SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Study of UV-induced cyclobutane pyrimidin dimer (CPD)-dependent processes

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1. Introduction

Ultraviolet radiation from the Sun is responsible for numerous physiological and pathological processes. Accumulations of UV-induced photolesions in the DNA [(6-4)photoproducts and cyclobutane pyrimidine dimers] can cause heritable mutations. In human cells, the repair of UV-induced DNA lesions is achieved by the nucleotide excision repair (NER) complex. The activity of NER can be modified chemically by numerous agents. Several molecules were proved to inhibit the activity of NER *in vitro*. Some of these inhibitors are used clinically. Among them veliparib and arsenic-trioxide are used in anticancer therapy and spironolactone as a diuretic. Nowadays there is also a growing interest in the investigation of different plant-derived phytophenols. Silymarin originating from the seeds of *Silibum marianum*, has a great antioxidant potential, which makes Silymarin a promising candidate in dermatological researches. However, its application is limited due to its low bioavailability.

1.1. Effects of UV radiation on the human skin

Both types of UV radiation reaching the surface of Earth (UVA and UVB) are photochemically active. The short-wavelength UVB is mainly absorbed by the epidermis, the outer layer of the skin, cells of the dermis are less affected. However, UVB can induce substantial damage also in moderate doses, due to its higher energy state. UVB typically induces (6-4)photoproducts (6-4PPs) and cyclobutane pyrimidine dimer photolesions (CPDs) in the DNA. Low energy UVA is absorbed by endogenous chromophore substrates (such as porphyrins, bilirubin, melanin, flavins), which can transfer the energy to other intracellular molecules resulting in the formation of reactive oxygen species (ROS).

High-dose UV exposure induces acute sunburn (dermatitis solaris), hyperplasia, pigmentation and immunosuppression in the skin. Long-term, chronic UV radiation contributes to skin photoaging and enhances the risk of melanoma and non-melanoma skin cancers.

1.2. Nucleotide excision repair

In human cells, UV-induced photolesions are repaired by the nucleotide excision repair (NER) complex. NER recognizes the distortions of DNA strand and restores its structure by the excision and re-synthesis of the lesion-containing nucleotide region. NER is efficient in the repair of 6-4PPs, but it works slowly in the case of CPDs. Because of this, the role of CPDs in UV-induced mutagenesis is well-known. Two types of NER subpathways can be distinguished based on the initiation of DNA damage recognition. The global-genome (GG)-NER is active

throughout the genome, while transcription-coupled (TC)-NER operates only in transcriptionally-active regions.

In eukaryote cells, numerous cellular mechanisms exist besides DNA repair, which are suitable for the prevention of the deleterious effects of UV radiation. Cell cycle arrest, apoptosis and autophagy can prevent the inheritance of the damaged genome to avoid the formation of irreversible alterations of the DNA.

1.3. Clinically applied NER inhibitor molecules

The activity of NER can be chemically modified by numerous agents. In the past few years, several molecules were proved to *in vitro* inhibit NER proteins, thus they prevent the repair of UV-induced DNA damage. Some of these molecules are used clinically. Because of their NER inhibitor activity, an enhanced risk for UV-induced tumorigenesis may be expected after their *in vivo* treatments.

Veliparib is a potent inhibitor of PARP1 protein. PARP1 regulates several cellular processes including DNA repair. Inhibition of PARP1 increases the sensitivity of cells to UVB radiation-induced apoptosis – thus, PARP1 may play a protective role in UVB-induced carcinogenesis. However, PARP1 overexpression was observed in different tumors, which often indicates a poor prognosis. Thus, veliparib and other PARP1 inhibitors are used in cancer therapy.

Resveratrol is a plant-derived compound with anti-inflammatory and antioxidant potential. It was shown that resveratrol enhances UV-induced apoptosis, thereby it may decrease the survival of malignantly transformed cells. However, resveratrol was found to inhibit the repair of 8-oxoguanine lesions, simple-strand DNA breaks and CPD photolesions. Based on the above, the effect of resveratrol in UV-induced mutagenesis is controversial.

Spironolactone is a mineralocorticoid antagonist, which is used as a potassium-sparing diuretic. It is clinically applied for the treatment of progressive heart failure and hypertension. In *in vitro* experiments, spironolactone was proved to induce the fast proteasomal degradation of XPB protein of NER. Although spironolactone is clinically approved, there is no data about the possibility of increased skin cancer formation by the treatment.

Inorganic arsenic compounds are well-known genotoxic and mutagenic agents. Their co-carcinogenic effect with UVB radiation was also proved – probably due to their ability to inhibit XPC protein of NER. Despite the above, *arsenic-trioxide* is used in the therapy of acute promyelocytic leukemia. Arsenic-trioxide enhances apoptosis in multidrug-resistant cell lines. Autophagy induction, ROS-mediated processes and immunological changes are also parts of

its complex effects. Because of this, the involvement of arsenic-trioxide in the UV-induced tumorigenesis remains controversial.

1.4. Dermatological application of silymarin

Silymarin, originating from milk thistle, contains several bioactive components, such as silibinin, silychristin, silydianine and taxifolin. The antioxidant, anti-inflammatory, anti-fibrotic and immunomodulatory effects of the complex is widely-known. Besides, its protective effects against UV-induced apoptosis and carcinogenesis were also shown. On the other hand, silymarin acts as a photosensitizer in UV-exposed keratinocytes. The poor solubility of silymarin in water, thus its low bioavailability makes the dermatological application of the compound difficult. However, the latter could be effectively compensated by the appropriate formulation of the extract.

2. Aim of study

The UV-induced CPD formation contributes to the mutagenesis of the damaged cells. Thereby, tumorigenic transformation of the skin may occur. The long-term effect of CPD lesions greatly depends on the function of intracellular repair mechanisms. Chemical inhibition, thus decreased activity of the repair proteins may result in enhanced UV-induced mutagenesis. Veliparib, resveratrol, spironolactone and arsenic-trioxide are clinically used molecules, which are proved to inhibit the nucleotide excision repair (NER) complex. Dermatological application of the plant-derived silymarin is also controversial: antioxidant, photoprotective and phototoxic effects of the compound were also shown.

In our study, we aim:

1. to demonstrate the NER inhibitor capacity of veliparib, resveratrol, spironolactone and arsenic-trioxide; and investigate their effects on UVB-induced mutagenesis of epithelial cells *in vitro*.

2. to investigate the effects of these compounds on UVB-induced apoptosis, cell cycle arrest, autophagy and protein expression changes.

3. to evaluate the effects of silymarin compounds with different origins on the viability of UVA and UVB-irradiated human keratinocytes.

4. to assess the effects of silymarin compounds with different origins and formulations on UVA-induced ROS production, CPD accumulation and mutagenesis.

3. Materials and methods

3.1. Cell cultures

CHO-K1 (Chinese hamster ovary) and HaCaT (immortalized human keratinocyte) cells were cultured in 4500 mg/L glucose-containing Dulbecco's modified Eagle media (DMEM) supplemented with L-glutamine, 10% heat-inactivated fetal bovine serum (FBS), and 0.5% antibiotic/antimycotic solution (penicillin-streptomycin-amphotericin B).

3.2. Cell treatments

Cells were pretreated with 25 μM ABT-888 (PARP1 inhibitor, veliparib), 10–50 μM resveratrol, 5–25 μM spironolactone, or 0.5–4 μg/mL As_2O_3 solution 120 min prior to UVB irradiation. Silymarin treatments were performed for 30 min immediately before UVA irradiation in different doses and composition. We applied 1. a silymarin extract ordered from Sigma-Aldrich dissolved in ethanol, 2. a commercially available silymarin compound (TEVA-Silegon) and 3. four different topical formulations of silymarin containing 250 μg/ml herbal extract (University of Debrecen, Department of Pharmaceutical Technology). Formulations were aimed to enhance cellular penetration of the compound.

3.3. UV radiation

Cells were covered with a thin layer of DPBS complemented with D-glucose and irradiated with 10-20 mJ/cm^2 UVB (TL-20W/12 RS) or 10-20 J/cm^2 UVA (PUVA 800, H. Waldmann GmbH & Co.).

3.4. CPD-specific Enzyme-Linked Immunosorbent Assay (ELISA)

Genomic DNA was extracted by an Invitrogen™ PureLink™ Genomic DNA Mini Kit 24 h after the UV irradiation. A CPD-specific ELISA was performed as previously described by our workgroup (Boros et al. 2013). Detection of CPD lesions were carried out by anti-CPD monoclonal antibody (clone TDM-2, dilution 1:1500, Cosmo Bio Co., Ltd.).

3.5. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay

24 h after UVA irradiation, cells were washed with PBS, then 100 μL/well DMEM without phenol red containing 200 μg/mL MTT were added. Cells were incubated for 3 h at 37 °C in 5% CO_2 atmosphere. Media was removed and 0.04 M HCl in isopropanol was added to

solubilize the formazan crystals. Absorbance was measured at 590 nm with background subtraction at 620 nm.

3.6. Apoptosis assay

Cell viability was measured 24 or 48 h after UV irradiation using Alexa Fluor 488–conjugated Annexin V/propidium iodide (PI) dual staining. Cells were labeled according to the manufacturer's instructions. Analyses was performed by flow cytometry using a FACS Calibur flow cytometer and CellQuestPro software 5.2. Fluorescence intensity was measured in the FL-1 (for Annexin V) and FL-3 (for PI) channels.

3.7. Reactive oxygen species (ROS) production

Intracellular ROS detection was carried out by dihydroethidium (DHE) staining followed by flow cytometric analysis. After UVA irradiation, cells were washed with PBS and 200 nM DHE in PBS was added for 30 min. Samples were analyzed by a FACSCalibur flow cytometer using CellQuestPro software, fluorescent signal was measured in FL3.

3.8. Cell cycle analysis

Cell cycle progression was quantified 1, 3, and 6 days after UVB irradiation. Cells were fixed with 80% ethanol. Equal numbers of cells were centrifuged at 3500 rpm, for 5 min and re-suspended in 50 μL DPBS containing 0.2 mg/mL RNase A, 0.1 μL Triton-X 100, and 5 mg/mL PI. Samples were incubated at 37 °C for 45 min and supplemented with 0.5% bovine serum albumin (BSA). Cell cycle progression was analyzed by flow cytometry with a FACS Calibur instrument and fluorescence was measured on the x-axis in the FL2-A channel. Doublet discrimination was performed for single-cell analysis. FlowJo software was used for analyzing the data.

3.9. HPRT gene mutation assay

CHO cells were cultured in DMEM containing HAT (hypoxanthine–aminopterin–thymidine) for a week to eliminate preexisting HPRT-mutant cells. After the previously specified treatments, cells were cultured for one more week in complete DMEM. Equal number of cells (1×10^6) were seeded into 100 mm Petri dishes in selective DMEM containing 5 μM 6-thioguanine (6-TG). The 6-TG-resistant cells were allowed to form visible clones for 10 days. Clones were fixed with 100% methanol, and stained with May–Grünwald–Giemsa. HPRT-mutant colonies were counted. 10 μM 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) was used as positive control.

3.10. Western blot

Cells were lysed in RIPA (Radioimmunoprecipitation assay) buffer containing protease-inhibitor cocktail (dilution 1:1000) 2, 6, or 24 h after UVB irradiation. Lysates were centrifuged at 15,000 rpm for 5 min at 4 °C. Protein samples were mixed with 5× loading buffer [bromophenol blue (0.25%), β-mercaptoethanol (5%), glycerol (50%), SDS (sodium dodecyl sulfate; 10%), Tris-HCl (0.25 M, pH 6.8)] then boiled at 100 °C for 10 min. Proteins were separated on 7.5%, 10% or 12.5% polyacrylamide gels, then transferred to nitrocellulose membranes. Membranes were blocked in 5% nonfat dry milk for 1 h, and incubated with the primary antibody overnight at 4 °C (anti-LC3I-II 1:100; anti-mTOR 1:1000; anti-p-mTOR 1:1000; anti-p53 1:1000; anti-p-p53 1:1000; anti-PAR 1:500; ß-actin 1:4000). Membranes were incubated with horseradish peroxidase (HRP)–conjugated goat anti-mouse or anti-rabbit IgG secondary antibodies (dilution 1:2000) for 1 h with gentle shaking. After visualization, protein bands were quantified by ImageJ 1.8.0 software.

3.11. Statistical analysis

The normality of the population was determined using the Shapiro–Wilk test. If two groups were compared, we used independent t-test (two tailed), as the Shapiro–Wilk test showed normal distribution. When we compared three or more groups, one-way ANOVA complemented by Dunnett's post-hoc test was used, if the data showed normal distribution. Kruskal–Wallis test complemented with Dunn's post hoc test was performed, if the distribution of the data was not normal. Statistical calculations were performed using GraphPad Prism 7 and SPSS 25 software.

4. Results

4.1. Mitigation of UVB-induced mutagenesis and modification of cellular UVB-response by chemically-induced NER inhibition

In previous studies, veliparib, resveratrol, arsenic-trioxide and spironolactone were shown to inhibit NER complex and induce the accumulation of UVB-induced CPD lesions *in vitro*. In our present work, HaCaT and CHO cells were pre-treated with the previously mentioned compounds for 2 hours prior to 10-20 mJ/cm^2 UVB irradiation, depending on the experiment.

4.1.1. Veliparib, resveratrol, arsenic-trioxide and spironolactone inhibit CPD removal

24 hours after UVB irradiation (20 mJ/cm^2), we quantified CPD lesions in the DNA of UV-exposed cells. After veliparib, resveratrol, arsenic-trioxide or spironolactone treatments, the relative amounts of CPDs were significantly higher compared to the control (UV-irradiated only) cells. Decreased CPD removal was detected both in HaCaT and CHO cells. These results suggest the inhibitory effect of the tested molecules on CPD elimination.

4.1.2. Investigation of the relationship between CPD accumulation and mutagenesis

To assess UVB-induced mutagenesis, HPRT gene mutagenesis assay was performed. Cells were cultured in selective media, thus single cells carrying heritable, loss-of-function mutations in HPRT gene were selected. By this assay, only viable and actively proliferating cells are detected. In our study, HPRT mutation rate of CHO cells were measured after different doses of UVB radiation. In the case of low-dose UVB (0-10 mJ/cm^2), the number of HPRT mutant cells increases linearly with UVB dose. After higher doses ($\geq$15 mJ/cm^2), the number of mutated cells decreased despite of higher CPD level. It suggests that UVB doses with lower cytotoxic effect may have greater potential to induce mutagenesis.

4.1.3. UVB-induced mutagenesis is attenuated by by veliparib, arsenic-trioxide and spironolactone

UVB-induced mutagenesis after inhibitory treatments were assessed on CHO cell line after 10 mJ/cm^2 UVB. Contrary to our expectations, three out of four inhibitor molecules (veliparib, arsenic-trioxide, spironolactone) significantly decreased UVB-induced mutagenesis. Higher concentrations of the compounds decreased the mutation rates almost to baseline. Resveratrol did not cause any changes in UVB-induced mutation formation.

4.1.4. Effect of NER inhibitors on UVB-induced apoptosis

CHO and HaCaT cells were pre-treated with the previous compounds and irradiated with 20 mJ/cm^2 UVB. 48 hours post-irradiation, cells were stained with Annexin V and propidium-iodide (PI). Viable, apoptotic and necrotic cell populations were distinguished by flow cytometry. Arsenic-trioxide and spironolactone treatments decreased cell viability with 40%, compared to control (UVB-irradiated only) cells. Resveratrol induced mild, ~20% decrease in cell viability. Veliparib had no effect on UV-induced cell death.

4.1.5. Effect of NER inhibitors on UVB-induced cell cycle arrest

Followed by inhibitor treatments and 20 mJ/cm^2 UVB, cell cycle progression by propidium-iodide (PI) staining was performed 1, 3 and 6 days after the irradiation. DNA content of single cells was measured by flow cytometry. Thus, cells in different cell cycle phases can be distinguished. One day post-UVB, a large fraction of cells was detected in the G_2/M phases in every UV-irradiated group. Restoration of cell cycle arrest began at 3 days after UVB exposure and was nearly indistinguishable from the non-irradiated group 6 days after UVB radiation. When cells were pretreated with veliparib, the percentage of cells in the G_2/M phase showed a statistically significant increase compared to vehicle control. The increase lasted up to 6 days after the exposure. Resveratrol caused a moderate elevation in G_2/M-block 3 days after the UVB. Arsenic-trioxide and spironolactone treatments did not affect cell cycle progression after UVB.

4.1.6. Effects of NER inhibitors on UVB-induced protein expression changes

In our further work, we aimed to identify general upstream regulators of UV response at the protein level. We measured total protein expression and phosphorylation status of p53, LC3I-II („microtubule-associated protein 1A/1B-light chain 3 protein") and mTOR („mammalian target of rapamycin"). Alterations were assessed 2, 6 and 20 hours after 20 mJ/cm^2 UVB irradiation. Total p53 expression level was increased 2-6 hours post-irradiation, then it decreased to the baseline after 20 hours. Veliparib pre-treatment caused significant changes in p53 phosphorylation level. Other treatments did not affect p53 activation. LC3-II autophagy marker was also significantly increased after veliparib treatment. Arsenic-trioxide and spironolactone enhanced mTOR phosphorylation after UVB irradiation, but statistically significant difference was only detected in the case of spironolactone treatment.

4.2. Effects of silymarin on UVA-response of human keratinocytes

In our study, the effects of silymarin compounds from different origins were assessed on UVA- or UVB-exposed human keratinocytes. Cells were pre-treated with the compounds for 30 min prior to UV exposure. We used silymarin extracts from three different origins: 1. silymarin powder ordered from Sigma-Aldrich, 2. commercially available, silymarin-containing dietary supplement (Silegon) and 3. four different topical formulations of silymarin containing penetration enhancers (University of Debrecen, Department of Pharmaceutical Technology).

4.2.1. Silymarin treatment increases UVA-induced apoptosis

Cell viability was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay 24 hours post UVA or UVB radiation. Silymarin treatment did not affect UVB-induced cell death, but it induced significant, dose-dependent decrease in cell viability after UVA treatment. Silymarin (Sigma) and Silegon had similar effect on cell survival. Silymarin dissolved in penetration enhancers showed the same effect, the extent of phototoxicity varied based on the composition of the different formulations. UVA-photosensitisation effect of silymarin was confirmed with Annexin V – PI dual staining followed by flow cytometry.

4.2.2. Silymarin decreased UVA-induced reactive oxygen species (ROS) production

To assess the effects of silymarin compounds on UVA-induced ROS production, DHE staining was performed by followed by flow cytometric analysis. Silymarin pre-treatment significantly decreased ROS production after high-dose (20 J/cm^2) of UVA. Decrease in ROS was observed in the case of all silymarin compounds. Silymarin did not affect ROS production of non-irradiated keratinocytes.

4.2.3. Effects of silymarin on UVA-induced CPD accumulation and mutagenesis

Relative amount of UVA-induced CPD lesions was quantified 24 hours after 10 J/cm^2 UVA by CPD-specific ELISA. Silymarin treatments significantly enhanced CPD accumulation after UVA. For the measurements, we used the two most cytotoxic concentrations of silymarin (Sigma).

Effects of the extracts on UVA-induced mutational burden was assessed by HPRT gene mutation assay. In our experiments, we used three different dilutions of silymarin powder ordered from Sigma. Despite the enhanced CPD accumulation, silymarin had no effect on the number of mutation-carrying cells after UVA.

5. Discussion

In our work, we aimed to investigate molecules, that are able to inhibit the activity of NER complex, thus impairs the removal of UV-induced CPD photolesions. We confirmed the NER inhibitor acitivity of veliparib, resveratrol, arsenic-trioxide and spironolactone after UVB irradiation and we showed that silymarin treatment increases UVA-induced CPD formation. We found that three of the tested molecules – veliparib, spironolactone and arsenic-trioxide – significantly attenuated UVB-induced mutation burden. The anti-mutagenic effect of these compounds may be related to their observed pro-apoptotic or anti-proliferative effects.

Reduced number of mutant cells after the inhibitor treatments could be interesting in the aspect of synthetic lethality, which is a promising therapeutical approach for the treatment of various cancers. Synthetic lethality occurs when the loss-of-function mutation of a single gene is tolerable for the cells, but two different gene function loss results in the apoptosis of the cell. Inhibitors of PARP1 are already investigated due to their synthetic lethal effects with the combination of pre-existing BRCA1/2 (Breast Cancer gene 1/2) loss-of-function mutations. Our findings that these inhibitors are able to reduce the survival of cells carrying mutations, also support the hypothesis that selective elimination of cancerous cells can be achieved by inducing defects in cellular repair pathways, supplementing the deleterious effects of other genetic deficiencies. The possibility of testing other anti-mutagenic chemicals (besides veliparib) as synthetic lethal compounds should be also considered - especially in the case of spironolactone, which has markedly less harmful side effects compared to arsenic trioxide.

These results suggest, that possible synthetic lethal properties of the chemicals can originate from their ability to inhibit specific proteins of the NER complex. PARP1 is involved in the recognition of CPDs and thus in the initiation of the NER process through the activation of several NER proteins (e.g. DDB2; XPC). Spironolactone inhibits NER by inducing the rapid proteasomal degradation of the XPB, which suggests a more specific interaction between pre-existing mutations and defective NER repair resulting in the elimination of genetically damaged cells. Arsenic trioxide was also shown to inhibit the XPC, but this molecule has other versatile effects. For instance, arsenic trioxide regulates the survival of damaged cells through mitochondrial apoptosis induction, ROS production and the downregulation of survivin. Thus, its lethal effects on UV-exposed cells cannot be merely explained by XPC inhibition. Resveratrol induced only moderate changes in the UVB-response besides NER inhibition, which may explain why resveratrol did not alter mutagenesis.

In our study, veliparib significantly enhanced UV-induced p53 phosphorylation. This is intriguing in the view of other studies, which found that the application of PARP1 inhibitors in cancer therapy is more effective in p53-deficient cells. Increased activation of p53 following chemically induced PARP1 inhibition may serve to protect cells from intensive genome instability due to the loss of PARP1 function. With the exception of veliparib, none of the other molecules caused significant alteration in p53 phosphorylation, suggesting that changes are at least partly mediated by p53-independent pathways in these cases. The phosphorylation of the cell survival regulator mTOR was increased by spironolactone, which contradicts the strong apoptotic response. We hypothesize that increased mTOR phosphorylation is a cellular strategy to counteract the cytotoxic effect of spironolactone treatment. Autophagy was also enhanced by veliparib treatment, which could contribute to the anti-mutagenic effect of the inhibitor. However, the role of autophagy in tumorigenesis is controversial. While autophagy helps to remove damaged cellular organelles and thereby prevents tumor formation, it can also fuel metabolism by recycling damaged molecules to promote the survival of pre-cancerous cells during metabolic stress in a nutrient-deficient environment.

We observed that the mutagenic effect of UVB and CPD accumulation did not exhibit parallel increase, as UVB-induced mutagenesis decreased at higher UVB doses. This shows that there is no obligate linear relationship between repair activity and UVB mutagenesis, but other factors should be also considered while assessing the possible risk of skin cancer induction by a chemical treatment.

We also observed enhanced UV-induced CPD accumulation and pro-apoptotic response by silymarin treatment. However, photosensitizing effect of the plant extract was only detectable in the case of UVA radiation. This highlights the importance of pyrimidine dimer formation and NER repair after UVA radiation, which is often thought to be less harmful due to its lower energy. The results of HPRT mutation assay suggests that mutagenesis induced by UVA is also significant.

Effect of silymarin on cellular UV-response is controversial: the photoprotective and photosensitizing abilities of silymarin were also shown in different studies. The former is probably due to the antioxidant activity of the plant extract, which may counteract the enhanced CPD accumulation in our experimental system. The diverse effects of silymarin likely originated from the various structure of its flavonoolignant components, their proportion and penetration ability through the cell membrane. In future researches, silymarin can be a promising photosensitizer molecule in photodynamic therapy (PDT) in the treatment of „pre-

cancerous” diseases. Although, the absorption maximum of recently used photosensitizers are mainly in infrared spectrum, low-dose UVA-PDT is also applied due to its stronger cytotoxic effect.

The reason of increased CPD accumulation by silymarin treatment after UVA radiation is currently unknown. However, neutral effect of silymarin on UVA-induced mutagenesis suggests that elevated number of CPDs does not necessarily leads to enhanced risk of skin cancer. According to our results, dermatological applications of natural flavonoids require careful testing and thoughtful assessment of their potential UV interaction to avoid possible adverse effects.

6. Summary

In this study, we investigated the effects of molecules that are able to prevent the removal of UVA- or UVB-induced CPD photolesions from the DNA and modify cellular stress-response upon UV radiation. All compounds used in our work are in clinical practice, thus their effect on skin carcinogenesis is of particular importance. In this work, we examined the effects of veliparib, resveratrol, spironolactone and arsenic-trioxide on UVB-induced apoptosis, cell cycle arrest, autophagy, mutagenesis, and protein expression. Besides, we investigated the effects of a well-known plant-derived extract, silymarin on UVA-irradiated keratinocytes.

In this study we have shown, that enhanced CPD formation does not necessarily associated with increased UV-induced mutagenesis. Despite their ability to inhibit DNA repair, none of the tested compounds augmented UV-induced mutation formation. Surprisingly, some of them even significantly reduced it. These data draw attention to the complex interplay between UV-induced carcinogenesis and DNA repair. In addition, it highlights the importance of other aspects of cellular stress-response beyond repair activity, which should be also taken into account when the mutagenic potential of a chemical treatment is evaluated.

7. Publication list

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Candidate: Eszter Fidrus
Doctoral School: Doctoral School of Health Sciences

List of publications related to the dissertation

1. **Fidrus, E.**, Hegedűs, C., Janka, E. A., Paragh, G., Emri, G., Remenyik, É.: Inhibitors of Nucleotide Excision Repair Decrease UVB-Induced Mutagenesis - an In Vitro Study.
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IF: 1.12

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