Infrared Radiation-Induced Matrix Metalloproteinase in Human Skin: Implications for Protection

Peter Schroeder[1], Juergen Lademann[2], Maxim E. Darvin[2], Helger Stege[3], Corinna Marks[1], Susanne Bruhnke[1] and Jean Krutmann[1]

Human skin is exposed to infrared radiation (IR) from natural and artificial sources. In previous studies, near IR radiation (IRA; 760–1,440 nm) was shown to elicit a retrograde mitochondrial signaling response leading to induction of matrix metalloproteinase-1 (MMP-1) expression. These studies, however, have exclusively employed cultured human skin fibroblasts *ex vivo*. Here, we have assessed the *in vivo* relevance of these observations by exposing healthy human skin *in vivo* to physiologically relevant doses of IRA. Eighty percent of the tested individuals responded to IRA radiation by upregulating of MMP-1 expression. Specifically, IRA irradiation caused increased expression of MMP-1 in the dermis, but not in the epidermis. Raman spectroscopy revealed that IRA radiation also caused a significant decrease in the antioxidant content of human skin. *In vitro* studies had previously shown that IRA-induced MMP-1 expression was mediated through an oxidative stress response, which originates from the mitochondrial electron transport chain. We now report that incubation of cultured human dermal fibroblasts or treatment of human skin with specific antioxidants prevented IRA radiation-induced MMP-1 expression *in vitro* and *in vivo*. Thus, IRA irradiation most likely promotes premature skin aging and topical application of appropriate antioxidants represents an effective photoprotective strategy.

Journal of Investigative Dermatology (2008) **128,** 2491–2497; doi:10.1038/jid.2008.116; published online 1 May 2008

INTRODUCTION

Infrared A (IRA) radiation (760–1440 nm) is a major component of natural sunlight. Approximately 30% of the total solar energy reaching the earth's surface is within the IRA range (Kochevar *et al*., 2007). In addition to natural sunlight, human skin is exposed to artificial IRA radiation from UV or IR irradiation devices, which are increasingly used for medical or purposes of well being (Schroeder *et al*., 2006). A careful analysis of the clinical relevance of IRA irradiation of human skin is lacking. There is, however, compelling evidence that IRA radiation does not simply penetrate through skin cells but instead has the capacity to elicit biological responses.

We, and others, have shown in previous studies that, similar to UVB and UVA radiation, IRA is able to activate mitogen-activated protein kinases and to induce gene transcription (Schieke *et al*., 2002, 2003; Kim *et al*., 2006a, b). More recent studies have demonstrated that the underlying signaling response completely differs from that induced by UV radiation. In contrast to UV, IRA radiation elicits a retrograde signaling response, which is initiated in the mitochondria through generation of reactive oxygen species (ROS) that originate from the mitochondrial electron transport chain (Schroeder *et al*., 2007).

One major biological consequence of this retrograde signaling response is the increased expression of matrix metalloproteinase (MMP)-1. This extracellular protease has the capacity to proteolytically degrade type-1 and type-3 collagen, as well as elastic fibers, and is thought to play a key role in the pathogenesis of photoaging of human skin (Rittie *et al*., 2006). It is now generally accepted that UVB as well as UVA irradiation lead to an increased expression and activity of MMP-1, but not of its tissue specific inhibitor tissue inhibitor of MMP-1 (TIMP-1), in the epidermal and dermal compartment of human skin (Fisher *et al*., 1997, 2002; Brenneisen *et al*., 2002). This imbalance is thought to be a major reason for the degradation and rarefication of collagen and elastic fibers, which is characteristic for photoaged skin and hold responsible for a number of clinical symptoms such as increased wrinkle formation and loss of skin elasticity and skin firmness (Rittie *et al*., 2006).

Similar to UV radiation, IRA irradiation of human skin fibroblasts was found to induce MMP-1 expression without a concomitant change in TIMP-1 expression, indicating the possibility that IRA radiation contributes to photoaging of human skin. Until now, however, these studies have exclusively involved cultured humans skin fibroblasts, and the *in vivo* relevance of these effects is unknown (Schieke

[1]*Cell Biology/Molecular Ageing Research, Institut fuer Umweltmedizinische Forschung (IUF) at the Heinrich-Heine-University Düsseldorf gGmbH, Duesseldorf, Germany;* [2]*Department of Dermatology, Charite, University of Berlin, Berlin, Germany and* [3]*Department of Dermatology, Heinrich-Heine-University Düsseldorf, Duesseldorf, Germany*

Correspondence: Dr Peter Schroeder, Cell Biology/Molecular Ageing Research, Institut für Umweltmedizinische Forschung (IUF) at the Heinrich-Heine-University Düsseldorf gGmbH, Auf'm Hennekamp 50, Duesseldorf 40225, Germany. E-mail: peet@gmx.de

Abbreviations: IR, infrared radiation; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of MMP

Received 6 October 2007; revised 5 March 2008; accepted 7 March 2008; published online 1 May 2008

et al., 2002). In this study, we have therefore exposed healthy skin of human volunteers *in vivo* to physiological doses of IRA radiation. We show that IRA irradiation of human skin causes an increased expression of MMP-1, but not TIMP-1 in the dermal compartment of human skin. We also provide evidence that this effect is mediated by an oxidative stress response.

RESULTS

Effects of IRA radiation on MMP-1 expression in human skin

Exposure of cultured primary human dermal fibroblasts has previously been shown to induce MMP-1 mRNA and protein expression. To assess the *in vivo* relevance of these observations, in this study normal buttock skin of 23 healthy human volunteers was irradiated with a single dose of 360 or 720 $J\,cm^{-2}$ IRA radiation (Tables 1–3) and subsequently assessed for MMP-1 mRNA ($n=15$) or protein ($n=8$; 4 by western blotting and 4 by immunohistochemistry) expression. These doses were chosen, because they correspond to the dose of IRA radiation, which can be achieved in a few hours on a summer day in central Europe. Sensitivity towards IRA radiation, as defined by increased MMP-1 mRNA (>1.5-fold upregulation) or protein expression (>1.5-fold upregulation) was observed in 19/23 volunteers, that is, approximately 80%. As is shown in Figure 1a and Table 1, IRA responsiveness, as well as the magnitude of the response, showed marked interindividual variability. The latter varied from 3- to 14-fold upregulation, as compared with sham-irradiated skin of the same individuals. This variability did not correlate with skin type, sex, or age of the respective volunteers (Tables 1 and 3).

To define whether IRA-induced MMP-1 mRNA expression occurred in the epidermal or dermal compartment of human skin, salt-split skin samples of irradiated buttock skin were prepared from biopsies of three volunteers (Table 1). Subsequent real-time PCR analysis revealed an IRA response in 2/3, which was confined to the dermal compartment (Figure 1b).

IRA radiation-induced upregulation of MMP-1 mRNA levels was associated with increased protein expression of MMP-1 and its precursor proMMP-1 in human skin, as was shown by western blot analysis of extracts prepared from biopsies of four volunteers (Figure 1c, Table 1). Analysis of salt-split skin samples of three of these four volunteers showed that increased MMP-1 protein expression was confined to the dermal compartment of IRA-irradiated human skin (Table 1; Figure 1d). These results were corroborated and extended by immunofluorescence microscopy. Staining of cryosections from IRA-irradiated or sham-irradiated human skin from four volunteers (Table 1) with antibodies specific

Table 2. IRA radiation-induced antioxidant depletion *in vivo* in human skin

No.	Sex	Age	Skin type	Investigation	Antioxidant content
15	Male	41	III	Raman of antioxidants	−30%
16	Male	21	II	Raman of antioxidants	−14%
17	Male	25	II	Raman of antioxidants	−9%
18	Male	25	II	Raman of antioxidants	−2%
19	Male	32	II	Raman of antioxidants	−22%
20	Male	23	II	Raman of antioxidants	−26%
21	Male	27	II	Raman of antioxidants	−39%
22	Male	22	II	Raman of antioxidants	−2%
23	Male	29	II	Raman of antioxidants	−11%

IRA, infrared A

Table 1. IRA radiation-induced MMP-1 expression *in vivo* in human skin

No.	Sex	Age	Skin type	Investigation	Response to IRA
1	Female	41	II	mmp1-PCR (whole skin)	Yes
2	Male	42	II	mmp1-PCR (whole skin)	Yes
3	Female	52	II	mmp1-PCR (whole skin)	Yes
4	Male	37	II	mmp1-PCR (salt-split)	Yes
5	Female	60	I	mmp1-PCR (salt-split)	Yes
6	Male	43	II	mmp1-PCR (salt-split)	No
7	Male	41	III	MMP-1, western blot (whole skin)	Yes
8	Female	29	III	MMP-1, western blot (salt split)	Yes
9	Male	64	II	MMP-1, western blot (salt split)	Yes
10	Male	42	II	MMP-1, western blot (salt split)	Yes
11	Male	34	II	MMP-1/TIMP-1, immunohistochemistry	Yes
12	Female	52	III	MMP-1/TIMP-1, immunohistochemistry	Yes
13	Male	27	IV	MMP-1/TIMP-1, immunohistochemistry	Yes
14	Male	50	III	MMP-1/TIMP-1, immunohistochemistry	Yes

IRA, infrared A; MMP, matrix metalloproteinase.

Table 3. Protection against IRA-induced MMP-1 expression in human skin by topical application of antioxidants

No.	Sex	Age	Skin type	Investigation	Response to IRA	Protection
24	Male	43	III	mmp1-PCR (whole skin)	Yes	Yes
25	Female	37	II	mmp1-PCR (whole skin)	No	NA
26	Male	45	II	mmp1-PCR (whole skin)	Yes	Yes
27	Female	50	II	mmp1-PCR (whole skin)	Yes	Yes
28	Male	26	II	mmp1-PCR (whole skin)	No	NA
29	Male	31	II	mmp1-PCR (whole skin)	Yes	Yes
30	Male	50	II	mmp1-PCR (whole skin)	No	NA
31	Male	33	II	mmp1-PCR (whole skin)	Yes	Yes
32	Female	38	II	mmp1-PCR (whole skin)	Yes	Yes

IRA, infrared A; MMP, matrix metalloproteinase; NA, not available.

for MMP-1 revealed increased MMP-1 protein expression in the dermis, but not in the epidermis (Figure 1e). This was not associated with a concomitant upregulation of TIMP-1 protein expression (Figure 1e).

Effects of IRA radiation on antioxidant content of skin

In vitro studies employing cultured primary human dermal fibroblasts have shown that IRA radiation-induced MMP-1 expression is mediated through a retrograde mitochondrial signaling pathway, which is initiated by the generation of ROS from the mitochondrial electron transport chain (Schroeder *et al.*, 2007). A well-known consequence of such an oxidative stress response is the consecutive alteration of antioxidative defence systems, which often show an initial depletion (Sies, 1985; Sies and Cadenas, 1985; Sies and Jones, 2007). Accordingly, we have previously observed that in cultured human dermal fibroblasts, a shift of the glutathione equilibrium toward the oxidized form occurs as a consequence of IRA irradiation (Schroeder *et al.*, 2007). We therefore speculated that IRA irradiation might elicit similar signaling events *in vivo* and thereby alter the antioxidative defence of human skin. Raman spectroscopy was therefore used as a sensitive and non-invasive method to assess the antioxidant capacity of IRA-irradiated human skin in nine volunteers (Table 2). As shown in Figure 2, IRA irradiation significantly decreased the antioxidant content of skin within 5 minutes after exposure ($P<0.05$). At later time points (24 hours post irradiation), skin antioxidant capacity fully recovered (Figure 2).

Effects of antioxidants on IRA radiation-induced MMP-1 expression

We have shown previously that IRA radiation-induced mitochondrial ROS production is of functional relevance for IRA radiation-induced gene expression, because treatment of cultured human dermal fibroblasts with the antioxidant MitoQ abrogated IRA radiation-induced MMP-1 expression (Schroeder *et al.*, 2007). We now corroborate and extend this observation by demonstrating that several other antioxidants also inhibit the IRA response. Among these were polyphenols such as EGCG and EC and vitamin C (Figure 3a). In contrast, vitamin E and phenylpropionic acid did not provide significant protection. We also tested an antioxidant mixture consisting of grape seed extract, ubichinone, vitamin C, and vitamin E. This mixture diminished IRA radiation-induced MMP-1 mRNA expression in a highly reproducible, dose-dependent manner, and was therefore next incorporated in a galenic preparation for *in vivo* studies in nine volunteers. In six out of these nine individuals, IRA radiation significantly upregulated MMP-1 mRNA expression, and topical application of the antioxidant mixture diminished this IRA response in 6 out of 6 individuals (Table 3). Protection showed a great interindividual variation and ranged from 25 to 100% (Table 3; Figure 4).

DISCUSSION

This study unambiguously shows that exposure of human skin to physiologically relevant doses of IRA radiation increases MMP-1 expression *in vivo*. This conclusion is based on three independent and complimentary methodological strategies: (i) demonstration of increased MMP-1 mRNA steady-state levels by real-time PCR, (ii) demonstration of of increased MMP-1 protein levels by western blot analysis, and (iii) demonstration of of increased MMP-1 protein expression by immunofluorescence microscopy in human skin. The magnitude of IRA radiation-induced MMP-1 upregulation showed considerable interindividual variability, and in 20% of the individuals tested, no IRA response was elicited. This observation is in line with previous *in vitro* results (Schieke *et al.*, 2002; Burri *et al.*, 2004), which showed comparable, donor-dependent degree of interindividual variability of the IRA response in cultured human skin fibroblasts. In this regard, IRA radiation resembles UVB and UVA radiation-induced MMP-1 expression; the underlying signaling mechanisms, however, are different (Schroeder *et al.*, 2007) and the factors determining the interindividual variability in MMP-1 responses may therefore be different as well. There is no obvious correlation between IRA responsiveness and skin type (Tables 1 and 3). In conclusion, these results show that IRA radiation-induced MMP-1 expression, which previously was only observed in cultured human dermal fibroblasts and in hairless mice (Kim *et al.*, 2006a), occurs in human skin as well and, thus, is of *in vivo* relevance.

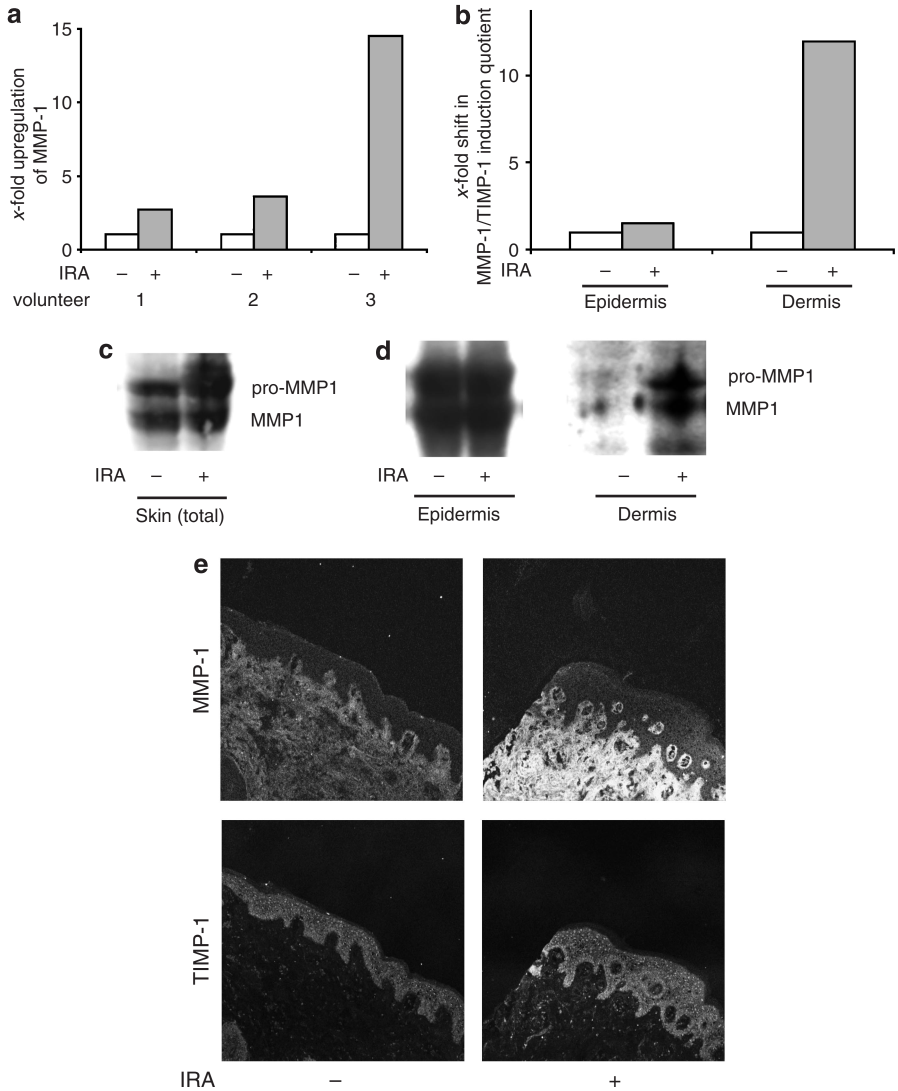

Figure 1. IRA radiation increases MMP-1 expression *in vivo* in human skin. (**a**) Punch biopsies were taken 24 hours after exposure to IRA (360 J cm^{-2}) or sham irradiation from healthy human skin. MMP-1 mRNA expression was analyzed by semi-quantitative real-time PCR. Data are means of two measurements per sample of three volunteers (Table 1, volunteer nos. 1–3). (**b**) Salt-split skin samples were prepared from punch biopsies obtained from IRA (360 J cm^{-2}) and sham-irradiated human skin 24 hours post exposure and analyzed by semi-quantitative real-time PCR for MMP-1 mRNA expression. Data are means of two measurements from two volunteers (Table 1, volunteer nos. 4 and 5). (**c**) Punch biopsies were taken from IRA (720 J cm^{-2}) and sham-irradiated gluteal skin 48 hours after exposure, homogenized, and analyzed for MMP-1 protein by western blotting. Data are from one volunteer (Table 1, volunteer no. 7). (**d**) Punch biopsies were taken from IRA (720 J cm^{-2}) or sham-irradiated skin 48 hours after exposure and salt-split skin samples were prepared for western blot analysis of MMP-1 protein expression. Data represent one of three volunteers (Table 1, volunteer nos. 8–10). (**e**) Punch biopsies were taken from IRA (360 J cm^{-2}) or sham-treated skin 24 hours after exposure. Cryosections were prepared and analyzed by immunohistochemistry for MMP-1 and TIMP-1 protein expression. Data represent one of four volunteers (Table 1, volunteer nos. 11–14).

The increase in MMP-1 has been observed in this study without a concomitant and comparable increase in the expression of its tissue specific inhibitor TIMP-1 (Figure 1e), thus confirming previous *in vitro* results (Schieke *et al*., 2002). It is, thus, likely that IRA irradiation of human skin leads to increased proteolytical degradation of dermal collagen fibers. Collagen degradation is a cardinal symptom of photoaged skin (Fisher *et al*., 1997, 2002; Krutmann and Gilchrest, 2006) and these observations, therefore, imply that IRA irradiation of human skin contributes to premature skin aging. This conclusion is in line with the seminal observation of Kligman (1982), who was first to show that IR exposure of guinea pigs caused skin changes similar to those observed in UV-induced actinic damage. It is also supported by recent studies in the hairless mouse model, which report that IRA irradiation causes two of the key symptoms of photoaging of skin, that is, an increase in dermal MMP-1 expression and enhanced neovascularization (Kim *et al*., 2006a). Artificial IRA irradiation devices of the same type as used in this study are currently promoted and sold for fat reducing and skin rejuvenation purposes (Möckel *et al*., 2006). In view of the studies performed by us and others mentioned above, these commercial activities seem at least paradoxical if not unethical and should be legally restricted.

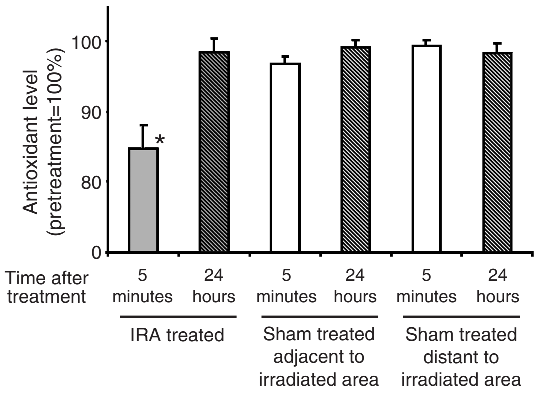

Figure 2. IRA decreases skin antioxidants *in vivo*. Healthy human volunteers ($n=9$) were irradiated with 360 J cm^{-2} IRA or sham-treated. Five minutes and 24 hours post exposure, skin antioxidant levels were measured by Raman spectroscopy. Data are means ± SEM of nine volunteers. *Significantly different to pretreatment ($P<0.05$).

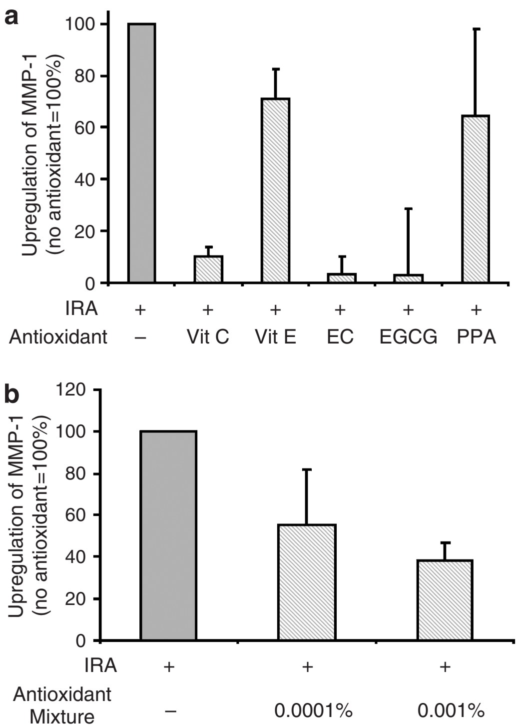

Figure 3. Inhibition of IRA-induced MMP-1 upregulation in cultured human skin fibroblasts by antioxidants. (**a, b**) Primary human dermal fibroblasts from three different donors were incubated with no antioxidants (–), 10 μM ascorbic acid (Vit C), 1 μM α-tocopherol (Vit E), 10 μM epigallocatechingallat (EGCG), 100 μM (–)-epicatechin (EC), or 100 μM phenylpropionic acid for 24 hours, exposed to IRA (360 J cm^{-2}) radiation, and incubated with the respective antioxidant for another 24 hours. MMP-1 expression was measured by real-time PCR; relative upregulation was defined as upregulation in the absence of antioxidants set to 100%. Data are means ± SEM of three independent experiments; average MMP-1 upregulation in the absence of antioxidant was 2.2 ± 0.4-fold. (**b**) Cells were incubated with the antioxidant mixture at the indicated concentrations and treated as described before. MMP-1 mRNA expression was analyzed 24 hours after IRA exposure by real-time PCR. Data are means ± SEM of nine independent experiments; average MMP-1 upregulation in the absence of antioxidant was 5.3 ± 1.3-fold.

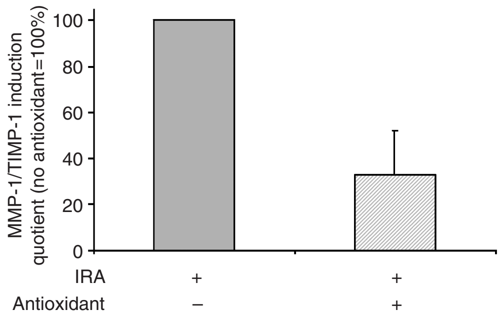

Figure 4. IRA radiation-induced upregulation of MMP-1 can be inhibited in human skin through topical application of antioxidants. Nine healthy volunteers (Table 3) were treated with a topical preparation containing 1% antioxidant mixture 20 minutes before irradiation (IRA 720 J cm^{-2}). Twenty-four hours post irradiation, punch biopsies were taken and MMP-1 and TIMP-1 expression was analyzed by real-time PCR. Six volunteers showed significant (>2-fold) IRA-induced upregulation of MMP-1, but not of TIMP-1 mRNA steady-state levels. For these six individuals, the relative increase of the MMP-1/TIMP-1 ratio was calculated based on the increase in MMP-1/TIMP-1 ratio in the absence of antioxidant treatment, which was set as 100%. Data are means ± SEM of six volunteers.

All three approaches, which were used in the present study, show that IRA-induced MMP-1 upregulation is restricted to the dermal compartment of human skin. This observation further emphasizes that primary human dermal fibroblasts are relevant and critical target cells for IRA radiation (Schieke *et al.*, 2002; Schroeder *et al.*, 2007). This is in contrast to UV radiation, which induces MMP-1 expression in both compartments of human skin (Tyrrell, 1996; Krutmann and Gilchrest, 2006). In this regard it should be noted that the underlying signaling pathways responsible for UV *versus* IRA radiation-induced MMP-1 expression completely differ. In contrast to UV, IRA radiation-induced MMP-1 expression involves a retrograde signaling response, which is initiated through generation of ROS from the mitochondrial electron transport chain. It is, thus, likely that IRA radiation, which is a potent inducer of this response in dermal fibroblasts (Schroeder *et al.*, 2007), fails to elicit the initial oxidative stress response and/or subsequent retrograde signaling steps in epidermal keratinocytes. Accordingly, the antioxidative capacity of keratinocytes is several fold higher than that of dermal fibroblasts (Applegate *et al.*, 1995) and the mitochondrial electron transport chain capacity of keratinocytes is smaller than that of fibroblasts (Hornig-Do *et al.*, 2007), which might make them less susceptible to IRA.

In our mechanistic studies, we have demonstrated that IRA radiation-induced MMP-1 expression can be inhibited by the antioxidant MitoQ (Schroeder *et al.*, 2007). In this study, we have screened the major antioxidant families in order to identify more antioxidants that are effective to inhibit IRA radiation-induced MMP-1 expression. We have shown that certain polyphenols and vitamins can be used for this purpose and that topical application of a mixture consisting of these antioxidants on human skin prior to IRA irradiation prevents IRA radiation-induced MMP-1 expression. To the best of our knowledge, this is the first report indicating that it is possible to protect human skin against IRA radiation-induced damage.

None of the active constituents of the galenic formulation is known to function as an IRA filter; therefore, we waived to burden the volunteers with an extra punch biopsy as placebo control. Future studies are required to further optimize this approach, for example, by identifying distinct antioxidants with the specific capacity to counter the initial mitochondrial oxidative stress and to abrogate the initial signaling step in IRA radiation-induced signal transduction.

The fact that topical application of antioxidants to human skin mitigates IRA radiation-induced MMP-1 expression indicates that the IRA-induced, intramitochondrial oxidative stress response that we have previously described *in vitro* (Schroeder *et al.*, 2007), also takes place *in vivo* in human skin. Moreover it was shown by Darvin *et al.* (2007) in an *in vivo* study that IR irradiation can produce free radicals in the skin, which was measured by the decreasing level of carotenoid antioxidants in human skin. This assumption is supported by the present observation that IRA radiation at early time points decreases the antioxidant content of human skin, as measured by Raman spectroscopy (Figure 2), whereas at later time points, antioxidative systems such as ferritin expression are increased (Applegate *et al.*, 2000). Both effects most likely reflect the consequence of an IRA-induced oxidative stress response in human skin, as they have been described to occur after stimulation of human tissues with other pro-oxidative stressors (Vile *et al.*, 1994; Pourzand *et al.*, 1999; Darvin *et al.*, 2007). Additional studies are required to identify the chromophore and the subsequent photochemical reactions responsible, and to answer the question how the initial IRA-induced signal, that is, intramitochondrial ROS production, is transferred from the mitochondria into the cytoplasm and subsequently into the nucleus.

MATERIALS AND METHODS

In vivo irradiation

All *in vivo* studies were carried out in adherence to the Declaration of Helsinki Principles and were approved by the local ethical committee of the Medical Faculty of the Heinrich-Heine-University in Düsseldorf, Germany. After obtaining informed consent, buttock skin of healthy human volunteers ($n=32$; non smokers, for further characteristics see Tables 1–3) were exposed to a single dose of IRA radiation from a water-filtered IR-A irradiation source (Hydrosun 500; Hydrosun Medizintechnik, Müllheim, Germany). This device emits wavelengths between 760 and 1440 nm (Figure S1) without any contaminating UV radiation as controlled by means of a UVAMETER (Mutzhas, Munich, Germany) and a UV-Dosimeter Type II equipped with a UV6 sensor (Waldmann Medizintechnik, Villingen-Schwenningen, Germany). The IRA output was determined with a Hydrosun HBM1 (Hydrosun Medizintechnik) measuring device and found to be 105 mW cm^{-2} at a lamp-to-target distance of 40 cm. The IRA radiation doses of 360–720 J cm^{-2} were applied in 57–114 minutes; none of the volunteers experienced extensive heating. For sham treatment, another area of buttock skin was treated completely identical, except that no IRA was applied. Twenty-four and 48 hours after irradiation, 4-mm punch biopsies were taken from IRA- and sham-irradiated skin sites. Samples were snap frozen and stored in liquid nitrogen until further analysis.

Split skin samples

For separation of the dermis from the epidermis, skin samples were immersed in 1 M NaCl for 48 hours at 4 °C ($n=3$) as described (Berneburg *et al.*, 2004).

RNA extraction and reverse transcriptase-PCR

Total RNA was isolated using RNeasy Total RNA kits (Qiagen, Hilden, Germany). Expression of MMP-1 was measured by quantitative reverse transcriptase-PCR based on the method described by Watanabe *et al.* (2004), using the reverse transcriptase-PCR core kit (Applied Biosystems, Darmstadt, Germany) and specific primer pairs for MMP-1 (5′-CATGAAAGGTGGACCAACAATTT-3′; 5′-CCAAGAGAATGGCCGAGTTC-3′) and TIMP-1 (5′-TCGTGGCTC CCTGGAACA-3′; 5′-CCAACAGTGTAGGTCTTGGTGAAG-3′). SEMi quantitative analysis of the reverse transcriptase-PCR products was done using the $\Delta\Delta C_t$ method using 18S RNA (primer pair: 5-GCCGCTAGAGGTGAAATTCTTG-3′; 5′-CATTCTTGGCAAATGC TTTCG-3′) for normalization. For analysis, the ΔC_t of MMP-1 or TIMP-1 *versus* 18S was set to 1.

Western blot analysis

For detection of MMP-1 protein expression, biopsies were homogenized under liquid nitrogen and resuspended in western blot sampling buffer. For western blotting, protein samples were separated by SDS–polyacrylamide-gel electrophoresis on gels containing 12% (w/v) acrylamide, followed by blotting onto a polyvinylidene difluoride membrane (Amersham, Buckinghamshire, UK). The membrane was blocked using 5% (w/v) non-fat dry milk (Bio-Rad, Hercules, CA) in TBST (20 mM Tris, 137 mM NaCl, 0.1% (v/v) Tween 20, pH 7.4). Immunodetection of MMP-1 was performed with a sheep anti-MMP-1 antibody (The Binding Site, Schwetzingen, Germany) and a peroxidase-coupled anti-sheep antibody (Calbiochem, Nottingham, UK), followed by chemiluminescent detection.

Immunofluorescence microscopy

Cryosections were prepared with a Cryostat CM3050 S (Leica Microsystems, Wetzlar, Germany) cryometer. Sections were stained with monoclonal antibodies specific for MMP-1 (Molecular Probes, Karlsruhe, Germany) or TIMP-1 (R&D Systems, Wiesbaden, Germany). As secondary antibodies, anti-rabbit Alexa Fluor 633 and anti-goat Alexa Fluor 488 (Molecular Probes) were used.

Raman spectroscopy

The cutaneous concentration of carotenoid antioxidant substances was determined non-invasively by means of resonance Raman-spectroscopic measurements.

The radiation of an argon laser, which operated alternately at 488 and 514 nm, and thus corresponded to the maximum absorption of carotenoid molecules, was used as a source of actuating light on the skin. The Raman signal from the skin, reflecting the concentration of the cutaneous carotenoids, was collected by a lens system, filtered, transferred to a spectrograph, and recorded by a CCD camera. The spectrum obtained was analyzed with specially developed software to obtain the concentration of the carotenoid molecules in the skin.

The measuring setup for the *in vivo* determination of carotenoids in the human skin, as well as the analytical mechanism, have been described in detail by Darvin *et al.* (2005) (2006).

Cell culture

Human dermal fibroblasts were isolated from neonatal foreskin obtained from three different donors. Cells were cultured in Eagle's minimum essential medium (Biochrom KG Seromed, Berlin, Germany) supplemented with 10% fetal bovine serum (Gibco, Karlsruhe, Germany), 1% antibiotics/antimycotics (penicillin, streptomycin, amphotericin B), and 1% glutamine (Gibco), and were grown on plastic Petri dishes (Greiner, Solingen, Germany) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C as previously described (Schieke *et al*., 2002). Cells were used between passages 4 and 12, grown to 100% confluence, and serum-starved before treatment.

Antioxidants

Ascorbic acid, α-tocopherol, epigallocatechingallat, epicatechin, retinoic acid, quercetin, and phenylpropionic acid were obtained from Sigma (München, Germany).

A galenic preparation containing an antioxidant mixture consisting of grape seed extract, ubichinone, vitamin C, and vitamin E was provided by Dr Peter Hansen (R & D Stada, Bad Vilbel, Germany).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by Deutsche Forschungsgemeinschaft (SFB 728), and the German Ministry for the Environment and the Research Committee of the Medical Faculty of the Heinrich-Heine-University, Duesseldorf. We thank Cornelia Mauch, Peter Hansen, and Kathrin Medve-Koenigs for technical support and stimulating discussion. We also thank the Foundation Skin Physiology of the Donor Association for German Science and Humanities for financial support.

SUPPLEMENTARY MATERIAL

Figure S1. Spectrum calculated from recording of Technical College Munich (Munich, Germany) of hydrosun 500 IRA source, and transmission recording of 10-mm water cuvette and blackfilter RG 830 by means of a Perkin Elmer Spectrophotometer Lambda 9 (information provided by the lamp manufacturer; Hydrosun, Muellheim, Germany).

REFERENCES

Applegate LA, Noel A, Vile G, Frenk E, Tyrrell RM (1995) Two genes contribute to different extents to the heme oxygenase enzyme activity measured in cultured human skin fibroblasts and keratinocytes: implications for protection against oxidant stress. *Photochem Photobiol* 61:285–91

Applegate LA, Scaletta C, Panizzon R, Frenk E, Hohlfeld P, Schwarzkopf S (2000) Induction of the putative protective protein ferritin by infrared radiation: implications in skin repair. *Int J Mol Med* 5:247–51

Berneburg M, Plettenberg H, Medve-Konig K, Pfahlberg A, Gers-Barlag H, Gefeller O *et al.* (2004) Induction of the photoaging-associated mitochondrial common deletion *in vivo* in normal human skin. *J Invest Dermatol* 122:1277–83

Brenneisen P, Sies H, Scharffetter-Kochanek K (2002) Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events. *Ann N Y Acad Sci* 973:31–43

Burri N, Gebbers N, Applegate LA (2004) Chronic infrared-A radiation repair: Implications in cellular senescence and extracellular matrix. *Recent Res Dev Photochem Photobiol* 7:219–31

Darvin ME, Gersonde I, Albrecht H, Meinke M, Sterry W, Lademann J (2006) Non-invasive *in vivo* detection of the carotenoid antioxidant substance lycopene in the human skin using the resonance Raman spectroscopy. *Laser Phys Lett* 3:460–3

Darvin ME, Gersonde I, Albrecht H, Zastrow L, Sterry W, Lademann J (2007) *In vivo* Raman spectroscopic analysis of the influence of IR radiation on the carotenoid antioxidant substances beta-carotene and lycopene in the human skin. Formation of free radicals. *Laser Phys Lett* 4:318–21

Darvin ME, Gersonde I, Meinke M, Sterry W, Lademann J (2005) Non-invasive *in vivo* determination of the carotenoids beta-carotene and lycopene concentrations in the human skin using the Raman spectroscopic method. *J Phys D Appl Phys* 38:2696–700

Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S *et al.* (2002) Mechanisms of photoaging and chronological skin aging. *Arch Dermatol* 138:1462–70

Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ (1997) Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 337:1419–28

Hornig-Do HT, Kleist-Retzow JC, Lanz K, Wickenhauser C, Kudin AP, Kunz WS *et al.* (2007) Human epidermal keratinocytes accumulate superoxide due to low activity of Mn-SOD, leading to mitochondrial functional impairment. *J Invest Dermatol* 127:1084–93

Kim MS, Kim YK, Cho KH, Chung JH (2006a) Infrared exposure induces an angiogenic switch in human skin that is partially mediated by heat. *Br J Dermatol* 155:1131–8

Kim MS, Kim YK, Cho KH, Chung JH (2006b) Regulation of type I procollagen and MMP-1 expression after single or repeated exposure to infrared radiation in human skin. *Mech Ageing Dev* 127:875–82

Kligman LH (1982) Intensification of ultraviolet-induced dermal damage by infrared radiation. *Arch Dermatol Res* 272:229–38

Kochevar IE, Taylor CR, Krutmann J (2007) Fundamentals of cutaneous photobiology and photoimmunology. In: *Fitzpatrick's Dermatology in General Medicine* (Wolff K, Austen KF, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, eds), New York: McGraw-Hill (in press)

Krutmann J, Gilchrest BA (2006) Photoaging of skin. In: *Skin Aging* (Gilchrest BA, Krutmann J, eds), New York: Springer, 33–44

Möckel F, Hoffmann G, Obermüller R, Drobnik W, Schmitz G (2006) Influence of water-filtered infrared-A (wIRA) on reduction of local fat and body weight. *Ger Med Sci Online* 4:Doc05

Pourzand C, Watkin RD, Brown JE, Tyrrell RM (1999) Ultraviolet A radiation induces immediate release of iron in human primary skin fibroblasts: the role of ferritin. *Proc Natl Acad Sci USA* 96:6751–6

Rittie L, Fisher GJ, Voorhees JJ (2006) Retenoid therapy for photoaging. In: *Skin Aging* (Gilchrest BA, Krutmann J, eds), New York: Springer, 143–56

Schieke S, Stege H, Kurten V, Grether-Beck S, Sies H, Krutmann J (2002) Infrared-A radiation-induced matrix metalloproteinase 1 expression is mediated through extracellular signal-regulated kinase 1/2 activation in human dermal fibroblasts. *J Invest Dermatol* 119:1323–9

Schieke SM, Schroeder P, Krutmann J (2003) Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. *Photodermatol Photoimmunol Photomed* 19:228–34

Schroeder P, Pohl C, Calles C, Marks C, Wild S, Krutmann J (2007) Cellular response to infrared radiation involves retrograde mitochondrial signaling. *Free Radic Biol Med* 43:128–35

Schroeder P, Schieke S, Morita A (2006) Premature skin aging by infrared radiation, tobacco smoke and ozone. In: *Skin Aging* (Gilchrest BA, Krutmann J, eds), New York: Springer, 45–54

Sies H (1985) Oxidative stress: introductory remarks. In: *Oxidative Stress* (Sies H, ed), London: Academic Press, 1–8

Sies H, Cadenas E (1985) Oxidative stress: damage to intact cells and organs. *Philos Trans R Soc Lond B Biol Sci* 311:617–31

Sies H, Jones D (2007) Oxidative stress. In: *Encyclopedia of Stress* (Fink G, ed), San Diego: Elsevier, 45–8

Tyrrell RM (1996) Activation of mammalian gene expression by the UV component of sunlight—from models to reality. *Bioessays* 18:139–48

Vile GF, Basu-Modak S, Waltner C, Tyrrell RM (1994) Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. *Proc Natl Acad Sci USA* 91:2607–10

Watanabe H, Shimizu T, Nishihira J, Abe R, Nakayama T, Taniguchi M *et al.* (2004) Ultraviolet A-induced production of matrix metalloproteinase-1 is mediated by macrophage migration inhibitory factor (MIF) in human dermal fibroblasts. *J Biol Chem* 279:1676–83