Short communication

Influence of skin melanisation and ultraviolet radiation on biomarkers of systemic oxidative stress

Barbara B. Shiha, Mark D. Farrarb, Andy Vailb, Donald Allanc, Mu-Rong Chaod, Chiung-Wen Huc, George D.D. Jonesf, Marcus S. Cookeg, Lesley E. Rhodese

a Centre for Dermatology Research, Division of Musculoskeletal and Dermatological Sciences, School of Biological Sciences, Faculty of Biology Medicine and Health, The University of Manchester and Salford Royal NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK
b Centre for Biostatistics, Division of Population Health, Health Services Research & Primary Care, School of Health Sciences, Faculty of Biology Medicine and Health, The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK
c Medical Physics Department, Salford Royal NHS Foundation Trust and The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK
d Department of Occupational Safety and Health, Chung Shan Medical University, Taichung, 402, Taiwan
e Department of Public Health, Chung Shan Medical University, Taichung, 402, Taiwan
f Leicester Cancer Research Centre, University of Leicester, Leicester, UK
g Oxidative Stress Group, Department of Cell Biology, Microbiology and Molecular Biology University of South Florida, Tampa, FL, 33620, USA

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ABSTRACT

Skin melanisation ranges widely across human populations. Melanin has antioxidant properties and also acts as a filter to solar ultraviolet radiation (UVR) incident upon the skin. In this study we firstly examined whether melanin level might influence baseline levels of systemic oxidative stress, in 65 humans in vivo from the same geographical area ranging from the lightest to darkest skin type (phototype I-VI). This was examined in winter-time (latitude 53.5°N). Remarkably, we found that urinary biomarkers of oxidatively-generated DNA damage (8-oxodG) and RNA damage (8-oxoGuo) were significantly correlated with skin lightness (L*), such that 14–15% of the variation in their baseline levels could be explained by skin colour. Next we exposed 15 humans at the extremes of skin melanisation to a simulated summer-time exposure of solar UVR (95% UVA, 5% UVB; dose standardised to sunburn threshold), following which they provided a sample of every urine void over the next five days. We found that UVR induced a small but significant increase in urinary 8-oxodG and 8-oxoGuo, with differing kinetics between skin types. Thus greater melanisation is associated with protection against systemic oxidative stress, which may reflect melanin’s antioxidant properties, and solar UVR exposure also influences systemic oxidative stress levels in humans. These novel findings may have profound implications for human physiology and health.

1. Introduction

Cutaneous melanin absorbs solar ultraviolet radiation (UVR), providing protection from skin cancer [1], although may also protect via its antioxidant properties. Reactive oxygen species (ROS) [2], and DNA strand breaks [3] both inversely correlate with melanocyte melanin levels in vitro. Levels of skin melanisation across the human skin colour range (phototype I-VI) also show differential distribution of the predominantly directly UVR-induced skin DNA lesion, the cyclobutane pyrimidine dimer (CPD), in vivo [4]. However, potential differences in the formation/repair of indirectly-generated, oxidatively-damaged DNA across human phenotypes remains unexplored, despite their likely contribution to skin cancer development [5]. Moreover, oxidatively-generated DNA and RNA damage have wider significance as biomarkers of systemic oxidative stress, with potentially detrimental cellular effects [6]. They can be measured non-invasively via their urinary excretion [7], permitting multiple measurements and human biology investigation in vivo.

* Corresponding author. Photobiology Unit, Dermatology Centre, University of Manchester and Salford Royal NHS Foundation Trust, Manchester, M6 8HD, UK.
** Corresponding author. Oxidative Stress Group, Department of Cell Biology Microbiology and Molecular Biology University of South Florida, Tampa, FL, 33620, USA.

E-mail addresses: bshih@ed.ac.uk (B.B. Shih), mark.farrar@manchester.ac.uk (M.D. Farrar), andy.vail@manchester.ac.uk (A. Vail), donald.allan@srf.nhs.uk (D. Allan), mrchao@csmu.edu.tw (M.-R. Chao), windyhu@csmu.edu.tw (C.-W. Hu), gdj2@le.ac.uk (G.D.D. Jones), cookem@usf.edu (M.S. Cooke), lesley.e.rhodes@manchester.ac.uk (L.E. Rhodes).

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Oxidatively-generated DNA damage can be induced in skin cells through ROS derived from UVR-mediated photosensitization [8,9], the main oxidation product being 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) [10–12]. As 8-oxodG may mispair and misincorporate with adenine, failure to remove this DNA lesion can result in G→T and A→C transversion mutations [13], with implications for carcinogenesis. Indeed, oxidatively-generated DNA damage contributes ~8% of longer wavelength UVA-induced mutations [14]. However, ROS generation is not unique to UVR; they are produced by oxidative phosphorylation in mitochondria, and other endogenous and exogenous processes. Urinary 8-oxodG is proposed as a ‘whole-body’ biomarker of oxidative stress; while its precise origins are unclear, sanitisation of 8-oxodGTP from the dGTP nucleotide precursor pool appears the prime candidate [15].

UVR-induced RNA oxidation has been demonstrated in human skin fibroblasts in vitro [16]. Formation, repair, measurement, and biological consequences of oxidatively-generated RNA damage are less studied than for DNA, but information is emerging [17]. Similar to 8-oxodG, RNA oxidation products such as 8-oxo-7,8-dihydroguanosine (8-oxoGuo) are conveniently and sensitively measured in urine, with analogous origins i.e. the ribonucleotide precursor pool [18]. RNA oxidation is also suggested as a disease marker, offering different prognostic value from DNA oxidation markers [19,20]. In several experimental systems, levels of oxidatively-generated damage were higher in RNA than DNA [21]. However, we are unaware of studies examining urinary RNA in relation to melanin level or as a marker of UVR-induced oxidative stress.

Our objectives were: (1) evaluate baseline urinary 8-oxodG and 8-oxoGuo levels across the human phototypes, from I (light white skin) to VI (black skin), examining relationship to skin melanisation; (2) examine these biomarkers in the lightest and darkest phototypes after a single, sub-sunburn exposure to UVR simulating summer sunlight.

2. Material and methods

2.1. Study design

The human study (Fig. 1) occurred at the Photobiology Unit, Dermatology Centre, Salford Royal Hospital, Manchester, UK (53.5°N), in November–March (2012–2013 or 2013–2014) when ambient UVR influence is minimal. Healthy volunteers, phototype I–VI, 20–49 y, from the Greater Manchester area, participated. Exclusions: history of skin cancer/photosensitivity, sunbathing/sunbed in prior three months/taking vitamin D supplements or phototoxic medication/pregnancy/breast-feeding/smoking. The study was approved by The University of Manchester Research Ethics Committee (ref 11266), registered at www.isrctn.org (ref 99738113) and adhered to Declaration of Helsinki principles; participants gave written informed consent.

2.2. Skin assessments

Detailed standardised phototype assessment was performed according to modified Fitzpatrick [22]. Volunteers described their (i) propensity to burn: virtually always/sometimes/rarely/never; (ii) propensity to tan: never/light/medium/heavy; (iii) response to first occasion of 30–40 min unprotected exposure to midday sun. Volunteers’ ethnicity, skin/hair/eye colour, and freckling presence/absence were recorded.

A spectrophotometer (CM-600D, Konica Minolta, Tokyo, Japan) recorded triplicate measurements of skin lightness (L*) from a sun-protected site (upper buttck, or upper inner arm if lighter) using the L*a*b* colour space, scale 0–100 (black-white) [23]. An individual’s minimal erythema dose (MED) was assessed as the lowest UVR dose producing visually discernible erythema at 24 h, as follows. A geometric series of 10 doses (~30% increments) of erythemally-weighted UVR was applied to unprotected skin (upper buttck, or upper inner arm if lighter) using a Philips (Amsterdam, Netherlands) TL-20W/12 lamp (280–400 nm, peak 312 nm). Thresholds in darker skin were confirmed by determining minimal flux dose, as described [4,24].

2.3. Simulated sunlight exposure in vivo

A single 0.8 MED of UVR was given to each volunteer using a horizontal whole-body irradiation cabinet (Phillips HB598) fitted with Arimed-B (Cosmedico GmbH, Stuttgart, Germany) fluorescent tubes emitting a UVR spectrum similar to UK midday summer sunlight (95% UVA: 320–400 nm; 5% UVB: 290–320 nm). Emission was characterised and monitored as described [4]. Volunteers wore standardized T-shirt and shorts, i.e. summer clothing exposing ~35% body surface area (BSA) [4].

2.4. Urinary sampling and analysis

All volunteers provided a morning baseline mid-stream urine sample. Following UVR, each subsequent void was collected for five days (additional to a sample immediately pre-UVR). Samples were stored at ~20 °C until analysis at Chung Shan Medical University, Taichung, Taiwan for creatinine, and 8-oxodG and 8-oxoGuo concentrations using validated LC-MS/MS methodology [18]. The limits of detection were 0.002 ng/mL for 8-oxodG and 0.003 ng/mL for 8-oxoGuo. Intraday/interday imprecisions in urine ranged from 1.4 to 5.0%.
for 8-oxodG and 2.9–13.7% for 8-oxoGuo; recoveries in urine were 94–101% and 109–117% respectively.

2.5. Statistical analysis

Outcomes were urinary 8-oxodG and 8-oxoGuo. Data were In-transformed for analysis. Pearson correlation coefficient examined relationship between L* and baseline 8-oxodG or 8-oxoGuo. Effect of time post-UVR and phenotype on urinary 8-oxodG and 8-oxoGuo post-UVR was analysed by linear mixed-effects regression. Analyses were adjusted for repeated measurements by treating volunteers as a random effect. As 8-oxodG and 8-oxoGuo were hypothesised to increase, or increase then decrease, over the five day collection period, both time post-UVR and (time post-UVR)^2 were explored as the independent variable.

3. Results

3.1. Volunteer characteristics

Sixty-five volunteers (mean 31 years; 34F/31 M; Table 1) participated, each providing a baseline urine sample. Fifteen of these received a UVR exposure, providing a total 460 post-UVR urine samples (Fig. 1).

3.2. Baseline urinary 8-oxodG and 8-oxoGuo across phototypes

Mean baseline urinary 8-oxodG and 8-oxoGuo for each phototype are shown (Table 2).

Little is known of the relationship between systemic oxidative stress and constitutive skin pigmentation. Moreover, influence of cutaneous UVR exposure on oxidative stress is poorly understood. Herein, we showed, for the first time, that baseline levels of both urinary 8-oxodG and 8-oxoGuo correlate with phenotype, suggesting that skin melanisation provides protection against oxidative stress. Levels of 8-oxodG were ~twice as high and 8-oxoGuo ~1.5 times higher in the lightest versus darkest skin type. To examine the impact of UVR exposure, individuals of the lightest and darkest skin received a near-sunburn dose of solar simulating UVR. Collection and analysis of every urine void for five days post-UVR revealed the single exposure to ~35% BSA was

| Table 1 | Characteristics of all volunteers (n = 65). |
|---------|--------------------------------------------|
| Skin type | n | Gender (F/M) | Age | L* | MED (mJ/cm²) | Skin colour | Hair colour | Eye colour |
| I | 12 | 3/9 | 36 (8) | 73.9 (2) | 21 (5) | Light white | Sandy/red (25%) | Light blue/green/grey (42%) |
| II | 14 | 9/5 | 29 (6) | 72.0 (3) | 26 (4) | Light white | Blonde (7%) | Light blue/green/grey (7%) |
| III | 19 | 11/8 | 31 (7) | 69.7 (3) | 42 (15) | White | Chestnut/dark blonde (22%) | Light blue/green/grey (17%) |
| IV | 6 | 4/2 | 30 (9) | 63.1 (5) | 57 (13) | Olive/light brown | Black (67%) | Black (33%) |
| V | 7 | 2/5 | 30 (8) | 50.2 (7) | 75 (19) | Mid-brown | Dark brown (29%) | Dark brown (100%) |
| VI | 7 | 5/2 | 31 (8) | 40.5 (5) | 21 (141) | Dark brown/black | Dark brown (29%) | Dark brown (100%) |

Data are mean (SD) unless otherwise stated.
* Missing information on hair and eye colour for one volunteer.

| Table 2 | Baseline urinary 8-oxodG and 8-oxoGuo levels grouped by skin type and ethnicity (n = 64). |
|---------|-----------------------------------------------|
| Skin type | n | 8-oxodG (ng/mg creatinine) | 8-oxoGuo (ng/mg creatinine) |
| I | 12 | 3.9 (1.6) | 5.6 (1.4) |
| II | 14 | 3.7 (1.0) | 4.9 (1.0) |
| III | 19 | 4.1 (1.9) | 5.5 (1.5) |
| IV | 6 | 3.4 (2.3) | 5.5 (2.2) |
| V | 7 | 2.1 (0.6) | 4.1 (0.6) |
| VI | 6 | 2.0 (0.6) | 3.5 (0.6) |

Data are mean (SD).
sufficient to produce detectable increases in both 8-oxodG and 8-oxoGuo in light and dark skin people (Fig. 4).

In a pilot study, higher urinary 8-oxodG in individuals of phototype II than V was incidentally observed [25]. Our investigation in groups of individuals over the entire phototype range has now revealed a significant positive correlation between skin lightness ($L^*$) and baseline 8-oxodG (r = 0.372, P = 0.002) and 8-oxoGuo (r = 0.386, P = 0.002). Data shown for n = 64 volunteers (missing 8-oxodG data for n = 1).

Since UVR can cause oxidatively-generated damage through ROS generation, we performed a post-UVR time-course study. This revealed that a solar-simulating UVR exposure provoked an increase in urinary 8-oxodG and 8-oxoGuo across light and dark skin types (P = 0.01, P = 0.001 respectively). In light skin individuals, levels of both species showed initial increase followed by return to baseline, with peak ~ day three. This pattern of response was less evident for 8-oxodG in dark skin individuals, while for 8-oxoGuo the kinetics significantly differed from light skin individuals, with no evidence of decrease during the five days. This indicates a longer period of cutaneous nucleic acid damage repair in the darker skin group, potentially reflecting the higher absolute UVR dose given and/or intrinsic difference in repair kinetics.

Studies of urinary biomarkers of oxidative stress following UVR exposure are scarce. Urinary 8-oxodG was examined following single-dose whole-body photopheresis (psoralen-UVA; PUVA); urinary 8-oxodG peaked ~ day four [35]. However, PUVA’s phototoxic reaction differs from sunburn. Pilot work exploring impact of low level UVR exposures showed no impact on urinary 8-oxodG [25]. However, the current, personalised UVR dose, close to the sunburn threshold (0.8 MED, median SED 1.68 and 7.2 in phototypes I and V/VI respectively) to ~35% BSA provided a level of insult that induced oxidative stress. Sun exposure recommendations are to keep below personal sunburn threshold; accordingly we UVR-exposed volunteers according to individual threshold. Pivotally, we mimicked natural conditions (UVR emission close to ambient summer sunlight: 5% UVB, 95% UVA; volunteer wearing summer clothing). In contrast, personal exposure to ambient UVR is extremely low in winter-time at 53.5°N (~0.1 SED/week to ~8% BSA) [36].

In conclusion, this original work notably reveals a linear relationship between skin lightness and baseline 8-oxodG and 8-oxoGuo levels, which we propose is principally due to an antioxidant effect of melanin. Further, sub-sunburn cutaneous UVR exposure can cause detectable levels of oxidatively-generated damage to nucleic acids, so simple avoidance of visible skin redness is insufficient to avoid tissue damage.

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**Table 3**

Characteristics of volunteers who underwent simulated sunlight exposure (n = 15).

| Skin type | MED, mJ/cm² | UVR dose, mJ/cm² (SED) | L* | Ethnicity |
|-----------|-------------|-------------------------|----|-----------|
| I         | 12          | 10 (1.0)                | 75.35 | White Caucasian |
| I         | 14          | 11 (1.1)                | 77.07 | White Caucasian |
| I         | 20          | 16 (1.6)                | 73.91 | White Caucasian |
| II        | 21          | 17 (1.7)                | 72.16 | White Caucasian |
| II        | 21          | 17 (1.7)                | 70.76 | White Caucasian |
| II        | 28          | 22 (2.2)                | 73.61 | White Caucasian |
| II        | 28          | 22 (2.2)                | 75.61 | White Caucasian |
| II        | 28          | 22 (2.2)                | 72.29 | White Caucasian |
| III       | 68          | 54 (5.4)                | 58.05 | South Asian |
| III       | 90          | 72 (7.2)                | 52.80 | South Asian |
| IV        | 90          | 72 (7.2)                | 46.84 | Black |
| IV        | 102         | 82 (8.2)                | 49.31 | South Asian |
| V         | 83          | 66 (6.6)                | 44.98 | Black |
| V         | 163         | 130 (13.0)              | 39.02 | Black |
| V         | 205         | 164 (16.4)              | 36.18 | Black |
| VI        |             |                        |      |            |

* South Asian volunteers were Indian or Pakistani; Black volunteers were Black African or Black British.
and solar UVR may contribute to systemic oxidative stress during summer-time. Biomarkers 8-oxodG and 8-oxoGuo behaved similarly in response to UVR, suggesting similarity in origin e.g. the nucleotide precursor pools, while differences in their kinetics were apparent between light and dark skin types.

Future studies could examine quantity/location of oxidatively-generated damage in the tissues of light and dark skin people, together with DNA repair capacity. Further perspectives for research include a comparison of the responses of oxidative stress biomarkers derived from other groups of compounds, e.g. lipid/protein that occur in urine. The

Fig. 3. Observed points and modelled curves (one per participant) for urinary 8-oxodG and 8-oxoGuo levels following 0.8 MED of UVR. Urinary 8-oxodG and 8-oxoGuo levels were measured in every urine void for five days post-UVR. (a) A statistically significant (P = 0.01) increase and decrease in 8-oxodG occurred overall, with dark skin types at much lower values throughout. The apparent difference in curvature between skin type groups was not statistically significant (P = 0.11). (b) A statistically significant (P = 0.001) change in 8-oxoGuo levels occurred overall with lower values and a significant difference (P = 0.006) in curvature in the dark skin group. Two data-points (8-oxodG values = 24.13 and 31.38) from a skin phototype V subject were clearly erroneous and were excluded from analyses.
finding of lower systemic oxidative stress levels with greater melanisation has important implications for human physiology and health.

Declaration of competing interest

The authors declare no conflict of interest.

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