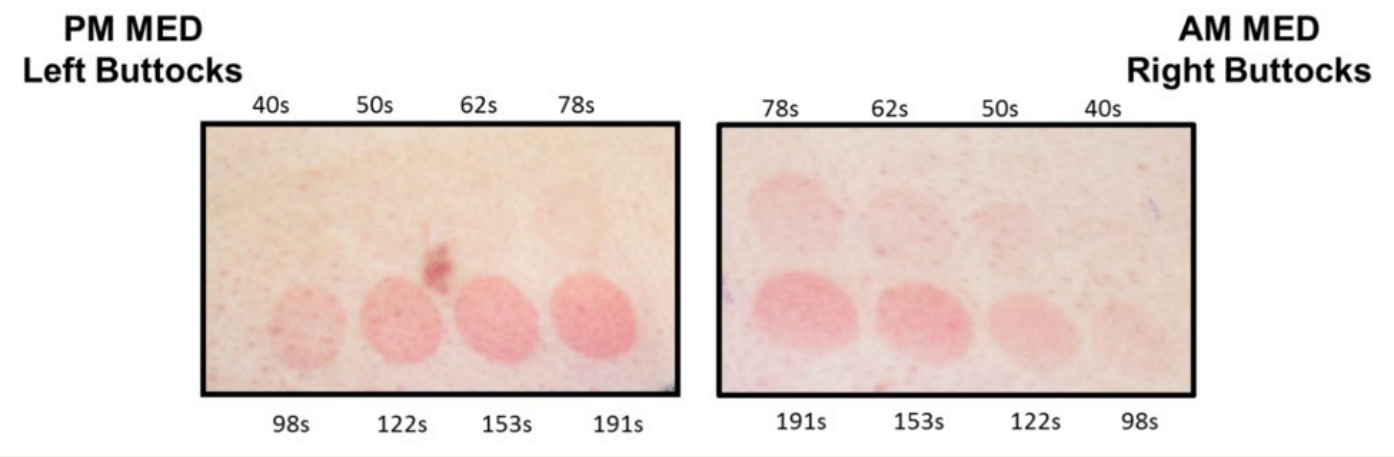
INTRODUCTION

The human body and its functions have a natural rhythm controlled by a region in the anterior hypothalamus known as the suprachiasmatic nucleus. This region modulates many physiological processes in a pattern that follows a 24-hour cycle, known as the circadian rhythm.[1] Circadian rhythm is influenced by light and dark cycles and regulates hormone levels, body temperature, and sleep.[2,3,4] This central clock also relays signals to peripheral organs and plays an integral role in skin function.[5,6] One of the main functions of the skin is to maintain homeostasis by acting as a barrier against water loss. The circadian rhythm affects the skin's role in water homeostasis by regulating the expression of aquaporin 3 in the epidermal stratum basalis for the modulation of water content and trans-epidermal water loss.[7] In addition, epidermal stem cells also display temporal oscillations in a circadian manner.[8] The stem cells' ability to undergo mitosis also assists in skin homeostasis through proliferation, differentiation, and response to UV damage. Furthermore, it has been reported that the skin displays time-dependent variations in temperature, pH, and barrier function.[9]

A recent mouse study demonstrated that UV-induced erythema in mouse skin was also affected by circadian rhythm.[10] It was shown that under the same UV conditions, mice irradiated in the AM showed significantly elevated erythemal response compared with those mice irradiated in the PM.[10] It is already established that ultraviolet radiation (UVR) causes many adverse effects on the skin from photoaging, increased susceptibility to skin cancer,

FIGURE 1. Results of visual MED 24 hours post SSR for a female subject of FST III: On the right (AM MED), there was visible full circle erythema at 62 seconds, with faint but discernible pink circles at 40 seconds and 50 seconds. On the left side (PM MED), full circle erythema occurred at 78 seconds.



and erythema.[11,12,13] The degree of erythema is directly correlated to degree of DNA damage.[14]

Our study was performed to determine whether erythema from a controlled dose of UV exposure demonstrates a circadian pattern in humans as well. We also aimed to determine whether these results were accompanied by changes in protein expression relevant to DNA damage, such as xeroderma pigmentosum, complementation group A (XPA), and cyclobutane pyrimidine dimers (CPD). XPA is a protein involved with recognizing damaged sites for nucleotide excision repair (NER) while CPD is a form of DNA damage most commonly induced by UV exposure. Both XPA and CPD are indicative of UV-induced DNA damage in the cell and the cell's efforts towards repair.[15,16]

RESULTS

7 subjects completed the study: 2 Fitzpatrick SkinType (FST) II, 4 FST III, and 1 FST IV. Sleep duration averaged 6.5-8 hours with a mean of 7.3 hours.

Similar to mice, our human subjects showed increased erythemal response to UVR in the AM compared to the UVR in the PM. Expression levels of both XPA and CPD were found to be significantly elevated in the biopsy samples irradiated in the AM compared to the biopsy samples irradiated in the PM.

Mean Erythema Dose (MED)

The mean erythema dose (MED) was performed to determine the subject's sensitivity to simulated solar radiation (SSR). MED is defined as the lowest dose of UVR that produces a change in erythema equivalent to 2.5 units in the a* axis by colorimetry.[21]

Visual MED showed increased erythema on skin exposed to SSR in the morning versus the afternoon in each subject. Figure 1 shows the results of visual MED for a female subject of FST III. On the right panel (AM MED), there was visible full circle erythema at 62 seconds, with faint but discernible pink circles at 40 and 50 seconds. In contrast, on the left side (PM MED), it took longer, 78 seconds to see a full circle erythema. Lower visual MED equates to less SSR required to produce the same amount of erythema. This trend of decreased MED in the AM versus the PM was seen in all patients.

Quantitative data on MED via chromameter readings are shown in Figure 2. The y-axis represents the MED in mJ/cm^2. The subject data along the x-axis are presented according to the order of enrollment in the study. In every subject, the MED was lower in the AM; therefore, it took a lower dose of SSR to produce erythema in the morning. Paired t-testing of the MED values in AM vs PM showed the difference to be statistically significant (P = 0.03). Figure 3 shows the mean of quantitative MED for all 7 subjects with SSR exposure in the AM versus the PM. The

FIGURE 2. Quantitative data on AM vs PM MED via chromameter readings: The y-axis represents the MED in mJ/cm^2. The subject data along the x-axis are presented according to the order of enrollment in the study.

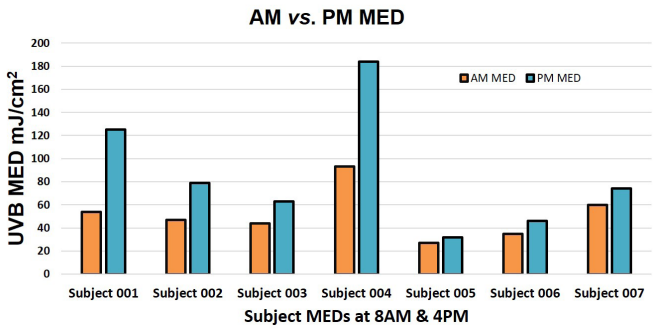
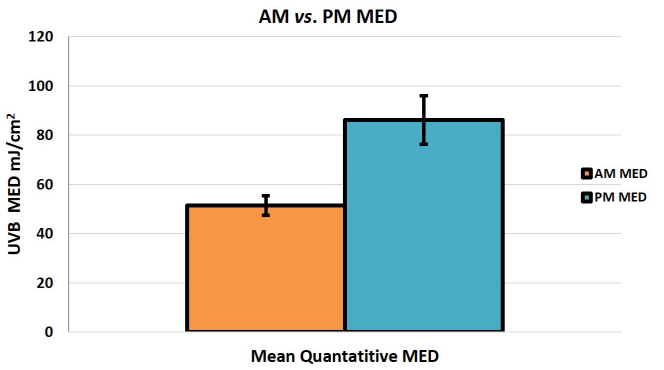


FIGURE 3. Average MED for all 7 subjects: The mean of the MED from SSR exposure in the AM was 51.43 mJ/cm^2 with standard error of the mean of 8.09 mJ/cm^2. The mean of the MED from SSR exposure in the PM was 86.14 mJ/cm^2 with standard error of the mean of 19.74 mJ/cm^2.



mean of the MED from SSR exposure in the AM was 51.43 with standard error of the mean of 8.09, while the mean of the MED from SSR exposure in the PM was 86.14 with standard error of the mean of 19.74.

XPA

Western blot was performed to determine if there was a difference in XPA expression between the AM and PM irradiated skin and compare them to unirradiated skin biopsy of subject. The unirradiated skin was taken in the PM. Western blot and quantification of data are shown in Figure 4. Actin was used as a loading control. XPA was barely detectable in the unirradiated (-UV) sample. XPA was clearly expressed in the PM UV-irradiated tissue, but showed significantly more expression in the AM UV-irradiated skin as demonstrated in Figure 4A. Compared to the unirradiated (-UV) sample, SSR exposure in the AM resulted in a 20-fold increase in XPA expression, while SSR exposure in the PM showed a less than 5 fold increase in XPA expression (Figure 4B).

Immunohistochemistal staining for XPA was performed on the corresponding biopsy sample to determine whether XPA was localized in the nucleus where it can participate in NER. As depicted in Figure 5, there was more nuclear fluorescence for XPA in the sample from the AM exposure versus the sample from the PM exposure. Both had a higher fluorescence signal compared to the unirradiated (-UV) sample.

Quantification using MetaMorph software confirms that the amount of XPA in the nuclei was significantly higher in the AM versus PM (Figure 5D). Nuclear localization was 46% higher in the AM irradiated skin compared to the unirradiated (-UV) skin, and 26% higher in the PM irradiated skin compared to the unirradiated (-UV) skin. Nuclear localization of XPA was 27% higher in the AM compared to the PM.

CPD

Immunohistochemical staining for CPD was performed on the corresponding biopsy sample to evaluate the level of UV-induced DNA damage in the nucleus. As seen in Figure 6A and 6B, there was significantly more CPD in the sample from the AM exposure versus the sample from the PM exposure. The unirradiated (-UV) sample showed slight autofluorescence with no visible CPD fluorescence in the nuclei (Figure 6C).

Quantification using MetaMorph software confirmed that the amount of CPD in the nuclei was significantly higher in the AM versus the PM exposure (Figure 6D). Nuclear localization was 31% higher in the AM irradiated skin compared to the unirradiated (-UV) skin, and only slightly higher (10%) in the PM compared to the unirradiated (-UV) skin. Nuclear localization of CPD was 25% higher in the AM compared to the PM.

FIGURE 4. Western blot comparing XPA expression level following AM vs PM UV exposure. (A) Using actin as a loading control, Western blot data for XPA expression (B) Quantification of XPA protein expression.

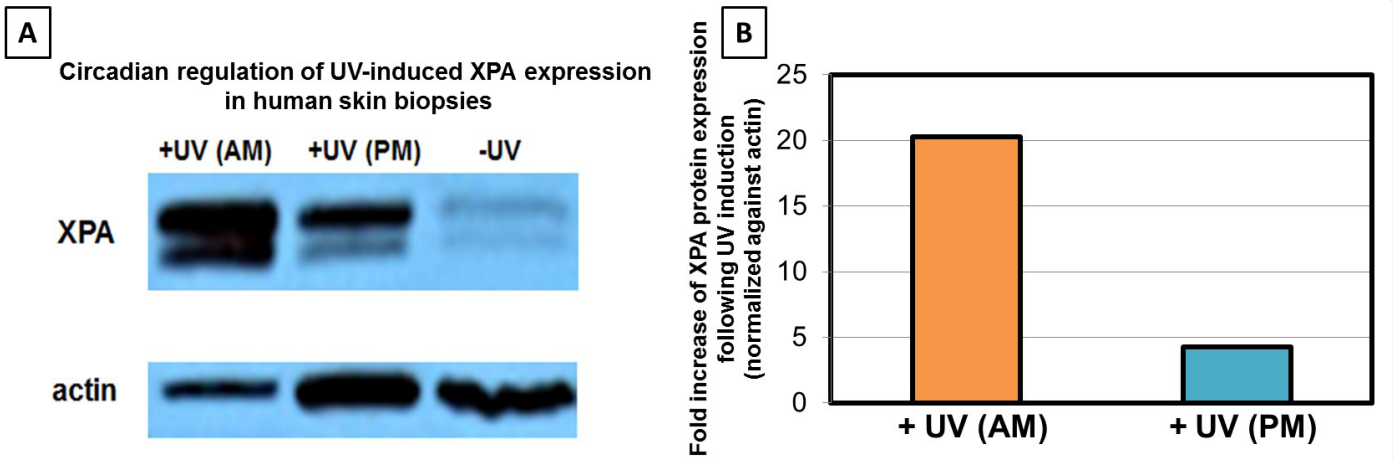
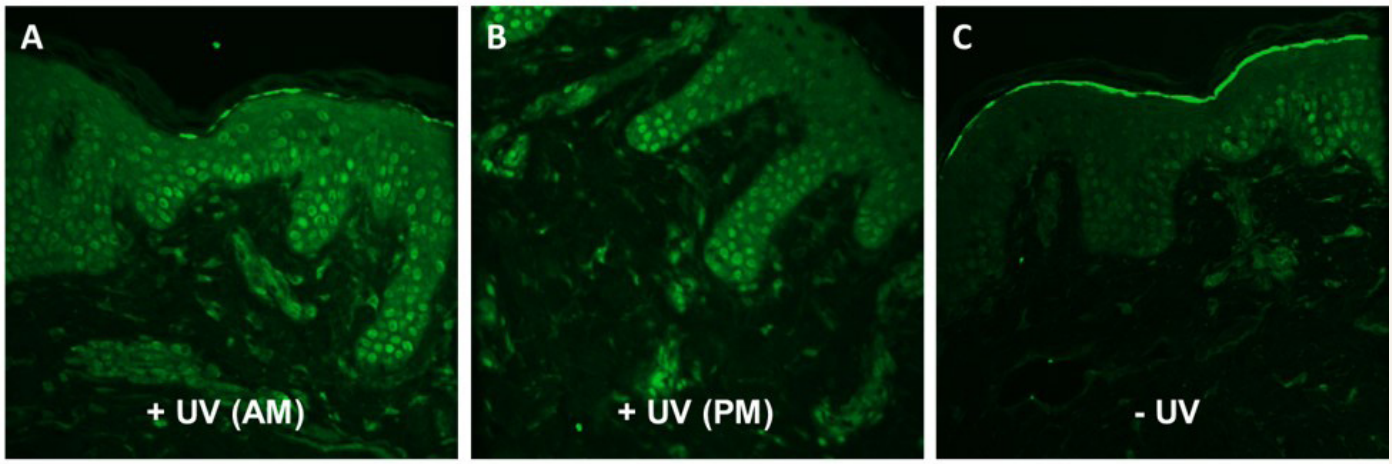
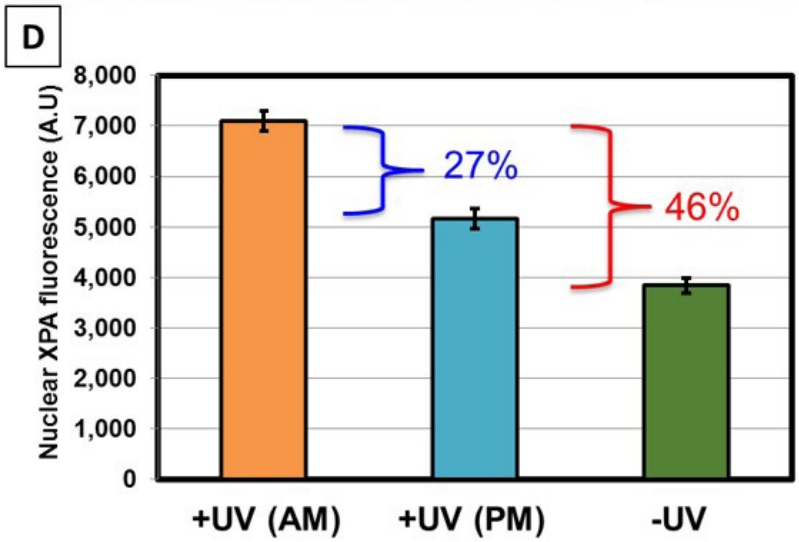


FIGURE 5. Immunofluorescence comparing XPA expression level following AM vs PM UV exposure: Representative CPD fluorescence images from skin biopsies following irradiation in the AM (A), in the PM (B), and without UV in the PM (C).





DISCUSSION

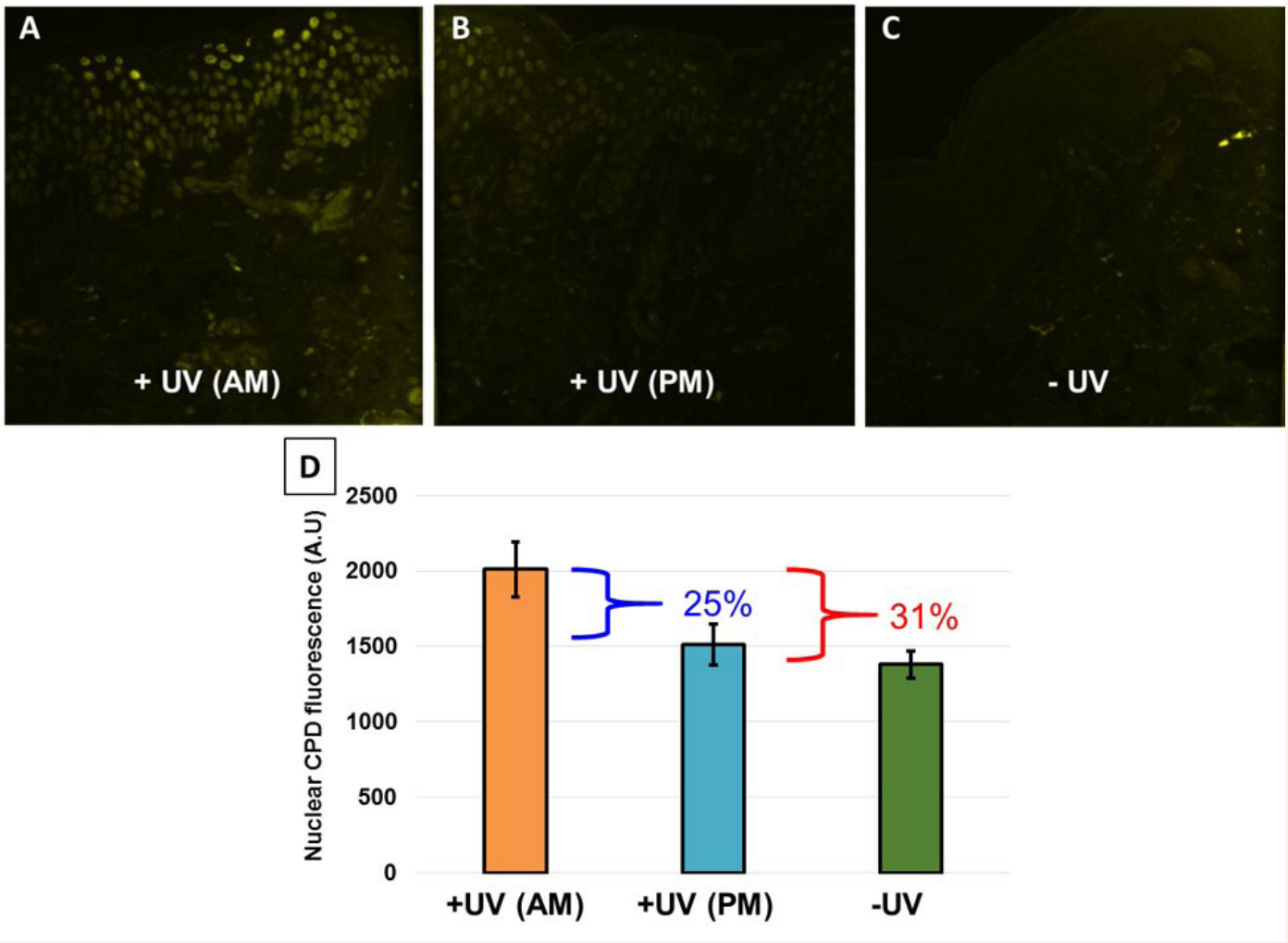
The circadian rhythm is known to affect inflammation and immunity. The pathogenesis of conditions such as diabetes, rheumatoid arthritis (RA), and cardiovascular disease have been at least partially associated with circadian rhythm dysfunction.[22] For example, the prominence of joint symptoms in the morning in RA coincides with the surge in proinflammatory IL-6 in the serum of RA patients.[22] Acute cardiac events have also been noted to be more severe in the morning.[22]

With regard to inflammation in the skin, such as in psoriasis, it has been shown that psoriatic mice subjected to sleep deprivation had increased levels of pro-inflammatory cytokines such as Kallikrein 5, IL-1, IL-6, IL-12.[23] It has also been reported that there is a higher incidence of psoriasis in American female nurses that work rotating night shifts versus those that reported no night shift work.[24] This suggests that disruption of the circadian rhythm plays a vital role in pathology.

In our study, we examined an acute and measurable event, the erythemal response to UV in the morning versus the late afternoon. At the molecular level, UV-exposed skin leads to erythema and causes breaks in DNA integrity, as well as CPDs and pyrimidine-(6,4)-pyrimidone photoproducts, which both disrupt the DNA helix.[25,26] It has been demonstrated that the circadian clock regulates DNA replication, and NER in an antiphase circadian rhythmicity.[10]

Comparing the human data from our study with the previously published mouse data,[10] it is interesting that the same trend of circadian pattern of erythema was observed. In both our study and the previous mouse study, AM UV exposure was more

FIGURE 6. Immunfluorescence comparing CPD expression level following AM vs PM UV exposure: Representative CPD fluorescence images from skin biopsies following irradiation in the AM (A), in the PM (B), and without UV in the PM (C).



erythemogenic than PM UV exposure –a contradiction to the hypothesis that humans would display the opposite pattern given that mice are nocturnal and humans are diurnal.[10]

Our study showed that the more erythemogenic AM exposure also correlated with increased XPA in skin irradiated in the AM versus the skin irradiated in the PM. This confirms the previous findings that XPA in humans peaks at 07:00 h.[27] XPA is 1 of 6 core factors in the human NER system[10,28] and is responsible for the rate-limiting step of excision.[27] It is essential for the formation of the pre-incision complex[29] and recognizing DNA damage.[27] XPA is regulated by the circadian clock as well as DNA damage. Upon DNA damage, XPA is transported to the nucleus.[30] Our findings of increased XPA localization in the nucleus in the more erythemogenic AM irradiated samples can be explained by both the increased UV-induced DNA damage in the AM leading to increased nuclear localization of XPA, as well as the effects of the circadian clock elevating XPA in the AM. The relationship between the elevated AM XPA and the increased erythema after AM UV irradiation seems to contradict the relationship observed in mice. In liver, brain, and skin from mice, XPA was found to increase during the day and decrease during the night, peaking between 16:00 and 18:00 and dipping to the minimum between 04:00 and 06:00.[27] Combining the results of our study and the mouse study, it seems that the relationship between the circadian clock regulation of XPA expression and the circadian clock regulation of erythema is confounded by other factors that need to be elucidated.

Since erythema is the outcome of interest in most experimental testing of photoprotective agents, this observed variation in AM versus PM susceptibility to UV exposure may be an indication to improve product testing protocols to account for this variability. This is similar to the methods of other specialties, timing the delivery of therapies to maximize efficacy based on known circadian cellular responses.

Furthermore, pinpointing when maximal erythema occurs in a circadian cycle can shed light on skin protection against UV damage. From the observations of our study, it may be important to change the timing of outdoor activities from the AM to the PM in order to avoid excessive UV-induced skin damage.

Experimental Section

Volunteer selection

All subjects were recruited through the Skin Study Center at University Hospitals Case Medical Center. Seven healthy, non-shift work, FST[19] II-IV adults were enrolled after informed consent was obtained.

Mean Erythema Dose (MED)

MED testing was performed at 8:00 h and 16:00 h using an 8-holed template and exposing 8, 1-cm^2 circles of buttock skin to increasing doses of SSR, a full spectrum light source that most closely resembles natural sunlight. This test was done on the right buttock for the AM MED exposure and the left buttock for the PM MED exposure. A 1000 W xenon arc solar simulator model 6271 (Oriel Instruments, Stratford, CT), with a dichroic mirror and 81017bis filter (WG320/1.5 mm), producing a spectrum of 290–400 nm was used for the irradiation at increasing length of time depending on the patient's FST. Those with lower FST were exposed to a lower starting dose. The setup for the SSR exposure is listed in previous literature.[17] The spectrum and integrated irradiance were measured with a calibrated Bentham DM 150 double monochromator spectroradiometer. Irradiance was measured routinely using an IL1700 radiometer (International Light, Newburyport, MA) equipped with a sensor for UVA (SED 033, UVA filter 19672) and UVB (SED 240, UVB filter 15541) positioned 10 inches from the light source.

After 24 hours, the areas were visually graded based on the degree of erythema to determine the visual MED. Areas that showed no redness were graded "0", incomplete circles of pink skin were graded "Trace", complete pink circles were graded "1", and complete dark pink to red circles were graded "2". The erythematous skin (full pink circle) that was exposed for the shortest duration is the visual MED. Calculating the MED is performed by measuring the amount of erythema on each exposed area as well as an adjacent non-exposed skin area, using the CR300 chromameter from Konica Minolta (Tokyo, Japan).

Linear regression was applied and 1 MED was calculated using Microsoft Excel program according to COLIPA recommendations as the dose of UV producing an increase in the redness parameter (a*) of +2.5.[18]

Tissue Analysis

6-mm punch biopsies were obtained from both SSR-irradiated skin and non-irradiated skin approximately 24 hours post SSR. The biopsies were obtained in the areas that received the highest dose of SSR and adjacent non-irradiated skin. Samples were then analyzed using Western blot and immunohistochemistry (IHC). Western blot for XPA was performed according to protocols previously listed in literature.[20] Actin was used as a control. For IHC, the fixed biopsies were embedded in paraffin and serially cut into 5-µm sections. After deparaffinization and dehydration, the skin sections were heated in epitope retrieval buffer at 95–97°C for 20 minutes then cooled for 30 minutes. They were then blocked in a dilution buffer containing 5% normal goat serum (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) and 0.5% saponin (Sigma, St. Louis, MO) in 1x phosphate buffered saline (PBS) and incubated 1 hour at room temperature with dilution buffer containing polyclonal anti-XPA antibody (ThermoFisher Scientific, Eugene, OR) or with polyclonal anti-CPD antibody (CosmoBio, Tokyo, Japan). After washing in PBS, Alexa Fluor 488- or 594-conjugated goat anti-rabbit secondary antibody (Invitrogen) was used to detect primary antibody and Vectashield Mounting Medium for Fluorescence with DAPI (Vector, Burlingame, CA) was used as a nuclear marker. To exclude nonspecific antibody staining, proper isotype controls were performed in every experiment. All images were acquired using an Ultra*VIEW* VoX spinning disk confocal system (PerkinElmer, Waltham, MA) mounted on a Leica DMI6000B microscope (Leica Microsystems, Inc., Bannockburn, IL) equipped with a HC PLAN APO 20×/0.7 objective. Confocal images of Alexa 488 or 594-conjugated anti-rabbit secondary antibody and DAPI were collected using solid-state diode lasers emitting 488-nm or 561-nm and 405-nm excitation light, respectively, and with appropriate emission filters. Images were then exported and quantitatively analyzed using MetaMorph Premier Software (Molecular Devices Corporation, Sunnyvale, CA). Quantification of XPA and CPD expression levels was performed by first tracing the nuclei of DAPI stained image and translocating the nuclei location to the corresponding antibody fluorescent image. The average pixel intensity of the circled areas were then measured and recorded via MetaMorph. Data was analyzed using Microsoft Excel.

Data Analysis

MED between skin that was irradiated in the morning versus afternoon were analyzed via T-testing and a difference of <0.05 was considered significant. Tissue data were quantified using Metamorph software and descriptive statistics were applied.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

All those who met authorship criteria were listed as authors. Authors listed contributed significantly to the manuscript.

E.B. and A.S. conceived and designed the experiments. A.S and J.S. conducted the clinical aspects of the study. L.G. performed the basic science experiments and wrote the manuscript. L.G., M.L., and A.S. analyzed the data. M.L contributed to reagents/materials/analysis tools. M.T. and S.A. aided in editing of the manuscript and compilation of the data.

DISCLOSURES

This is an investigator-initiated study where the sponsor had no role in the design of the study.

REFERENCES

1. Bell-Pedersen, D., Cassone, V.M., Earnest, D.J., Golden, S.S., Hardin, P.E., Thomas, T.L., Zoranm, M.J. Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nat Rev Genet* 2005; 6(7), 544-556.
2. Hastings, M., O'Neill, J.S., Maywood, E.S. Circadian clocks: regulators of endocrine and metabolic rhythms. *J Endocrinol* 2007; 195(2), 187-98.
3. Refinetti, R., Menaker, M. The circadian rhythm of body temperature. *Physiol Behav* 1992; 51(3), 613–637.
4. Purves, D., Augustine, G., Fitzpatrick, D., Katz, L.C., LaMantia, A.S., McNamara, J.O., Williams, M. Circadian Cycle of Sleep and Wakefulness. In *Neuroscience*, 2nd ed; Purves, D.; Augustine, G.; Fitzpatrick, D.; Katz, L.C.; LaMantia, A.S.; McNamara, J.O.; Williams, M. Eds.; Sinauer Associates: Sunderland, MA, 2001.
5. Zanello, S.B., Jackson, D.M., Holick, M.F. Expression of the Circadian Clock Genes Clock and Period in Human Skin. *J Invest Dermatol* 2000; 115(4), 757-760.
6. Geyfman, M., Andersen, B. Clock genes, hair growth and aging. *Aging* 2010; 2(3), 122-128.
7. Matsunaga, N., Itcho, K., Hamamura, K., Ikeda, E., Ikeyama, H., Furuichi, Y., Watanabe, M., Koyanagi, S., Ohdo, S. 24-hour rhythm of aquaporin-3 function in the epidermis is regulated by molecular clocks. *J Invest Dermatol* 2014; 134(6), 1636-44. doi: 10.1038/jid.2014.13.
8. Peggy, J., Toufighi, K., Solanas, G., Luis, N.M., Minkwitz, S., Serrano, L., Lehner, B., Benitah, S.A. Human Epidermal Stem Cell Function Is Regulated by Circadian Oscillations. *Cell Stem Cell* 2013; *13*(6), 745-53. doi: 10.1016/j.stem.2013.09.004.
9. Yosipovitch, G., Xiong, G.I., Haus, E., Sackett-Lundeen, L., Ashkenazi, I., Maibach, H.I. Time-Dependent Variations of the Skin Barrier Function in Humans: Transepidermal Water Loss, Stratum Corneum Hydration, Skin Surface pH, and Skin Temperature. *J Invest Dermatol* 1998; 110(1), 20-3.
10. Gaddameedhi, S., Selby, C.P., Kemp, M.G., Ye, R., Sancar, A. The Circadian Clock Controls Sunburn Apoptosis and Erythema of Mouse Skin. *J Invest Dermatol* 2015; 135(4), 1119-27.
11. Wlaschek, M., Tantcheva-Poór, I., Naderi, L., Ma, W., Schneider, L.A., Razi-Wolf, Z., Schüller, J., Scharffetter-Kochanek, K. Solar UV irradiation and dermal photoaging. *J Photochem Photobiol B* 2001; 63(1-3), 41-51.
12. Ziegler, A., Jonason, A.S., Leffell, D.J.; et al. Sunburn and p53 in the onset of skin cancer. *Nature* 1994; 372(6508), 773–6.
13. Harrison, G.I., Young, A.R. Ultraviolet radiation-induced erythema in human skin. *Methods* 2002; 28(1), 14-9.
14. Woollons, A., Kipp, C., Young, A.R., Petit-Frère, C., Arlett, C.F., Green, M.H., Clingen, P.H. The 0.8% ultraviolet B content of an ultraviolet A sunlamp induces 75% of cyclobutane pyrimidine dimers in human keratinocytes in vitro. *Br J Dermatol* 1999; 140(6), 1023-30.
15. Toshiro, M., Saijo, M., Kuraoka, I., Kobayashi, T., Nakatsu, Y., Nagai, A., Enjoji, T., Masutani, C., Sugasawa, K., Hanaoka, F., Yasui, A., Tanaka, K. DNA Repair Protein XPA Binds Replication Protein A (RPA). *J Biol Chem* 1995; 270, 4152-4157.
16. Woollons, A., Clingen, P.H., Price, M.L., Arlett, C.F., Green, M.H. Induction of mutagenic DNA damage in human fibroblasts after exposure to artificial tanning lamps. Br J Dermatol 1997; 137(5), 687-92.
17. Baron, E.D., Fourtanier, A., Compan, D., Medaisko, C., Cooper, K.D., Stevens, S.R. High Ultraviolet a Protection Affords Greater Immune Protection Confirming that Ultraviolet A Contributes to Photoimmunosuppression in Humans. *J Invest Dermatol* 2003; 121, 869–875. doi:10.1046/j.1523-1747.2003.12485.x.
18. Ferguson, J., Brown, M., Alert, D., Bielfeldt, S., Brown, J., Chardon, A., Hourseau, C., Mazilier, C., Cuthbert, J., D'Arcy-Burt, K., Jolley, J., Murdoch, M., Finkel, P., Masson, P., Merot, F., Maclennan, A., Poret, J., Siladgi, S. Collaborative development of a sun protection factor test method: A proposed European standard. *Int J Cosm Sci* 1996; 18, 203–218.
19. Bickers, D.R. Photosensitivity and other reactions to light. In *Harrison's Principles of Internal Medicine*, 18th Edition; Mc-Graw Hill: New York, NY, 2005.
20. Lam, M., Lee, Y., Deng, M., Hsia, A.H., Morrissey, K.A., Yan, C., Azzizudin, K., Oleinick, N.L., McCormick, T.S., Cooper, K.D., Baron, E.D. Photodynamic Therapy with the Silicon Phthalocyanine Pc 4 Induces Apoptosis in Mycosis Fungoides and Sezary. *Adv Hematol* 2010.
21. Iordanou, E., Berneburg, M. Phototherapy and photochemotherapy. *J Dtsch Dermatol Ges* 2010; 8(7), 533-541.
22. Hochberg, M.C., Silman, A.J., Smolen, J.S. *Rheumatology*, 5th edition.; Elsevier Health Sciences: Philadelphia, PA, 2010.
23. Hirotsu, C., Rydlewski, M., Araújo, M.S., Tufik S., Andersen, M.L. Sleep Loss and Cytokines Levels in an Experimental Model of Psoriasis. *PLoS One* 2012, 7(11). doi: 10.1371/journal.pone.0051183.
24. Li, W., Qureshi, A.A., Schernhammer, E.S., Han, J. Rotating night shift work and risk of psoriasis in US women. *J Invest Dermatol* 2013; 133(2), 565–567.
25. Baron, E.D., Suggs, A. Introduction to Photobiology. *Dermatol Clin* 2014; 32(3), 255-66.
26. de Lima-Bessa, K,M., Armelini, M.G., Chiganças, V., Jacysyn, J.F., Amarante-Mendes, G.P., Sarasin, A., Menck, C.F. CPDs and 6-4PPs play different roles in UV-induced cell death in normal and NER-deficient human cells. *DNA Repair* 2008; 7(2), 303-12.
27. Gaddameedhi, S., Selbya, C.P., Kaufmannb, W.K, Smarte, R.C, Sancar A. Control of skin cancer by the circadian rhythm. *PNAS* 2011; 108(46), 18790-18795.
28. Kang, T.; Reardon, J.T.; Sancar, A. Regulation of nucleotide excision repair activity by transcriptional and post-transcriptional control of the XPA protein. *Nucleic Acids Res* 2011, 39(8), 3176–3187.
29. Sugasawa, K. Xeroderma pigmentosum genes: functions inside and outside DNA repair. *Carcinogensis* 2008, 29(3), 455-465.
30. Li, Z.; et al. UV-induced nuclear import of XPA is mediated by importin-α4 in an ATR-dependent manner. *PloS One* 2013, 8(7).

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