THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

UV-IRRADIATION INDUCED DNA-DAMAGE AND REPAIR IN SKIN CELLS. IN VITRO METHODS

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INTRODUCTION

The UV-radiation is responsible for several physiological and pathological effects of sunlight (vitamin D synthesis, antigenspecific immunotolerance, sunburn, photoallergy, phototoxicity, photoaggravated skin diseases, photoaging, photocarcinogenesis, etc.). The background cellular effects: photochemical reactions, changes of membrane receptors, lipid- and protein-modifications and DNA-damage. On the basis of that their type and extent are dependent on wavelength, three spectral regions are defined: UVC (200-280 nm), UVB (280-320 nm) and UVA (320-400 nm). The DNA-damage has a key role. On one hand, it is important for carcinogenesis because of the possibility of change in genetic content, on the other hand, the DNA-lesions are signals for activation of DNA-repair pathways, cell cycle regulation, apoptosis and also for induction of immunomodulation, immunosuppression, photoallergy, or melanin synthesis in melanocytes (MC). The effects of UV-irradiation on DNA depend on cell type, cell-proliferation status, DNA-repair capability, and on the presence of endogenous and exogenous photosensitizers. Therefore studies considering the influence of several different factors on the effects of UV-irradiation on human skin are important.

The UVC- and UVB-photons are absorbed by the DNA and induce cyclobutane-pyrimidine-dimers (CPD) and pyrimidine-(6-4)-pyrimidone photoproducts ((6-4)-PD). The UVC-irradiation is absorbed by the ozone layer, therefore it does not reach the atmosphere and the human skin. Because of the damage of the ozone layer the proportion of shorter wavelength UVB-radiation reaching the earth is higher, with relevant biological effects.

The DNA-lesions mentioned are repaired by nucleotide excision repair (NER). Incomplete repair of dimers will be resulted in formation of DNA-loops during replication, which form single stranded DNA-gaps, which are very sensitive toward endonucleases. Consequently DNA-double strand breaks are formed, then chromatide type chromosomal

aberrations. The recognition of CPD and (6-4)-PD is accompanied by the activation of cell cycle check points, which provides more time to finish the DNA-repair, or induces programmed cell death, respectively.

The long-wavelength UVA is hardly absorbed by the DNA. Therefore the extent of direct DNA-damage is much less, whereas the probability of excitation of cell membrane lipids and cytoplasmic polymers producing different types of free radicals is increasing. The consequence of it is induction of DNA-single and double strand breaks, base modifications (8-oxo-7,8-dihydro-2'-deoxyguanozine, abasic sites), DNA-protein crosslinks (DPC). The single strand breaks may be rejoined very fast, the base damage can be repaired by base excision repair, DPC may be removed enzymatically. Because of the lack of CPD and (6-4)-PD the DNA-damage caused by UVA seems to be less severe, but there are point mutations induced, even chromosomal damage, though the type and extent of them is much less known. Molecular analysis of skin tumors shows a broad spectrum of genetic modifications, some of them are induced by UV. It is not clear, which part of the UV-spectrum causes these DNA-lesions, and to what extent chromosomal aberrations are induced by UV-irradiation. The UVA-induced DNA-damage is important, on one hand, because the proportion of UVA within the UV-spectrum of sunlight is high (95 %). On the other hand, the extended sunbathing using UVB-blocker sunscreens and solarium result in much more exposure to UVA. Furthermore, the penetration of UVA into the skin is deeper than of UVB. Taking all things into consideration the UVA-irradiation can not be thought to be harmless.

Epidemiological and experimental data show that the increasing incidence of human skin cancers, at least in part, is associated with the increasing sun exposure of skin. This relationship is rather convincing for non-melanoma skin tumors (actinic keratosis, basalioma, squamous cell carcinoma). The role of UV in the development of melanoma is less understood. Recent models of photocarcinogenesis suggest a multistep process in the

background of tumor development, in which not only the UVB, but also the UVA may play a role, and possible additive or synergistic interactions between drugs, cosmetics, other environmental factors and UV-irradiation should be considered as well. There are experimental data showing that chemicals absorbed by skin can induce direct DNA-damage, delay DNA-repair, replication or transcription, can induce photochemical reactions, and may disturb the cell cycle regulation. All these are able to modify the tumor-initiator efficacy of solar radiation.

Chemicals get into the skin in different ways. For instance, with therapeutic purpose, such as photochemotherapy (PUVA=8-methoxy-psoralen /8-MOP/+UVA). This therapeutic modality is widely used for adjuvant treatment of several skin diseases (psoriasis, vitiligo, atopic dermatitis, parapsoriasis, and mycosis fungoides). Its mechanism of action is not completely clear. It is known that 8-MOP participates in type I. photochemical reaction, i.e. an 8-MOP molecule intercalates between the DNA-strands and absorbing a UV-photon it forms 3,4- or 4',5'-monoadduct with a pyrimidine base, then with another photon DNA-DNA crosslink. The repair of these DNA-lesions is not clear. In Escherichia coli, in yeast cell and in plasmid DNA the complex interaction of NER and recombinational repair seems to be involved. In human lymphoid cells different DNA-repair time periods were observed after UVB and PUVA treatment. It is known from the clinical practice, that the efficacy of the photochemotherapy is higher or different from that of UVB or UVA alone in many cases. It may derive from the different kinds of DNA-damage. Since PUVA-treatment increases the risk of skin cancers, the study of the DNA-damage induced is important for better understanding of PUVA-carcinogenesis.

Chemicals get into our skin also from our environment. It is well known, that formaldehyde (FA) is a major source of pollution and there are negative effects of FA on skin: irritation, contact allergy, and photosensitivity. Furthermore, it is known that the FA is

cytotoxic, mutagenic and clastogenic. Recently positive correlation was reported between cumulative low-dose occupational exposure to FA and risk for nasopharyngeal squamous cell carcinoma in humans. There is no known epidemiological evidence for an increased skin cancer risk up to now. It is thought that the persistence of DPC formed between nuclear DNA and histone proteins are responsible for the cytotoxic and genotoxic effects. On the other hand, the FA-induced DPC can block either DNA-polymerase or the entire replication complex and may inhibit the DNA-structure modulating topoisomerase II as well. On the basis of these it can be expected that FA influences on UV-irradiation induced DNA-damage and repair. But, although there are experimental data regarding the ability of FA to arrest the repair DNA-synthesis of NER, these results were not reproducible in another study.

AIMS

Through our experimental work we were planned to answer the following questions:

1.1. Can the PUVA-induced DNA-damage and repair be measured by means of comet-assay?

1.2. Are different the DNA-damage and repair induced by PUVA from those induced by UVB and UVA in hyperproliferative keratinocyte (KC) model HaCaT (immortalized human KC) cultures?

The second part of my work deals with:

2.1. Can clinically relevant low doses of UVB- and UVA-irradiation induce micronuclei (MN) indicating chromosomal damage in human fibroblasts (FB) and in melanocytes (MC)?

2.2. Can a previously described multiparameter flow cytometric (FCM) method be applied to our cell cultures for measuring MN-induction or can it be adapted with modifications? Besides the objectivity, time sparing, statistical reliability of this method whether the

measuring of the DNA-content of particles is able to give additional information about UV-induced cell cycle disturbances?

The third part of my study was:

3.1. Can FA cause DNA-damage detectable by comet-assay (DPC, DNA-single strand breaks) in human, non-transformed, adult skin KC and FB? Do long-term exposure- which can be easily reached during 8 h occupational exposure or using make-up- to low concentrations of FA induce significant DNA-damage in these cells?

3.2. By means of comet-assay is there any difference between FB and KC regarding DNA-damage and repair time kinetics following UVC-, UVA-, and solar simulated UV (UVB+UVA)-irradiation?

3.3. Is there any effect of FA on UV-induced DNA-damage and repair? If it is the case, can FA enhance the UV-induced MN-formation?

MATERIALS AND METHODS

Cell cultures

For comparative studies we obtained MC and FB from newborn praeputium-skin. The culture medium for MC was Ham's F-10 supplemented with 12-O-tetra-decanoyl-phorbol-13-acetate, cholera-toxin, isobuthyl-methyl-xanthine, 5 % fetal calf serum (Hyclone, Logan UT), penicillin and streptomycin. FB obtained from dermis were grown in Ham's F-10 supplemented with 10 % fetal calf serum, penicillin and streptomycin.

HaCaT cell cultures were maintained in DMEM medium containing 10 % fetal calf serum, penicillin and streptomycin.

KC and FB were derived from normal adult skin obtained from breast plastic surgery. The epidermal cell suspension was seeded on mitomycine-treated (23.9 μM) human FB-culture in FAD2 growth medium (DMEM and Ham's F-12 (3:1) supplemented with fetal calf

serum, adenine, insulin, trijod-thyronine, hydrocortisone, epidermal growth factor, cholera-toxin, penicillin and streptomycin), then the KC were maintained in serum-free KC medium (KGM, Clonetics, US&Can). FB were seeded in 10 % fetal calf serum, penicillin and streptomycin containing RPMI 1640 medium (Biochrom KG, Berlin, Germany), then they were grown in serum-free FB medium (FGM, Promocell, Heidelberg, Germany).

UV-irradiation

UVB-irradiation was performed by Philips TL-01 fluorescent lamps (Philips Nederland BV, Eindhoven, Netherlands), and by an ORIEL xenon arc solar simulator (Oriel Corporation, Stratford, CT, Model 81160) containing UVC blocking filter. UVA-irradiation was provided by Sellas Sunlight lamp (type 2001, Sellas, Gevelsberg, Germany), by Waldmann PUVA 800 lamp (H. Waldmann GmbH&Co., Germany), and by CAMAG Deluxe lamp (Muttenz, Switzerland).

Comet-assay

Cells were harvested by trypsin, then embedded in low melting point agarose layer (0.5 %) on normal melting point agarose (0.5 %) pre-coated frosted glass slides. The cells were lysed (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1 % N-lauroyl-sarcosine, 1 % Triton, 2 % DMSO, pH 10) overnight at 4 °C. Thereafter, the cells were allowed to be exposed to a strong alkali solution (300 mM NaOH, 1 mM EDTA, pH 13.0) or in the case of neutral assay to a pH 8.0 solution (89 mM Tris, 89 mM boric acid, 2 mM EDTA) for 25 min in an electrophoresis chamber, then electrophoresis was performed (21 V (0.86 V/cm), 300 mA, 25 min). After neutralization (0.4 M Tris, pH 7.5) the DNA was stained with ethidium-bromide (EB, 20 μg/ml). We evaluated the length and intensity of comet tails by fluorescence microscopy (Leica, Leica Microsystems, Wetzlar, Germany), representative samples were photodocumented. The nuclei were classified into 4 categories, at least 1x100 nuclei were scored per slide.

Micronucleus (MN)-assay

For fluorescence microscopy scoring exponentially growing cells were seeded on glass slides, for FCM measurements in Petri dishes. The MN-formation was studied in FB 4, in MC 8 days after irradiation.

Glass slides with cultured cells were washed in phosphate-buffered saline (PBS), then treated with a hypotonic solution (0.1 M NaCl, 1.7 mM KCl), and fixed with methanol:acetic acid:PBS 1:3:4. The DNA of the nuclei and MN was stained with propidium-iodide (5 μg/ml) in Vectashield (Vector Laboratories, Inc., USA). Within intact interphase cells the particles that were observed to be completely separated from the main nucleus were scored as MN. Their diameters ranged between an eighth and a half of the diameter of the nucleus. At least 4x500 cells were scored in each sample.

For FCM measurement 0.5 ml solution I. (10 mM NaCl, 3.4 mM Na-citrate, 10 mg/l bovine pancreas derived RNase A and 0.03 % (v/v) Nonidet P-40) was added to the pellets of cells, followed by the addition of EB (Sigma) at a final concentration of $1.3x10^{-5}$ M. After 1.5 h incubation at room temperature in the dark, 0.5 ml solution II. (71.4 mM citric acid, 0.25 M sucrose and 2 mM EDTA) was added. The samples were stored at 4 °C until the analysis was performed the next day. Cellular membranes were stained with the fluorescent dye 1,6-diphenyl-1,3,5-hexatriene (DPH, Sigma), which was added 3-4 h before measurements to a final concentration of $1.1x10^{-4}$ M.

The FCM measurements were carried out with an Epics Elite (Coulter, Luton, UK) flow cytometer equipped with two Ar+-ion lasers tuned at 480 nm and 351/363 nm for the excitation of the fluorochromes EB and DPH, respectively. For excitation of the two fluorochromes by the two lasers, a time delay (~40 μsec) was applied, based on the temporal separation of the two laser beams. The arrival of signals from the first laser at the computer was delayed in order to coincide with the arrival of signals from the second laser. The optical

filters used for EB and DPH fluorescence were a long wavelength pass filter at 630 nm and a 450 nm bandpass filter in combination with a long pass dichroic mirror at 550 nm. Particles with certain EB- and DPH-fluorescence intensity were sorted, collected on glass slides and identified as nuclei, MN or debris by fluorescence microscopy.

Proliferation assay

At the time of investigation the cell culture medium was replaced by fresh medium containing 10 % (v/v) alamarBlue^{TM}-t (Biosource, Nivelles, Belgium). The dye reduction, as denoted by increase in fluorescence intensity was monitored spectrofluorimetrically (FL1000^{TM}, Dynatech Laboratories, Sullyfield, Virginia, USA). The relation between alamarBlue^{TM} fluorescence and cell number was determined by performing a correlation analysis (Excel 97).

RESULTS

1. Photochemotherapy (PUVA) induced DNA-damage and repair

Investigating HaCaT cells by means of alkaline comet-assay, comet-formation (DNA-single strand breaks, alkali labile sites) was observed immediately after irradiation only in the case of UVA-irradiation (5 J/cm^2). The DNA-migration decreased to the control level 1.5 h after the irradiation indicating a fast repair of induced DNA-damage. Following UVB-irradiation (60 mJ/cm^2) we detected DNA-migration 0.5 h after exposure. It means that the comet-assay shows not a direct DNA-damage in this case, but the DNA-single strand breaks are resulted from the induced NER. Because of the DNA-resynthesis and ligation the comets disappeared in time. The alkaline comet-assay alone was not suitable to follow the DNA-damage after PUVA. By means of neutral comet-assay, where DNA-double strand breaks can be demonstrated, it seemed to be that neither UVA nor UVB can induce this kind of DNA-lesions. Opposite, treatment of cells with 8-MOP (300 ng/ml) before UVA-irradiation (2

J/cm^2) caused formation of DNA-double strand breaks 1.5 h after irradiation as detected by the neutral comet-assay.

2. Chromosomal damage in MC compared to FB following γ-, UVB- and UVA-irradiation

We first modified a previously employed method described by Wessels and Nüsse in 1995 for FCM measurement of MN-formation for the cell types under study. We provided a longer cell lysis incubation time (1.5 h) to establish improved separation of the MN and nuclei from the cytoplasm, adding 1 mM EDTA to the free MN after cell lysis we protected their DNA from endonucleases, and we applied a time delay to the dual laser FCM measurement.

In a pilot study we determined the post-irradiation period required for the development of MN. Using microscopic determination of MN, we found that for FB 4 d, for MC 8 d incubation time was required after irradiation to obtain an optimal MN-frequency.

After γ-irradiation we found a sharp increase in MN-formation with doses up to 9 Gy in MC and up to 4 Gy in FB. After UVB-irradiation with doses up to 2 J/cm^2 in MC and up to 1,1 J/cm^2 in FB we observed also linear dose-response relationship. In MC higher UVB-doses were needed to induce approximately the same percentage of MN as in FB. The maximal MN-induction was in FB 1.27±0.24 %, in MC 1.1±0.24 %. The proportion of G2/M-phase cell nuclei was also increased according to the dose. The increment accompanying the same ionizing irradiation- or UVB-dose was higher in FB than in MC.

For comparison and validation of the FCM method, we determined the MN-induction by means of fluorescence microscopy. In FB we observed linear dose-response relationship for MN-formation after UVA-irradiation (0-30 J/cm^2) as well. The maximum MN-frequency amounted to approximately twice the control value ($p<0.05$). The induction level observed for MN was higher when scored by microscopy than by FCM. The proportion of the cells in G2/M-phase also increased in a dose-dependent manner, up to approximately 20 %. In MC there was no MN-induction or cell cycle-arrest after UVA-irradiation.

3. DNA-damage caused by FA and the effect of FA on UV-induced DNA-damage and repair in normal human KC and FB

0-100 μM FA did not induce DNA-strand breaks, alkali labile sites in KC or FB. Opposite, we observed significant DPC-induction. Short-term MMS-exposure (250 μM, 30 min) caused significant ($p<0.001$) DNA-damage i.e. comet-formation in both cell types. Pretreatment of cells with FA (0-100 μM, 4 and 8 h incubation periods) induced a linear decrease ($R^2>0.95$) of DNA-migration caused by MMS because of the formation of DPC, therefore the DPC-induction could be defined by extent of this decrease.

Following 3 mJ/cm^2 UVC-irradiation we did not see immediate DNA-damage detectable by comet-assay (DNA-single strand breaks, alkali labile sites) in KC or in FB, just as in our previous experiments on HaCaT cells with UVB. DNA-migration became visible 30 min after irradiation, most likely from the induction of NER. Comet-formation was then detected the next 1-2 h, but in a decreasing fashion reaching the control level during 6 h. There was no significant difference between the two cell types regarding DNA-repair kinetics. Opposite, the presence of FA in KC 0.5 h after irradiation, in FB 4.5 h after irradiation resulted in significantly longer comet-migration compared to that is caused by UVC alone. At 20 h after irradiation comet-formation of cells was the same as for control independently from the presence of FA.

Following 3 J/cm^2 UVA-irradiation significant DNA-damage was detected immediately in both cell types, just as in our previous experiments with HaCaT cells. This DNA-damage disappeared very fast from the cells, during 1 h. Exposure to FA did not influence on the extent of DNA-damage or on the kinetics of DNA-repair.

30 mJ/cm^2 UVB- and 0.24 J/cm^2 UVA-irradiation together (solar simulated UV) resulted in similar changes as it was seen after UVC-irradiation. Comet-formation was seen 0.5-2 h after irradiation, although in this case it decreased to the control level in a shorter

time, during 3 h. Exposure of cells to 10 μM of FA prior to solar irradiation induced significantly ($p<0.05$) longer comet tails in FB as well as in KC 0.5-3 h after UV-exposure than in cells only exposed to solar-simulated irradiation.

Exposure of cells to 10 μM FA did not cause significant change in the proliferation of cells compared to the control. After UVC-irradiation the proliferation of cells decreased, it was more pronounced in FB, less obvious in KC. Considering the proliferation rates, 4 and 24 h after irradiation the FA-pretreatment enhanced the negative effect of UVC on cell proliferation. Opposite, in KC the toxicity was stronger immediately and 1 h after irradiation because of the presence of FA based on alamarBlue^{TM} fluorescence intensity data. The UVA-irradiation did not influence significantly on the proliferation. The proliferation of cells only slightly decreased because of FA. Solar UV-irradiation (UVB+UVA) did not disturb significantly the cell proliferation. FA-pretreatment decreased only slightly the cell growth.

We studied the MN-formation after UVC-irradiation in FB. 72 h following the UVC-irradiation (4 mJ/cm^2) there were significantly more MN in cell cultures compared to the control. 12,5 μM FA alone did not cause increased MN-induction. Opposite, 6 h incubation of cells with this concentration of FA significantly ($p<0.05$) enhanced the frequency of UVC-induced MN.

DISCUSSION

1. DNA-damage and repair after photochemotherapy (PUVA)

In this study we could demonstrate that the mechanism of the effects of UVB and UVA for the formation and repair of DNA-damage is quite different from that of PUVA. Following UVA-irradiation mainly DNA-single strand breaks are formed via oxidative pathways. Their fast rejoining results in a fast decreasing of comet tails in comet-assay. The UVB-irradiation induces CPD and (6-4)-PD, which can be repaired by NER. Therefore the DNA-single strand

breaks showed by comet-assay are most likely repair intermediary products. These results are in agreement with literature data. The DNA-damage (monoadducts, DNA-DNA crosslinks) caused by PUVA and its repair could not be demonstrated by means of alkaline comet-assay. It seems to be likely that the addition of psoralen to the cells impedes the detection of UVA-induced DNA-migration because of the induced DNA-DNA intra- and interstrand crosslinks. Using neutral comet-assay we could demonstrate DNA-double strand breaks, which can be DNA-repair intermediary products considering the time kinetics of their presence. For the repair of PUVA-induced DNA-monoadducts and crosslinks the NER and recombinational repair are described, even an interaction between them is also possible. In conclusion our assay used seems to be suitable for a bedside follow-up of clinical effect of photo(chemo)therapy.

2. Comparison of chromosome-damage (MN-induction) following different wavelength UV-irradiation in normal human MC and FB

MN-formation is a very sensitive parameter for induced chromosomal damage. MN represent genetic material that can not be incorporated into daughter cell nuclei at the time of cell division. In previous studies it was found that the solar UV-irradiation induced MN-formation is higher in cultured human FB from xeroderma pigmentosum and familial malignant melanoma patients than in cells derived from healthy individuals. It is in accordance with the enhanced cancer risk of these patients from sun exposure.

By means of a modified multiparameter FCM method we found dose-response relationship for MN-formation and G2/M-phase cell cycle delay following irradiation with low doses of UV and with γ-rays in MC and in FB.

Regarding UV-induced MN-formation there have been very few data available until recently. Considering literature data, for the induction of endonuclease-sensitive sites in human FB using UVB and UVA the ratio in efficacy (UVB versus UVA) was found to be

125:1. The same high ratio was also observed in hamster cells. In our experiments with FB we found a smaller ratio (25:1). A similar small yield ratio was also reported for the induction of DNA-double strand breaks. It can be explained by the fact that the UVA-induced MN are generated not only from the induced endonuclease-sensitive sites, but also from other lesions (e.g. direct DNA-strand breaks). Taken together, these results highlight the risk of UVA-induced chromosome damage, which can not be neglected in the photocarcinogenesis.

The extent of MN-induction was much less in MC than in FB after γ-, and UVB-irradiation. There was no significant MN-induction following UVA-irradiation. One possible explanation of it is the longer cell cycle of MC.

By means of FCM at the time of measurement of MN-induction we obtain information also about cell cycle delay, namely the G2/M-phase delay could be analysed characterising the cell nuclei in the same sample. The positive correlation between the MN-induction and proportion of G2/M-phase nuclei indicate a common origin of MN-formation and cell cycle delay, for instance, these can be DNA-double strand breaks.

Equitoxic doses (D50) of FB have been reported as 1 J/cm^2 of UVB (313 nm)- and 15 J/cm^2 of UVA-irradiation. The corresponding MN-frequencies are 3 % and 1 %. It is to be mentioned that 1 J/cm^2 UVB and 15 J/cm^2 UVA correspond to approximately 1 and 0.2 minimal erythemal dose. Such UV-doses are received easily by sun exposure in summer, and similarly high UVA-doses can be received from artificial UVA sources such as those employed in tanning salons or during UVA1-therapy. The significant clastogenic effects of UVB and UVA in skin cells, which were presented in our experiments, provides us with arguments to suggest that exposure of human skin to UVB as well as to UVA can contribute to development of genetic instability, e.g. loss of heterozygosity, which is found frequently in several types of human skin cancer cells.

3. FA-induced DNA-damage and modification of UV-induced DNA-damage and repair by FA in normal human KC and FB

FA induces preferentially DPC. Upon 4 h of exposure to FA in FB 50 μM, in KC 25 μM FA caused significant ($p<0.05$) DPC-formation. To this effect 25 μM FA was enough with an 8 h of exposure in both cell types. On the basis of literature data, in lymphoblasts 2 h incubation with 50 μM FA caused significant DPC-formation. In contrast, in human SV40-transformed FB and V79 cells 100 μM was the effective FA-concentration (4 h of exposure). Higher concentrations induced dose-dependent DPC-induction, in association with cytotoxicity. It was independent on cell type. One hour exposure to 100 μM FA in bronchial FB and KC was efficient to induce DPC, but this dose was also cytotoxic. It seems to be that primary cultures of normal human skin derived KC and FB are more sensitive to FA than bronchial cells or V79 cells. Following exposure to FA we could not detect DNA-single strand breaks, and the proliferation of FB and KC after 10 μM FA was not changed significantly. Therefore we choose this concentration to study the effect of FA on UV-induced DNA-damage. By means of comet-assay the UVA-, UVB- and UVC-irradiation induced wavelength –specific and time dependent changes in DNA-migration (comet-formation) were in agreement with our earlier experiments on HaCaT cells. If the cells were irradiated with low dose of solar simulator (UVB+UVA), the time kinetics of DNA-migration was the same as for NER-activation after UVB-irradiation. In this work for the first time we report the comparison of human primary KC with FB regarding UV-induced repair-kinetics by the same type of wavelength and doses of irradiation. Our results did not show significant differences between repair kinetics of FB and KC after any type of UV-irradiation. bronchial epithelial cells and FB from the same donor showed similar repair kinetics after UVC-induced DNA-damage measured by alkaline elution technique.

If the cell cultures were exposed to 10 μM FA before irradiation, after UVA we did not observe any delay of resealing of DNA-single strand breaks. But after UVC and solar UV-irradiation FA modified the time characteristic of DNA-repair, namely FA delayed the decreasing of comet-formation after irradiation. It may be explained by the difference of amount of induced DNA-damage, then the longer comet tails derived from more incision events by NER after co-exposure to FA. But photochemical reactions between FA and UV-irradiation leading to additional DNA-damage have not been described yet. The impaired activity of DNA-resynthesis/ligation of the single stranded DNA-repair patches during NER might be responsible for higher and slower decreasing comet tails. Inhibitory effects of FA on some DNA-repair pathways were previously reported, but the reason for this is only partly characterised. In our experiments we demonstrated the induction of DPC by FA, which might be responsible for repair inhibition and for the depression of cell proliferation. In bronchial cells FA inhibited the incorporation of thymidine and uridine into nucleic acids. It is likely that microenvironmental factors e.g. polyamine depletion, interference with nucleotide uptake and synthesis, resulting in a delay of repair DNA-synthesis, are important for the effects of FA.

The inhibition of NER may give rise to accumulation of single stranded DNA-gaps vulnerable toward endonuclease attack, resulting in higher frequency of DNA-double strand breaks, and followed by chromosomal damage. Our results showed that exposure of cells to FA before irradiation in a concentration (12.5 μM) not inducing a higher MN-frequency, enhanced UVC (4 mJ/cm^2)-induced MN-formation. As the genetic information and gene expression can be altered by chromosomal rearrangements, and a high number of chromosomal aberrations is characteristic for tumor cells, we suggest that chronic FA-exposure may contribute to skin carcinogenesis initiated by UV.

SUMMARY OF RESULTS

In the present work we studied the DNA-damaging effect of solar UV-radiation at different biological endpoints after exposure. Our results demonstrate the usefulness of the comet-assay and the micronucleus (MN)-assay in this research field.

1. Using comet-assay we could differentiate between UVA- and UVB-irradiation induced DNA-damage and repair in cultured human HaCaT keratinocytes. Our results are in agreement with literature data. Furthermore, we have found that the alkaline comet-assay is not able to show the effect of PUVA (8-MOP+UVA) on DNA.
2. We could demonstrate by means of neutral comet-assay the formation of DNA-double strand breaks during DNA-repair following PUVA-induced DNA-damage.
3. A flow cytometry method we modified and applied made it possible to measure MN-induction with differentiation between nuclei in G0/G1- and G2/M-phase at the same time in cultured normal human fibroblasts (FB) ad keratinocytes (KC). We observed positive correlation between MN-induction and proportion of G2/M-phase nuclei in the sample.
4. We were the first to report that UVA is able to induce chromosomal damage in a dose dependent manner in human FB.
5. We have found that UVB-irradiation also induces chromosomal damage in a dose dependent manner in human melanocytes (MC) and FB, but the extent of the DNA-damage is different in the two cell types regarding the same radiation doses.
6. By means of comet-assay we have found that upon 4 h and 8 h of exposure to very low concentrations of formaldehyde (FA) significant DNA-protein crosslink-formation is present in normal human FB and in normal human KC.
7. We were the first to describe DNA-damage and repair kinetics induced by UV-irradiation in cultured normal human KC in comparison with FB using comet-assay. The DNA-damage and repair following UV-irradiation were dependent on the wavelength of UV and the repair kinetics was similar in the two cell types.
8. We have found that FA at a low concentration level interfered with DNA-resynthesis/ligation step of nucleotide excision repair following UVC- and UVB-irradiation. FA had no effect on the DNA-repair after UVA-irradiation.
9. We have found that the delay in DNA-repair resulted in an increase of chromosomal damage. FA at a concentration not inducing MN caused significant increase of UVC-induced chromosomal damage.

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