Faculty of Pharmacy
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Protective effects of cannabidiol on skin keratinocytes in an oxidative microcellular environment induced by UVA/B radiation or exposure to hydrogen peroxide

PhD thesis
in the field of pharmaceutical sciences

Bialystok 2022

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Chapter 1

Introduction

The effect of hydrogen peroxide and ultraviolet radiation on the skin metabolism

The skin is the main barrier that protects the body against the influence of chemicals such as hydrogen peroxide (H_2O_2) and physical factors, including ultraviolet radiation (UVR). A chemical that induces oxidative stress in skin cells is hydrogen peroxide (H_2O_2), a compound widely used in clinical practice for its antiseptic and wound healing properties [Murphy et al., 2019]. This compound easily penetrates the epidermis, even reaching the dermis and influencing the metabolism of cells in both layers of the skin [Bito et al., 2010]. Moreover, H_2O_2, as the main signaling molecule in the regulation of redox homeostasis [Sies, 2017], enhances the generation of other reactive oxygen species (ROS), and consequently also induces oxidative stress and macromolecular oxidative modifications and inflammation of skin cells [Di Marzo et al., 2018]. Through oxidative modifications of proteins, it can modulate the level and effectiveness of redox-sensitive transcription factors, including nuclear factor erythroid 2 related factor 2 (Nrf2) and nuclear factor kappa B (NF-κB) [Di Marzo et al., 2018]. Consequently, both the metabolism and the functionality of skin cells may be altered. Thus, despite the beneficial clinical effects of using H_2O_2, its effect on skin cell metabolism can also lead to serious side effects in clinical practice [Murphy et al., 2019].

On the other hand, the physical factor disturbing the metabolism of skin cells is UV radiation (UVR). UVR, being a component of solar radiation, consists of three types of radiation, such as UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm) [Ivanov et al., 2018]. UVC radiation is almost completely absorbed by the ozone layer of the atmosphere and hardly reaches the surface of the earth [Ivanov et al., 2018]. Therefore, the human skin is exposed daily mainly to UVA and UVB radiation, which, in skin cells shifting the redox balance towards oxidation, causes oxidative stress, inflammation and changes in the pathways of cell proliferation and death [Panich et al., 2016]. Due to its energetic properties, UVA radiation intensifies oxidative stress and oxidative damage to macromolecules (proteins, lipids and nucleic acids) in the dermis. However, UVB radiation, absorbed mostly by the epidermis, more intensely promotes oxidative damage and inflammation in this layer of the skin [D'Orazio et al., 2013]. Moreover, changes in skin cell metabolism induced by UVA/B radiation increase the risk of malignant transformation of skin cells [Khan et al., 2018]. Despite the characteristic effect of UVA/B radiation on the skin, UV phototherapy is relatively often used in the treatment of skin diseases such as psoriasis due to its inhibitory effect on the cell cycle [Raone et al, 2018].

Alterations in skin cells metabolism under oxidative stress

The oxidative stress accompanying the action of both chemical and physical factors on keratinocytes and fibroblasts promotes oxidative modifications of proteins as well as phospholipids, which are the basis of the structure of biological membranes. The overproduction

of ROS favors their interactions, especially with lipid polyunsaturated fatty acids (PUFAs). As a result, oxidative fragmentation occurs with the formation of α,β-unsaturated aldehydes, such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), which due to the electrophilic nature resulting from the double bonds and the carbonyl moiety are chemically very reactive and interact with the nucleophilic elements of other compounds that play an important role in cell signaling and survival, such as DNA, lipids and proteins [Gaschler et al., 2017; Gęgotek et al., 2019]. Another type of phospholipid modification is oxidative cyclization with the formation of F_2- or D_2/E_2-isoprostanes [Milne et al., 2015; Ayala et al, 2014]. Changes in the structure and function of macromolecules and the associated changes in intracellular signaling can lead to damage to cell metabolism and even cell function and survival dynamics [Gęgotek et al, 2019].

On the other hand, the excessive generation of ROS by H_2O_2/UVR exposure of skin cells stimulates the cellular antioxidant response to counteract oxidative stress via alterations in the activity of redox-sensitive transcription factors Nrf2 and NF-κB. Under normal physiological conditions, the cytoplasmic inhibitor Keap1 by binding to Nrf2 directs Nrf2 towards cullin-catalyzed ubiquitination (the core complex of the E3 ubiquitin ligase complex) and to proteasomal degradation [Gęgotek et al., 2015]. Under the influence of oxidative stress, as a result of oxidation of Keap1 cysteine residues, the Nrf2-Keap1-Cul3 complex dissociates, and Nrf2 translocates to the nucleus [Bellezza et al., 2018], where Nrf2 interacts with a small Maf protein and can bind to genes involved in the antioxidant-responsive element (ARE), leading to transcription of genes encoding cytoprotective proteins, including antioxidant enzymes such as glutathione reductase (GR), thioredoxin reductase (TrxR), catalase (CAT) and superoxide dismutase (SOD) [Gęgotek et al., 2015].

Similarly to Nrf2, also the transcription factor responsible for inflammation - NF-κB (a family consisting of p50, p52, p65, RelB, c-Rel), under physiological conditions is inactivated by proteins of the IκB family. Under oxidative conditions, activation of NF-κB occurs, mediated by two molecular pathways: canonical and non-canonical. In the canonical pathway, IκBα is degraded through the ubiquitin-dependent proteasomal degradation due to its site-specific phosphorylation induced by the IκB kinase (IKK) subunit complex. Thus, the members of NF-κB (mainly p50/RelA and p50/c-Rel dimers) translocate to the nucleus to express pro-inflammatory mediators [Liu et al., 2017]. In contrast, in the non-canonical pathway, the p52 precursor protein - p100 is phosphorylated by IKKα (activated by the NF-κB inducing kinase - NIK) and ubiquitinated. After degrading its C-terminal IκB-like structure of p100, mature p52 translocates to the nucleus for the transcription of pro-inflammatory genes [Sun et al, 2011]. NF-κB, regulates the inflammatory response via the expression of pro-inflammatory mediators such as cytokine - tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) [Liu et al, 2017].

The crosstalk between Nrf2 and NF-κB has been indicated, due to the regulatory role of Keap1/Cul3 complex in both Nrf2 and the NF-κB activity via ubiquitin-related proteasomal degradation, and the stimulative role of Keap1 for IKKβ ubiquitination [Gęgotek et al, 2015]. The activity of these transcription factors and their molecular interplay play a critical role in the regulation of redox homeostasis and the balance between cell death and survival under oxidative

stress. In addition, due to the high level and hyperactivation of Nrf2 in skin cancer cells, including melanoma, particular attention is paid to the cytoprotective role of Nrf2 in the development of these cancers, and cell resistance to cancer therapies [Zimta et al, 2019; Jessen et al, 2021]. In addition, chronic inflammation initiated by H_2O_2 through oxidative stress-mediated activation of NF-κB may lead to pathological metabolic changes [Di Marzo et al., 2018, Zhu et al., 2017]. Along with the changes in the proteasomal system - which plays a regulatory role in several biological pathways, including not only the activation of Nrf2 and NF-κB, but also apoptosis - it has a large impact on the dynamics of the regulation of cellular metabolism in the oxidative environment [Höhn et al, 2014].

A cytoprotective phytocannabinoid: cannabidiol

Oxidative stress and chronic inflammation caused by H_2O_2 or UVR exposure can damage intracellular molecular signalization and cell metabolism and even result in cell death or the development of pathologies such as carcinogenesis [de Jager et al, 2017; Murphy et al, 2019]. To protect skin cells from these metabolic disorders, there is a need to identify compounds that can act as cytoprotective agents. The search is directed especially towards compounds of natural origin. Since it is known that lipid mediators, such as endocannabinoids, which, by activating membrane receptors associated with G proteins, model the levels of both ROS and TNFα, it is believed that phytocannabinoids may have anti-inflammatory properties that could be used in therapeutic activities. One of the widely studied phytocannabinoids found in *Cannabis sativa* L. and having no psychoactive properties is cannabidiol (CBD), which has antioxidant and anti-inflammatory properties [Peres et al., 2018].

CBD is a terpenophenol compound that exhibits antioxidant activity due to its hydroxyl groups and pentyl chain linked to the phenolic ring as well as the methyl group in the cyclohexene ring (Fig. 1) [Borges et al, 2013]. Due to its chemical structure, CBD reveals a direct antioxidant effect as well as other beneficial properties, such as anti-inflammatory, antidepressant, antipsychotic and anticonvulsant [Lim et al., 2017]. It has been shown that the effectiveness of the antioxidant and anti-inflammatory action of CBD - which is the main motivation behind this work - is the result of its direct antioxidant activity as well as its indirect action by altering the activation of membrane receptors and/or by modulating the levels of endocannabinoid components (anandamide, AEA; 2-arachidonylglycerol, 2-AG) (Fig. 2) [Peres et al., 2018]. Therefore, the regulatory role of cellular homeostasis makes CBD appear to be a promising element of pharmacological activities.

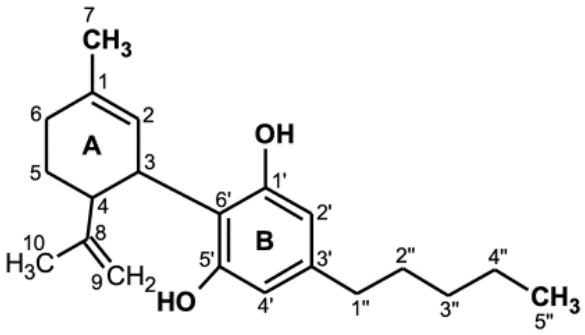

Figure 1. Chemical structure of cannabidiol (CBD)

Even though CBD, due to its biochemical properties, shows promising pharmacotherapeutic effects, knowledge about the molecular mechanism of its action is still limited. The study of the detailed elements of this mechanism using proteomic approaches is therefore of key importance in the pharmacological application of this compound, whose action is highly specific to the cell and the cellular environment.

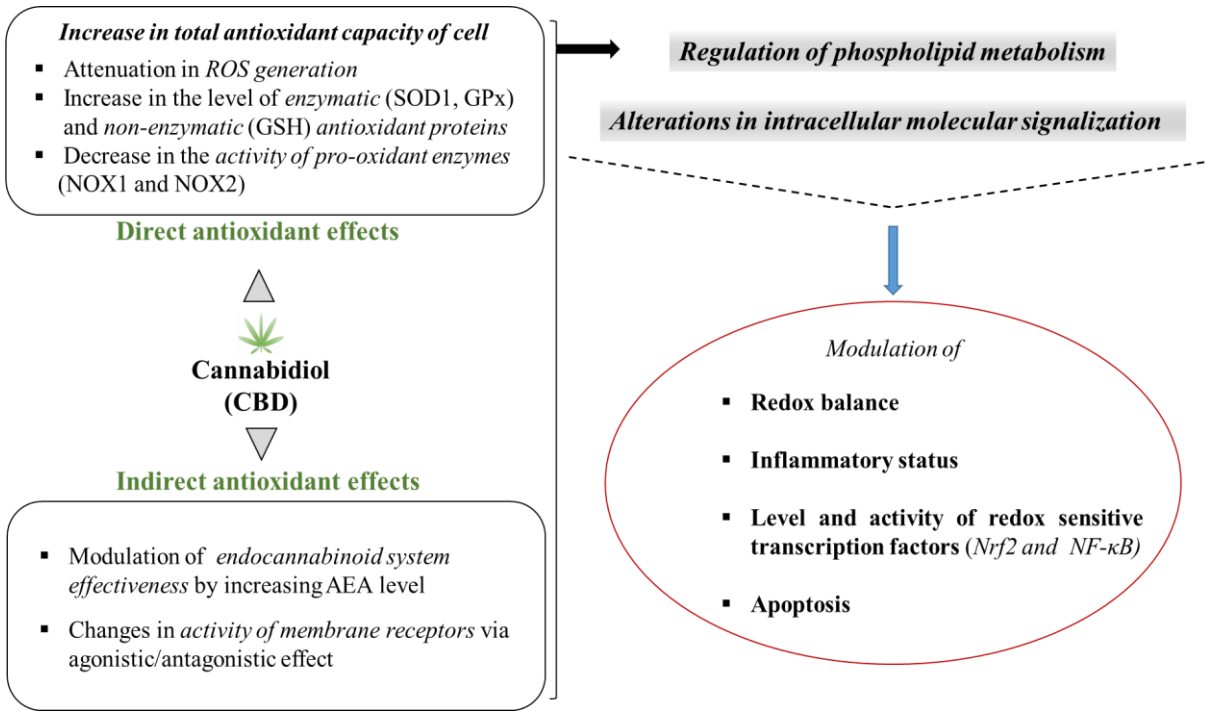

Figure 2. Antioxidant effects of cannabidiol, mediated by its direct/indirect activity in the regulation of cellular homeostasis. (SOD1, superoxide dismutase 1; GPx, glutathione peroxidase; GSH, glutathione, NOX 1 and 2, NADPH oxidase 1 and 2; AEA, anandamide; Nrf2, nuclear factor erythroid 2 related factor 2; NF-κB, nuclear factor kappa B)

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Chapter 2

The aim and outline of the thesis

Since H_2O_2 and UV radiation are external agents widely used in dermatology practice, but which nevertheless can promote oxidative stress and inflammation of skin cells, there is a need for antioxidant cytoprotective compounds to protect skin cells from metabolic damage.

Therefore, the aim of this thesis was to determine the effects of cannabidiol (CBD), a non-psychoactive phytocannabinoid, on keratinocyte metabolism under oxidative microenvironment conditions caused by exposure to H_2O_2 or UV radiation. Therefore, an evaluation of the effects of CBD was carried out in relation to intracellular redox homeostasis and the associated changes in cellular metabolism, in particular regarding the phospholipid and protein profile of the cell membrane.

Five publications have been presented to describe the metabolic changes induced by CBD. The first publication presents the biological effects of CBD [*Antioxidant and anti-inflammatory properties of cannabidiol*], emphasizing the possible antioxidant and anti-inflammatory effects due to the direct and indirect effects of CBD on cellular metabolism. This study indicates the possible antioxidant and anti-inflammatory activities of CBD due to its direct and indirect effects on cellular metabolism.

H_2O_2 and UV radiation damage phospholipid metabolism and membrane integrity, which play an important role in the development of oxidative stress-induced pathogenesis. The second publication [*Cannabidiol protects keratinocyte cell membranes after exposure to UVB and hydrogen peroxide*] shows differences in the protective effect of CBD used in the two experimental systems (administration of CBD only after or before and after exposure to stress factors) on keratinocytes exposed to H_2O_2 or UVB radiation. This chapter also shows that CBD prevents changes in redox balance and cell membrane integrity in a chemically/physically induced oxidative microenvironment.

Due to the highly lipophilic nature of CBD and its protective activity on membrane proteins against oxidative stress-induced modifications, as well as the critical role of biological membranes in molecular signaling, the following publication [*Protective effects of cannabidiol on the membrane proteins of skin keratinocytes exposed to hydrogen peroxide via participation in the proteostasis network*] focuses on CBD-mediated changes in the proteomic profile of keratinocyte membranes under an oxidative microenvironment caused by H_2O_2 exposure. This publication examines the potential effect of CBD on the survival of keratinocytes exposed to H_2O_2 by demonstrating its activity in reducing oxidative stress and maintaining the proteostasis network by modulating protein expression and modifying them by lipid peroxidation products.

Moreover, the next publication [*Protective effects of cannabidiol on the membrane proteome of UVB-irradiated keratinocytes*] indicates the modulatory activity of CBD in the proteomic profile of biological membranes obtained from keratinocytes exposed to UVB. This particular research paper also shows the potential pro-apoptotic action of CBD through its effects on the expression and lipid peroxidation-mediated modification of proteins involved in the

regulation of protein translation and cell proliferation (ribosomal proteins) and maintenance of redox balance (peroxiredoxin-1 and aldo-keto reductase family 1 members).

In turn, the last publication presented in this doctoral dissertation [*Therapeutic application of cannabidiol on the skin of a rat irradiated with UVA and UVB radiation. Proteomic study*], shows the protective effect of CBD *in vivo*. CBD has been shown to maintain proteostasis in keratinocytes of nude rats whose skin has been chronically exposed to UVA/B radiation. Also, CBD has been shown to regulate expression levels of proteins involved in redox balance (Nrf2) and apoptosis (Bcl-2) under oxidative conditions associated with UVB irradiation.

Chapter 3

Publication no 1

Antioxidative and anti-inflammatory properties of cannabidiol

Atalay S, Jarocka-Karpowicz I, Skrzydlewska E.

Antioxidants (Basel); 2019: 9, 21.

Publication no 1

Author declaration
_ SA _ publication 1

Author declaration
_ IJ-K _ publication 1

Author declaration
_ ES _ publication 1

Chapter 4

Publication no 2

Cannabidiol protects keratinocyte cell membranes following exposure to UVB and hydrogen peroxide

Atalay S, Dobrzyńska I, Gęgotek A, Skrzydlewska E.

Redox Biology; 2020: 36, 101613.

Publication no 2

Author declaration
_ SA _ publication 2

Author declaration
_ ID _ publication 2

Author declaration
_ AG _ publication 2

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_ ES _ publication 2

Chapter 5

Publication no 3

Protective effects of cannabidiol on the membrane proteins of skin keratinocytes exposed to hydrogen peroxide via participation in the proteostasis network

Atalay S, Gęgotek A, Domingues P, Skrzydlewska E.

Redox Biology; 2021: 46, 102074.

Publication no 3

Supplementary figure 1_Pub. 3

Supplementary figure 2_Pub. 3

Supplementary figure 3_Pub. 3

Supplementary table 1_Pub. 3

Supplementary table 2 _Pub. 3

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Chapter 6

Publication no 4

Protective effects of cannabidiol on the membrane proteome of UVB-irradiated keratinocytes

Atalay S, Gęgotek A, Skrzydlewska E.

Antioxidants (Basel); 2021: 10, 402.

Publication no 4

Supplementary file_Pub. 4

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_ AG _ publication 4

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_ ES _ publication 4

Chapter 7

Publication no 5

Therapeutic application of cannabidiol on UVA and UVB irradiated rat skin. A proteomic study

Atalay S, Gęgotek A, Wroński A, Domigues P, Skrzydlewska E.

Journal of Pharmaceutical and Biomedical Analysis; 2021: 192, 113656.

Publication no 5

Supplementary file_Pub. 5

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Bioethical approval
for publication 5

Statement

Chapter 8

Discussion

Human skin cells are constantly exposed to external factors, both physical (UV radiation) and chemical (including a disinfectant - hydrogen peroxide), which disrupt the physiological metabolism of these cells, which can lead to various types of skin pathologies. Both H_2O_2 and UV radiation can promote oxidative injury of the skin due to stimulation of excessive ROS generation [Sies, 2017; D'Orazio et al 2013], but the mechanism of action of these two factors on the cell is not the same (Fig. 1). H_2O_2, as a second messenger in the regulation of redox homeostasis, directly participates in intracellular ROS signaling. It is converted into $HO^{\bullet}$ radicals and then participates in modulating the inflammatory response and the healing process [van der Vliet et al, 2014; Collin, 2019]. On the other hand, UV radiation - with its high energetic characteristic and associated photosensitization mechanisms - stimulates the generation of ROS through the changes in the activity of enzymes such as catalase and nitric oxide synthase and also exhibits a subset of signature mutations participating in the development of skin carcinogenesis [Sullivan et al., 2012; Brash, 2015; de Jager et al., 2017].

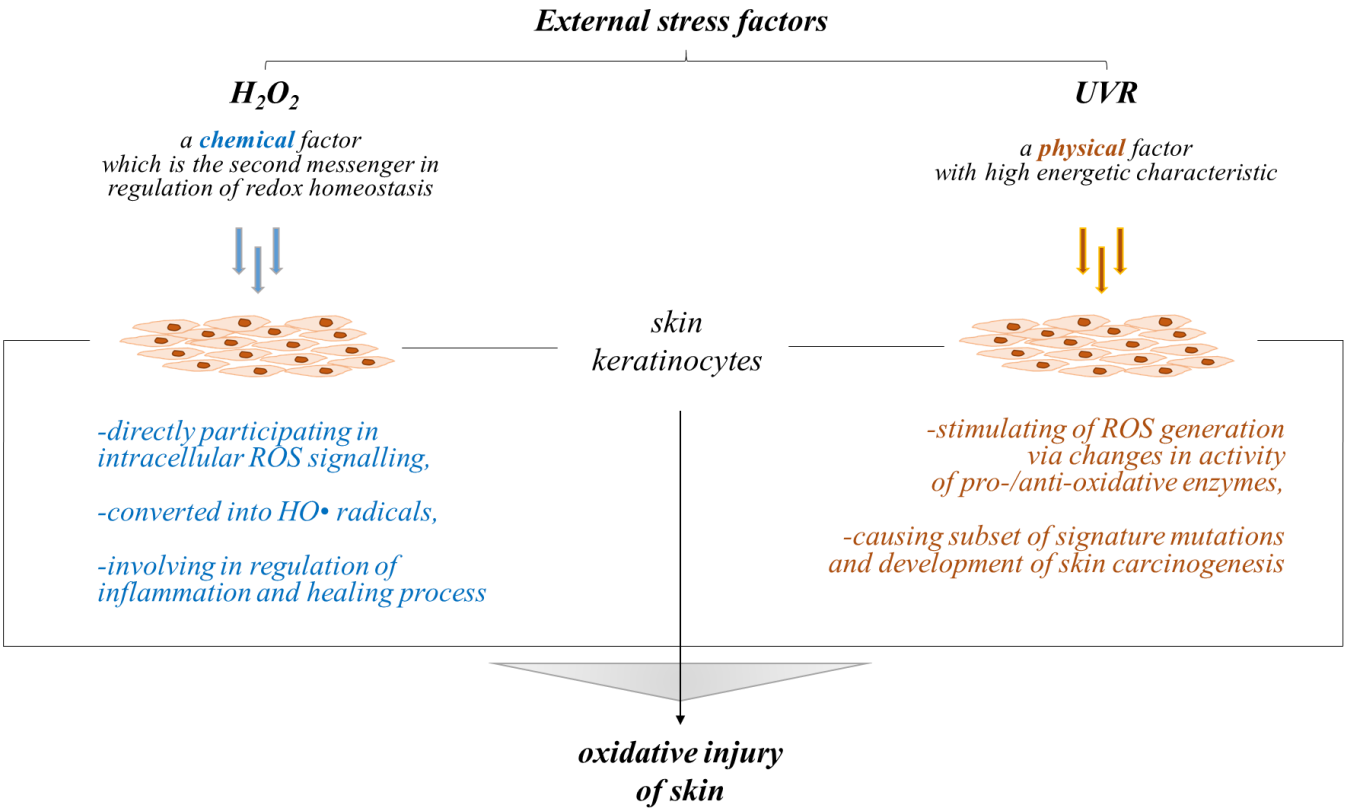

Figure 1. General mechanism of the pro-oxidative action of hydrogen peroxide (H_2O_2) and UV radiation (UVR) on skin keratinocytes.

Therefore, in order to prevent the causes or to heal the effects of the disorders that arise, substances/compounds are sought to counter, first of all, the oxidative stress and inflammation that accompany the body's response to metabolic disorders. The group of natural compounds that have been the subject of intensive research in recent years include phytocannabinoids, among which cannabidiol (CBD). CBD is the basic representative of *Cannabis sativa* L., with antioxidant and anti-inflammatory properties but without psychoactive effect. The literature data constituting the basis of the review [*publication no 1,* Atalay et al., 2019] which begins the cycle of publications belonging to the doctoral dissertation indicate that cannabidiol (CBD) can regulate intracellular molecular signaling by modulating the intracellular level of ROS. And, consequently, the structure, metabolism and function of lipids and proteins could be modulated due to the direct and indirect prevention of changes in the redox balance of cells. First of all, CBD can regulate the level and transcription efficiency of the redox-sensitive transcription factors Nrf2 and NF-κB, which play a key role in the regulation of cellular homeostasis [Jastrząb et al., 2019], including the regulation of inflammation [Iffland et al., 2017] and apoptosis [Sultan et al., 2018]. These are the key biological processes for the development of treatments for diseases accompanied by oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases [Liguori et al., 2018]. Therefore, it was decided to assess the effect of CBD on the metabolic changes in keratinocytes, the basic epidermal cells, most exposed to environmental factors, including those conducive to the formation of oxidative conditions, such as H_2O_2 - a chemical factor and UV radiation as a physical factor. For this purpose, modifications of the metabolism and function of phospholipids and proteins, both membrane and cytoplasmic, have been linked to changes in the redox balance at the transcriptional level. The results of the research showed significant changes at both the level of the lipidome and proteome.

Due to the potentially different effects of hydrogen peroxide and UV radiation on cellular metabolism, the effects of CBD on the cellular response triggered by these factors will be discussed below as specific to each factor. Since the studies evaluated two different durations of use of CBD, henceforth the term "*short*-term" will be used to describe CBD treatment only after exposure to a stress factor, and the term "*long*-term" will be used to denote the use of CBD before and after exposure to the stress factor.

The effect of CBD on skin keratinocytes exposed to H_2O_2

CBD has been found to prevent redox imbalance in keratinocytes treated with H_2O_2 [*publication no 2*; Atalay et al., 2020]. This has been shown for both the *short*-term and *long*-term effects of this phytocannabinoid. CBD was effective in reducing the activity of pro-oxidative enzymes (xanthine oxidase and NADPH oxidase), which increased rapidly after exposure of cells to H_2O_2 and consequently limited the production of superoxide anions. Moreover, both durations of using CBD (*short* and *long*) significantly increased the antioxidant capacity of keratinocytes, which is confirmed by literature data [Iffland et al., 2017]. Moreover, especially *long*-term use of CBD largely prevented changes in the structure and function of the cell membrane as a result of the action of H_2O_2. By protecting the structure of the cell membrane,

CBD prevented its increased permeability and leakage of LDH from keratinocytes and a reduction in keratinocyte size and membrane zeta potential as a result of exposure to H_2O_2. These results confirm the previously considered ability of CBD to regulate the structure of biological membranes by CBD while inhibiting the release of membrane vesicles [Kosgodage et al., 2019].

In addition to differences in the effectiveness of CBD's antioxidant effects, resulting from *short*- or *long*-term use, it was also found that CBD to a varying degree (depending on the method of application) accumulates in biological membranes, which suggests the possibility of an intense effect on membrane components. Furthermore, the levels of CBD in keratinocytes, including both membranes and the cytosol, decreased further with the prolonged application of CBD. Although it is difficult to unequivocally interpret these changes based on the results of previous studies, it can be suggested that they could result from the oxidative action of H_2O_2 and/or CBD metabolism [Hložek al, 2017]. In addition, a similar direction of changes was demonstrated for another lipophilic compound – rutin, which is a polyphenolic flavonoid with antioxidant activity [Gęgotek et al. al., 2017].

Considering the important role of biological membranes in cell functionality and molecular signaling [Watson, 2015; Dias et al., 2021], assessing the effects of CBD on modifications of the membrane composition, including changes in phospholipid and proteomic profiles, which play structural and functional roles in cell biology [Watson, 2015], is very important for understanding the ability and effectiveness of the biological activity of the mechanism of action of this phytocannabinoid. The results obtained indicated that CBD modulates the profile of unsaturated phospholipid fatty acids, including linolenic, linoleic, oleic, γ-linolenic, eicosapentaenoic and arachidonic acids. CBD acts on unsaturated fatty acids by protecting, at least partially, lipid peroxidation. And, consequently, this specific action of CBD decreases the level of oxidative fragmentation (determined by the level of MDA) and cyclization products (determined by the level of F_2-isoprostanes), which indirectly demonstrates lower oxidative stress [Ayala et al., 2014]. Also, recent data from the literature on phospholipids confirm such an effect of CBD [Jarocka-Karpowicz et al., 2020; Łuczaj et al., 2020]. This may indicate that the protective activity of CBD on membrane phospholipids may promote the integrity of cell membranes, thereby protecting the physiological functioning of cells upon hydrogen peroxide treatment.

The results of another study [*publication no 3;* Atalay et al., 2021] show that oxidative stress resulting from the action of hydrogen peroxide and its consequences in the form of an increased level of lipid peroxidation products also affect the proteome of keratinocyte membranes, which is also partially prevented by the action of CBD. *Long*-term treatment of keratinocytes, which was found to be more effective in maintaining the membrane integrity mentioned above, significantly decreased the expression of proteins involved in proteasomal degradation (*beta proteasome subunit type 1 and proteasome regulatory subunit 26S -6A and 60 kDa chaperonin*) which levels were particularly high under oxidative stress to protect cells against the negative effects of protein aggregation [Ikwegbue et al, 2017]. CBD also significantly reduced the level of 60 kDa chaperonin adducts with MDA. However, data from the literature indicate that the formation of 4-HNE/4-ONE-protein adducts can lead to a loss of activity of the

chaperone proteins Hsp70 and Hsp90 [Viedma-Poyatos et al, 2021]. Thus, the CBD-induced decrease in the MDA adduct formation may indicate a protective effect of CBD against the loss of function of 60 kDa chaperonin under oxidative conditions.

In addition, the CBD-induced decline of proteins involved in the degradation of proteasomes may also be indicative of the protective effect of CBD against chronic inflammation caused by oxidative stress. The literature shows that inflammation causes a significant increase in the level of proteins involved in the regulation of the NF-κB pathway [Höhn et. al 2014; Khilji et al., 2020; Song et al., 2021]. Therefore, a decrease in the levels of these proteins following the action of CBD may indicate a reduction in the inflammatory response mediated by NF-κB. CBD-induced lowering of the 27a ribosomal protein, which may support NF-κB activity [Vieira et al., 2020], further enhances the anti-inflammatory potential of CBD. Data from the literature also indicate other routes of regulatory action of CBD on the NF-κB pathway [Jastrząb et al., 2019]. The changes observed in this study, therefore, indicate a protective role of CBD by reducing oxidative stress and the associated chronic inflammation caused by hydrogen peroxide and maintaining the proteostasis network (Fig. 2).

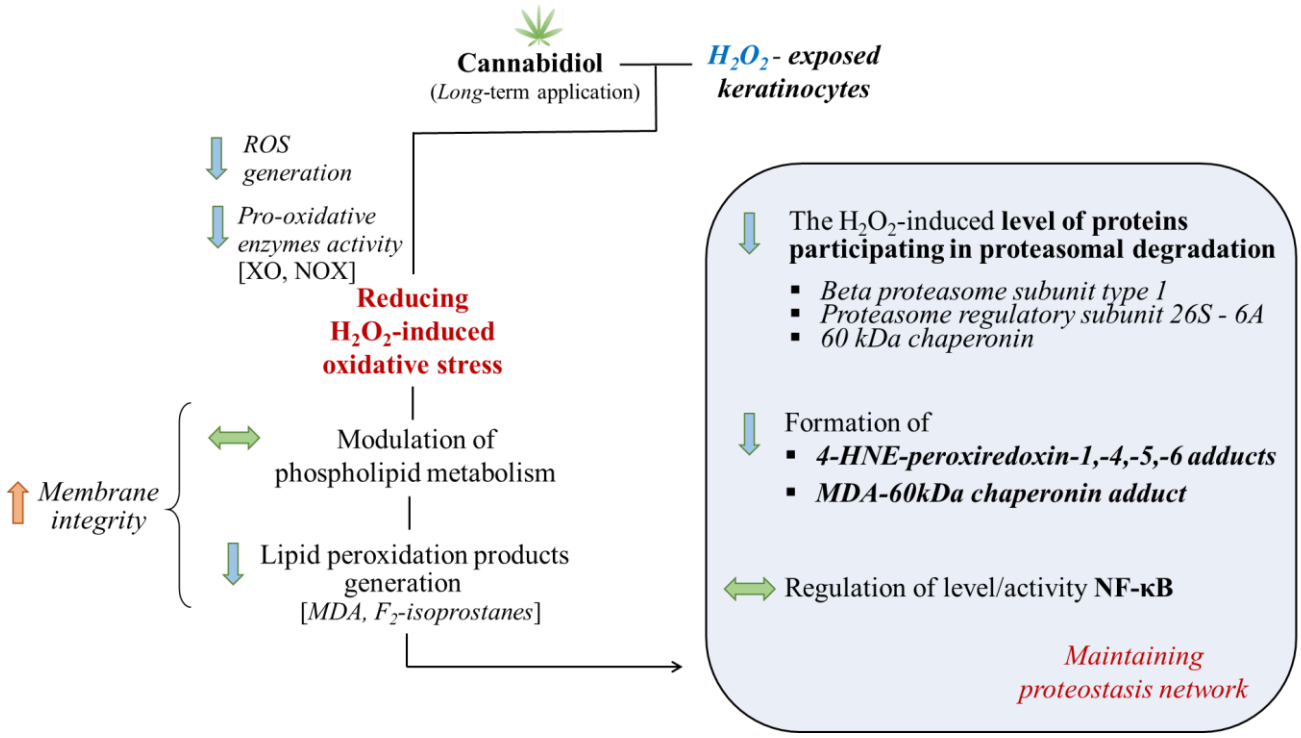

Figure 2. The effects of CBD in skin keratinocytes exposed to hydrogen peroxide (H_2O_2). (Arrow-up is used for increase, arrow-down is used for decrease, two sided-arrow is used for modulation and whole description in the text).

The effects of CBD on keratinocytes exposed to UV radiation

Since skin cells are subjected to both exogenous chemical and physical factors, the effect of CBD on the redox balance disturbed by UV radiation (*in vitro* and *in vivo*) was also assessed. *In vitro* experiments have shown that CBD reduces the UVB-induced increase in the generation of superoxide anions and the activity of pro-oxidative enzymes (xanthine oxidase and NADPH oxidase) as well as increases the total antioxidant status of keratinocytes [*publication no 2*; Atalay et al., 2020]. In addition, CBD increased the level of polyunsaturated phospholipid fatty acids (including linolenic, linoleic, oleic, γ-linolenic, eicosapentaenoic and arachidonic acids), which decreased significantly due to exposure to UVB radiation. This was accompanied by a reduction in the generation of lipid peroxidation products (MDA and F_2-isoprostanes) whose level increased due to UV radiation. It is known that the increased generation of lipid peroxidation products is an important marker of oxidative stress pathologies [Forman et al., 2021; Gęgotek et al., 2021]. A significant relationship between the activity of CBD and lipid metabolism has recently been demonstrated in the omics study of phospholipids and sphingolipids [Łuczaj et al., 2020; Charytoniuk et al., 2021]. Thus, the results of this work show that the antioxidant and lipid protective effect of CBD also applies to the oxidative microenvironment caused by exposure to UVB radiation.

The multidirectional protective effect of CBD on biological membranes exposed to UV radiation results not only from its antioxidant activity [Brand et al., 2020], but also from the accumulation of lipophilic CBD in the membranes. It was found that CBD - especially in its *long*-term action – protects the integrity of keratinocyte membranes by preventing the increase in permeability caused by exposure to UVB radiation, as evidenced by the reduction in LDH leakage induced by CBD-, and an increase in keratinocytes size and negative zeta potential. This indicates not only the effect of CBD on membrane protection but also a potential regulatory role of CBD in molecular signaling involving cell membranes and the consequent modulation of critical biological processes such as apoptosis or cell differentiation, strongly influenced by the high energy of UV radiation [Cheng et al., 2019; Addison et al., 2021].

Independently of the evaluation of the effect of CBD on the redox balance and the metabolism of phospholipids in keratinocytes, a specific proteomic analysis of biological membranes was performed [*publication no 4*; Atalay et al., 2021], demonstrating the protective effect of CBD (especially *long*-term treatment) on the balance of protein expression damaged by exposure to UVB radiation. This work indicates that the regulation of the proteome, both at the level of protein expression [Casares et al, 2020] and post-translational protein modifications [Vrechi et al., 2021], can have a significant influence on the modulation of intracellular signalling induced by CBD [Gęgotek et al, 2019]. L*ong-term* CBD treatment reduced the level of antioxidant proteins identified as aldo-keto reductases A1, B1, B10 and C3 in keratinocytes membranes, while *short-term* CBD treatment significantly increased their levels. However, peroxiredoxins 1, 4, 5, and 6 formed adducts with 4-HNE in *long-term* CBD-treated keratinocyte membranes. Moreover, in the membranes of UVB-irradiated keratinocytes, the total level of 4-HNE-protein adducts was not reduced as much as that of MDA-protein adducts, through CBD

activity (both *short*- and *long*-term treatments). Considering the suggested pro-apoptotic role of 4-HNE as a result of the formation of protein adducts under the influence of oxidative stress [Dalleau et al., 2013], as well as the significant decrease observed in the level of peroxiredoxin 1, an antioxidant enzyme with an anti-apoptotic role [Zhang et al., 2016] and an increase in the levels of pro-apoptotic ribosomal proteins (S3, s19, L13a and L7a) [Xu et al., 2016], the *long*-term effects of CBD may play a role in promoting a pro-apoptotic signal in keratinocytes exposed to UVB by regulating products of lipid peroxidation and associated changes in molecular signaling. It is known that these specific adducts (lipid peroxidation products-proteins) can drastically alter the stability, functionality and even molecular signaling of proteins [Martín-Sierra et al, 2019; Iuchi et al, 2021].

The results of the *in vitro* studies obtained on the ATCC keratinocyte line were confirmed by *in vivo* studies in which the skin of nude rats was exposed to UV radiation and CBD for 4 weeks. The whole proteome analysis of keratinocytes obtained from nude rat skin exposed to UVA/B showed significant activity of CBD in maintaining protein homeostasis, which was disrupted by the effect of UVA/UVB [*publication no 5*; Atalay et al., 2021]. In this work, CBD (applied topically following exposure of UVA/B every 48 h for 4 weeks) normalized the expression of Nrf2, which was significantly increased by UVA/B radiation due to the cellular antioxidant response [Gęgotek et al, 2015]. This may indicate the protective role of CBD against malignant transformation caused by radiation. Additionally, data from the literature indicate a strong link between high Nrf2 activity and malignant transformation, cell resistance to oxidative stress and cancer therapy [Gęgotek et al, 2015; Zimta et al, 2019]. Moreover, increased expression of Nrf2 was also presented in the case of keratinocytes differentiation [Piao et al., 2011] and keratinocytes proliferation in psoriasis [Yang et al., 2017]. In addition, mentioned CBD application also changed the anti-apoptotic Bcl-2 levels in rat keratinocytes. Under the influence of CBD, the level of Bcl-2 in keratinocytes obtained from nude rats decreased after UVA skin irradiation and increased after UVB irradiation. Although the role of CBD in the regulation of the apoptotic network and its consequences on the cellular response cannot be interpreted solely on the basis of the proteomic data presented in this article. This study clearly demonstrates the modulating effect of CBD on apoptotic signaling involving changes in the levels of Nrf2 and Bcl-2 in the cellular microenvironment due to exposure to UV radiation.

Moreover, given the different effects of CBD on the levels of Bcl-2 in keratinocytes exposed to UVA or UVB radiation, it can be concluded that the cellular response induced by CBD treatment may differ depending on the type of radiation (UVA or UVB). Literature data also show a variable effect of CBD on apoptosis, depending on the cell type and the characteristics of the microcellular environment, as well as the concentration of CBD used. The activity of CBD can have a positive [Zhang et al., 2019] or negative effect [da Silva et al., 2018]. Moreover, the modulation of Bcl-2 levels directly by CBD [Suvarna et al., 2019], as well as the upregulation of Bcl-2 via Nrf2 [Niture et al., 2012], confirm the effect of CBD on apoptotic signaling involving independent modulations of the Bcl-2 and Nrf2 pathways (Fig. 3).

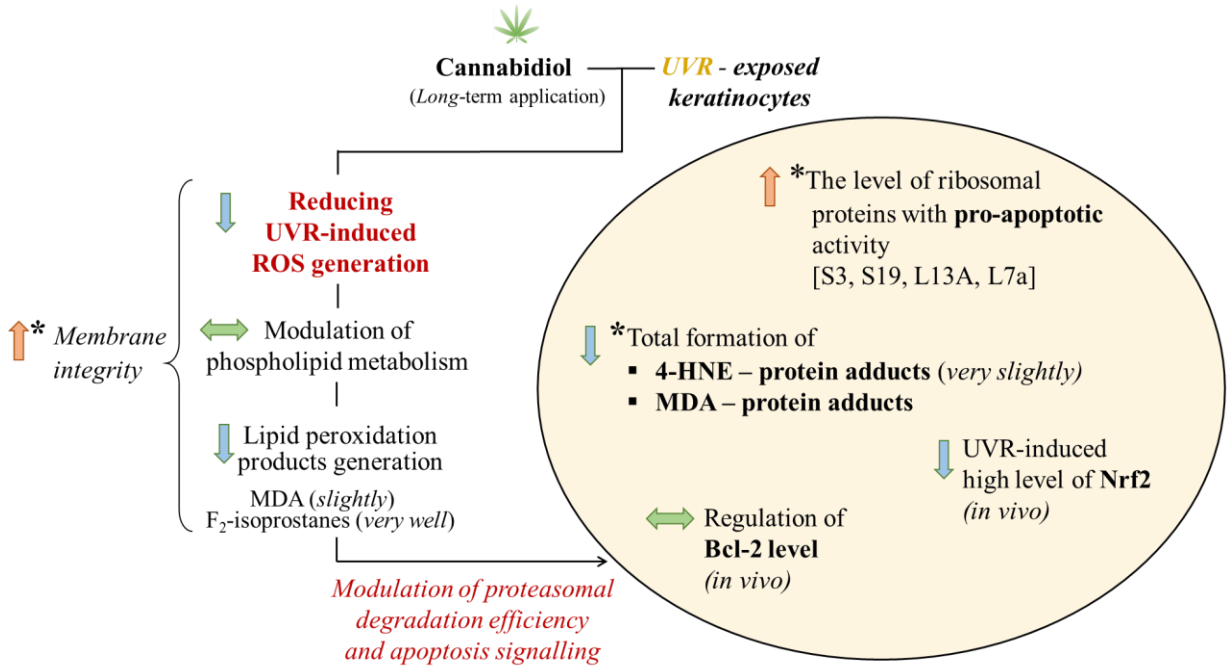

Figure 3. The effects of CBD on skin keratinocytes exposed to UV radiation (UVR). (Arrow-up is used for increase, arrow-down is used for decrease, two sided-arrow is used for modulation, star sign (*) is used for only the case of UVB radiation and whole description in the text).

The results of this dissertation show the multidirectional effect of CBD on keratinocytes exposed to two different stress factors (hydrogen peroxide and UV radiation). When hydrogen peroxide is used, CBD reduces oxidative stress and the related oxidative modifications of lipids and proteins, which helps maintain the proteostasis network and the stability of cell membranes (Fig. 4). In contrast, when CBD is applied to UV-exposed keratinocytes, CBD participates in the normalization of the excessive UV-induced antioxidant response through negative regulation of Nrf2 levels. Moreover, this study also demonstrates the modulating effects of CBD on apoptotic pathways and its potential to negatively regulate UV-induced inflammation.

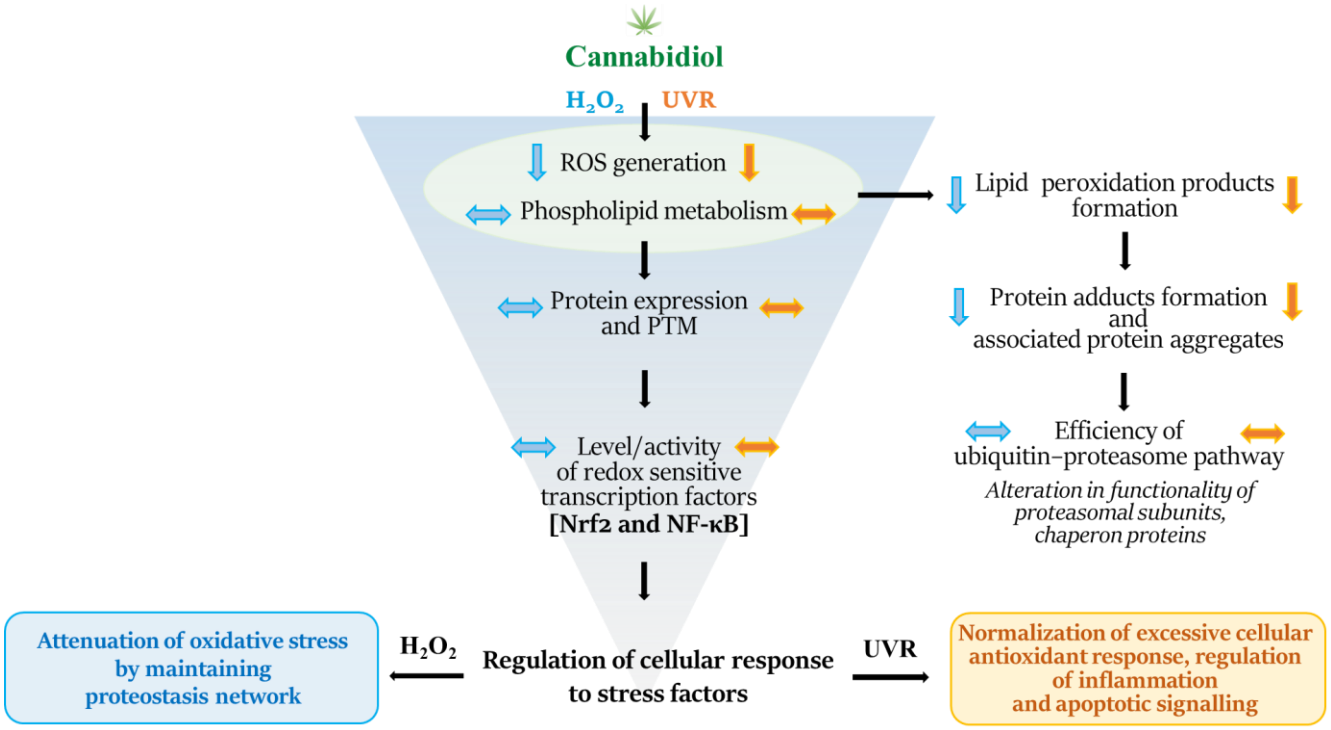

Figure 4. The flow of cannabidiol (CBD)-mediated modulation for intracellular signaling in keratinocytes under conditions of oxidative microenvironment caused by exposure to H_2O_2 or UV radiation. **Black** arrows are used for cascade direction while all the rest of the arrows indicate CBD effect on keratinocytes exposed to H_2O_2 (**blue**) or UV radiation (**orange**). *Arrow-up*, *arrow-down* and *two sided-arrow* represent an increase, decrease and regulation, respectively. (PTM: post-translational modification)

Given the regulatory role of CBD on redox balance and the dynamics of inflammation in skin keratinocytes under oxidative conditions, CBD can be used as a protective compound against oxidative damage caused by skin exposure to UV radiation and chemical agents. Moreover, due to its protective effect in maintaining the proteostasis network and redox balance, CBD can be suggested as a protective agent of healthy/unchanged keratinocytes in UV phototherapy in the treatment of skin diseases such as psoriasis. Also, in the case of hydrogen peroxide, CBD reveals a strong potential to protect keratinocytes by preventing the adverse effects of H_2O_2, in particular oxidative stress and chronic inflammation. These data are of great practical importance due to the use of hydrogen peroxide in medical practice as a skin disinfectant. Considering all the research results obtained, cannabidiol can be proposed as the compound of choice for the protection of the basic epidermal cells most exposed to external physicochemical factors.

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Chapter 9

Conclusions

1. The exposure of keratinocytes to both chemical (H_2O_2) and physical (UVA/B radiation) factors, causes disturbances in cellular metabolism:
   A. H_2O_2 stimulates the generation of ROS and reduces the antioxidant potential of keratinocytes, which contributes to the formation of oxidative stress and increased lipid peroxidation and, therefore, the permeability of the cell membrane. This is accompanied by a modification of the proteomic profile linked mainly to the regulation of the proteostasis network.
   B. UV radiation promotes dysregulation of the oxidant-antioxidant balance of keratinocytes with a shift towards redox imbalance accompanied by an excessive increase in oxidative status. Moreover, UVA/B promotes changes in the proteomic profile of keratinocytes, including increased levels of pro-apoptotic proteins and proteins involved in inflammatory signaling. Additionally, the permeability of cell membranes increases as a result of changes in the structure of membrane phospholipids resulting from increased lipid peroxidation.

2. Cannabidiol (CBD) acting as an antioxidant, both *in vitro* and *in vivo*, counteracts the pro-oxidative effects of hydrogen peroxide and UVA/B radiation and the formation of oxidative stress. This prevents structural and functional changes at the protein and lipid level, and therefore metabolic changes in keratinocytes.

3. Cannabidiol *in vitro* generates different intensities of cellular responses in keratinocytes, depending on the *short*- or *long*-term action of the chemical and physical stressors used:
   A. *Short*-term treatment of keratinocytes with CBD (after use of the stress factors only) increases the acute cellular antioxidant response to H_2O_2 or UV radiation. This leads to the reduction of lipid peroxidation and protein modifications by lipid peroxidation products.
   B. *Long*-term use of CBD (before and after stressors) is more effective than *short*-term use in preventing oxidative stress. This specific treatment limits more severely the modification of lipids and preserves the integrity of the cell membrane. In addition, it is more effective in maintaining the proteostasis network as well as protecting protein homeostasis, disturbed by the oxidative effect of exposure to both H_2O_2 and UVB.

4. CBD applied *in vivo* to the skin of rats exposed to 4 weeks of UVA/B treatment affects the proteomic profile of keratinocytes, including proteins involved in the regulation of redox balance, inflammation, and apoptosis, which is significantly altered by UVA and UVB radiation. CBD normalizes the UVA/B-induced overexpression of proteins, especially by regulating protein biosynthesis and degradation.

5. Considering the results obtained in *in vitro* and *in vivo* experiments, it can be concluded that CBD can be used to counteract the formation of oxidative stress and its effects when applied to skin exposed to chemical factors and to the daily solar radiation.

Chapter 10

Summary

Hydrogen peroxide (H_2O_2) and ultraviolet (UV) radiation, including UVA and UVB, are two types of external oxidative stressors, one chemical and the other physical, which are widely used in the clinical practice due to their antiseptic and cell-arresting/anti-proliferative properties, respectively. These two factors cause oxidative stress and associated chronic inflammation on skin cells, in particular keratinocytes, which are the cells most exposed to external factors. However, overproduction of reactive oxygen species (ROS), it is known to cause changes in cellular metabolism that can lead to changes in differentiation or cell death signalling.

The results of this study indicate that H_2O_2 and UV radiation shift the redox balance towards oxidative stress, intensifying the process of lipid peroxidation, causing changes in the structure and functions of membrane phospholipids. This process is also accompanied by changes in the proteome of cell membranes, which ultimately changes the integrity of these membranes. It was found that H_2O_2 mainly affects the level of proteins involved in the regulation of the proteostasis network, while UVA/B radiation primarily induces changes in the level of proteins mainly involved in the regulation of apoptotic and inflammatory signaling.

In order to prevent the harmful effects of H_2O_2 and UV radiation on skin cells, there is a need for protective compounds, especially natural ones, to counteract adverse metabolic changes. Therefore, the effect of cannabidiol (CBD), the main non-psychoactive phytocannabinoid of *Cannabis sativa* L., on metabolic changes in skin keratinocytes was investigated. The main goal was to evaluate the effect of CBD on cellular metabolism linked to redox homeostasis, particularly with regard to changes in the phospholipid and protein profiles of the cell membrane, under the conditions of the oxidative microenvironment caused by the exposure to H_2O_2 or UV radiation. Two CBD treatments were also compared: *short*-term (using CBD after exposure to stressors) and *long*-term (using CBD before and after exposure to stressors).

The results obtained using electron spin resonance (ESR) spectroscopy and liquid chromatography-mass spectrometry (LC-MS) indicate the protective effect of CBD in counteracting the oxidative stress caused by the action of H_2O_2 and UVA/B radiation. CBD has been shown to prevent over-production of ROS and augmentation of lipid peroxidation products and their adducts with proteins such as MDA/4-HNE/4-ONE-protein adducts, evaluated by changes in intensity of adducted proteins obtained from nano-high performance LC-tandem mass spectrometry (nanoHPLC-QorbiTrap). By decreasing the oxidative metabolism of phospholipids, CBD reduces the structural and functional changes of phospholipids, as observed in the phospholipid profile obtained by GC-MS, which helps to maintain the integrity of damaged keratinocyte membranes following the pro-oxidative action of H_2O_2 and UV radiation. This situation has been confirmed by the significant decrease in lactate dehydrogenase (LDH) leakage from keratinocytes induced by CBD.

Moreover, the results of this study comparing two types of CBD use show that *long*-term use of CBD is more effective in preventing oxidative stress compared to *short*-term use of CBD, due to more intense reduction of lipid modifications and preservation of membrane integrity. Also, the data showing alterations in the membrane proteome indicate that this specific treatment is found to be more effective in maintaining protein homeostasis in biological membranes, by preventing changes in protein expression induced by the effect of H_2O_2 or UVB.

In order to assess the impacts of the protective effect of CBD closer to real medical practice, the effectiveness of CBD used *in vivo* on the skin of nude rats subjected to a 4-week UVA/UVB treatment, often used in the treatment of skin diseases, was studied to uncover changes in the membrane and cytosolic proteome. Comparison of the protein intensities obtained with nanoHPLC-QorbiTrap shows that CBD significantly prevents the UVA/B induced overexpression of proteins involved in the regulation of redox balance, inflammation, and apoptosis.

Finally, the results presented in this study indicate two potential effects of CBD on keratinocytes under oxidative conditions induced by H_2O_2 or UV: pro-survival activity of CBD in keratinocytes exposed to H_2O_2 by reducing in oxidative stress and maintaining the proteostasis network; modulatoring effect of CBD on apoptotic signaling and regulation of redox balance and inflammatory signaling in keratinocytes exposed to UV radiation. Thus, it is possible to suggest that CBD may be used as a protective compound for skin keratinocytes against oxidative damage of cell metabolism caused by the effect of H_2O_2 or UV radiation.

Chapter 11

Summary in Polish

Streszczenie

Nadtlenek wodoru (H_2O_2) i promieniowanie ultrafioletowe (UV), w tym UVA i UVB, to dwa rodzaje zewnętrznych prooksydacyjnych czynników stresogennych, chemicznego i fizycznego, szeroko stosowanych w praktyce klinicznej z uwagi na ich działanie antyseptyczne i unieczynniające komórki/hamujące proliferację komórek. Oba czynniki wywołują stres oksydacyjny i towarzyszący mu przewlekły stan zapalny komórek skóry, w szczególności keratynocytów, które są najbardziej narażone na czynniki zewnętrzne. Jednakże nadprodukcja reaktywnych form tlenu (RFT) indukuje zmiany w metabolizmie komórkowym, powodujacych zaburzenia w różnicowaniu komórek lub szlakach sygnalizacyjnych prowadzących do śmierci komórki.

Wyniki niniejszych badań wskazały, że H_2O_2 i promieniowanie UV przesuwając równowagę redoks w kierunku stresu oksydacyjnego, nasilając proces peroksydacji lipidów, skutkujacy zmianami w strukturze i funkcjach fosfolipidów błonowych. Procesowi temu towarzyszą również zmiany w składzie białkowym błon komórkowych, co ostatecznie wpływa na integralność tych błon. Stwierdzono, że H_2O_2 wpływa głównie na poziom białek zaangażowanych w regulację sieci proteostazy, natomiast promieniowanie UVA/B indukuje przede wszystkim zmiany w zawartości białek zaangażowanych głównie w regulację sygnalizacji apoptotycznej i zapalnej.

Aby zapobiec szkodliwemu wpływowi H_2O_2 i promieniowania UV na komórki skóry, potrzebne są związki ochronne, zwłaszcza pochodzenia naturalnego, przeciwdziałające niekorzystnym przemianom metabolicznym. Dlatego oceniono wpływ kannabidiolu (CBD), głównego niepsychoaktywnego fitokannabinoidu występującego w *Cannabis sativa* L., na zmiany metaboliczne w keratynocytach skóry. Głównym celem niniejszej pracy była ocena wpływu CBD na metabolizm komórkowy związany z homeostazą redoks, szczególnie w odniesieniu do zmian profilu fosfolipidowego i białkowego błony komórkowej, w warunkach mikrośrodowiska oksydacyjnego wywołanego ekspozycją na H_2O_2 lub promieniowanie UV. Porównano również dwa warianty działania CBD: krótkoterminowy (przy podaniu CBD po ekspozycji na czynniki stresogenne) i długoterminowy (przy zastosowaniu CBD przed i po ekspozycji na czynniki stresogenne).

Wyniki uzyskane metodą elektronowego rezonansu paramagnetycznego (EPR) oraz chromatografii cieczowej ze spektrometrią mas (LC-MS) wskazują na ochronne działanie CBD, polegające na przeciwdziałaniu powstawaniu stresu oksydacyjnego wywołanego działaniem H_2O_2 i promieniowania UVA/B. Wykazano, że CBD zapobiega nadprodukcji ROS i produktów

peroksydacji lipidów oraz ich adduktów z białkami, takich jak addukty MDA/4-HNE/4-ONE-białka, oceniane z wykorzystaniem spektrometrii mas w połączeniu z nano-chromatografią cieczową (nanoHPLC-QorbiTrap). Na podstawie wyników analiz profilu fosfolipidowego ocenianego przy uzyciu GC-MS stwierdzono, że CBD poprzez zahamowanie procesów peroksydacji fosfolipidów zapobiega zmianom w strukturze oraz funkcji tych związków, co pomaga w utrzymywaniu integralności błon keratynocytów poddanych prooksydacyjnemu działaniu H_2O_2 i promieniowania UV. Efekt ten potwierdzony został ograniczonym wyciekiem dehydrogenazy mleczanowej (LDH) z keratynocytów będący wynikiem działania CBD.

Wyniki przeprowadzonych badań pokazują ponadto, że długie stosowanie CBD jest bardziej skuteczne w zapobieganiu stresowi oksydacyjnemu w porównaniu z krótkim stosowaniem CBD, ze względu na bardziej efektywne hamowanie oksydacyjnych modyfikacji lipidów i zachowanie integralności błon. Ponadto wyniki analiz proteomicznych wykazały, że długie stosowanie CBD, poprzez zapobieganie zmianom ekspresji białek wywołanym działaniem H_2O_2 lub UVB, jest bardziej skuteczne w utrzymywaniu homeostazy białek błonowych.

W celu oceny realnego, z punktu widzenia medycznego wpływu ochronnego działania CBD, sprawdzono skuteczność CBD stosowanego *in vivo* na skórę bezwłosych szczurów poddanych 4-tygodniowej terapii UVA/UVB, często stosowanej w leczeniu chorób skóry. Badania przeprowadzono aby ocenić zmiany w proteomie błon i cytozolu. Porównanie poziomu białek uzyskanego za pomocą nanoHPLC-QorbiTrap pokazuje, że CBD znacząco zapobiega wywołanej przez UVA/B nadekspresji białek biorących udział w regulacji równowagi redoks, zapaleniu i apoptozie.

Podsumowując, wyniki przeprowadzonych badań wskazują na dwa potencjalne efekty działania CBD na keratynocyty w warunkach stresu oksydacyjnego będącego skutkiem ekspozycji na działanie H_2O_2 lub UV. Pierwszy to zwiększenie przeżywalności keratynocytów poddanych działaniu H_2O_2 związane z zahamowaniem stresu oksydacyjnego i utrzymaniem proteostazy. Drugi efekt działania CBD związany jest z modulowaniem homeostazy redoks w keratynocytach wystawionych na działanie promieni UV. Zatem uzyskane dane sugerują, że CBD może być wykorzystany w roli związku chroniacego komórki skóry przed szkodliwymi skutkami stresu oksydacyjnego wywołanego wystawieniem skóry na działanie H_2O_2 lub UVB.

Chapter 12

List of publications

A. List of publications included in PhD thesis

1. **Atalay S**, Jarocka-Karpowicz I, Skrzydlewska E.
   Antioxidative and Anti-Inflammatory Properties of Cannabidiol.
   Antioxidants; 2019; 9, 21. DOI: 10.3390/antiox9010021.
   IF: 6.312.

2. **Atalay S**, Dobrzyńska I, Gęgotek A, Skrzydlewska E.
   Cannabidiol protects keratinocyte cell membranes following exposure to UVB and hydrogen peroxide.
   Redox Biology; 2020; 36, 101613. DOI: 10.1016/j.redox.2020.101613.
   IF: 11.799.

3. **Atalay S**, Gęgotek A, Wroński A, Domigues P, Skrzydlewska E.
   Therapeutic application of cannabidiol on UVA and UVB irradiated rat skin. A proteomic study.
   Journal of Pharmaceutical and Biomedical Analysis; 2021; 192, 113656. DOI: 10.1016/j.jpba.2020.113656.
   IF: 3.935.

4. **Atalay S**, Gęgotek A, Skrzydlewska E.
   Protective Effects of Cannabidiol on the Membrane Proteome of UVB-Irradiated Keratinocytes.
   Antioxidants; 2021; 10, 402. DOI: 10.3390/antiox10030402.
   IF: 6.312.

5. **Atalay S**, Gęgotek A, Domingues P, Skrzydlewska E.
   Protective effects of cannabidiol on the membrane proteins of skin keratinocytes exposed to hydrogen peroxide via participation in the proteostasis network.
   Redox Biology; 2021; 46, 102074. DOI: 10.1016/j.redox.2021.102074.
   IF: 11.799.

B. List of other publications

1. Gęgotek A, **Atalay S**, Domingues P, Skrzydlewska E.
   The Differences in the Proteome Profile of Cannabidiol-Treated Skin Fibroblasts following UVA or UVB Irradiation in 2D and 3D Cell Cultures.
   Cells; 2019; 8, 995. DOI: 10.3390/cells8090995.
   IF: 4.366.

2. Gęgotek A, **Atalay S**, Rogowska-Wrzesińska A, Skrzydlewska E.
   The Effect of Cannabidiol on UV-Induced Changes in Intracellular Signaling of 3D-Cultured Skin Keratinocytes.
   International Journal of Molecular Sciences; 2021; 22,1501. DOI:10.3390/ijms22031501.
   IF: 5.924

3. Gęgotek A, **Atalay S**, Skrzydlewska E.
   UV induced changes in proteome of rats plasma are reversed by dermally applied cannabidiol.
   Scientific Reports; 2021; 11, 20666. DOI: 10.1038/s41598-021-00134-8.
   IF: 4.380.

4. Gęgotek A, **Atalay S**, Wroński A, Markowska A, Skrzydlewska E.
   Cannabidiol Decreases Metalloproteinase Activity and Normalizes Angiogenesis Factor Expression in UVB-Irradiated Keratinocytes from Psoriatic Patients.
   Oxidative Medicine and Cellular Longevity; 2021;2021,7624389. DOI: 10.1155/2021/7624389.
   IF: 6.543.

C. List of abstracts presented at international conferences

Oral Presentations

1. **Atalay S**, Gęgotek A, Wroński A, Domingues P, Skrzydlewska E. *Proteomic profile of skin keratinocytes from rats exposed to UVA/B radiation and treated with cannabidiol*. 7th Metabolomics Circle 2020; online; 04.11.2020 - 6.11.2020.
2. **Atalay S**, Gęgotek A, Skrzydlewska E. *Membrane proteins of keratinocytes protection by the cannabidiol applied before and after UVB irradiation*. 1st International E-Conference on Antioxidants in Health and Disease, from the Journal Antioxidants; online; 01.12.2020 - 15.12.2020.
3. **Atalay S**, Gęgotek A, Domingues P, Skrzydlewska E. *Cannabidiol effect on the proteomic profile of keratinocytes isolated from rats skin exposed to UVA and UVB radiation*. 20th Biennial Meeting of SFRR International; online; 15.03.2021 - 18.03.2021.

Poster Presentations

1. Gęgotek A, **Atalay S**, Skrzydlewska E. *Rutin and ascorbic acid cooperation in protection against UV-induced oxidative PTMs of proteins in 3D cultured keratinocytes*. Advances in the Study of Lipid and Protein Oxidation: From Methods to Targets; Ghent, Belgium, 13.03.2019 - 15.03.2019, 15B, p.48.
2. **Atalay S**, Gęgotek A, Skrzydlewska E. *Cannabidiol cytoprotective effect against protein oxidative PTMs in UV irradiated human skin keratinocytes*. Advances in the Study of Lipid and Protein Oxidation: From Methods to Targets; Ghent, Belgium, 13.03.2019 - 15.03.2019, 3B, p.45.
3. Niemiro A, Zeliaś W, **Atalay S**, Tutor: Gęgotek A. *Age-dependent changes in proteomic profile of skin fibroblasts exposed to UV radiation*. 14th Bialystok International Medical Congress for Young Scientists; Białystok, Poland, 17.05.2019 - 18.05.2019, p.35.
4. Gęgotek A, **Atalay S**, Domingues P, Skrzydlewska E. *Proteomic analysis of cannabidiol effect on human skin fibroblasts exposed to UVA or UVB irradiation*. SFRR-E 2019 annual meeting "Redox homeostasis: from signaling to damage"; Ferrara, Italy, 19.06.2019 - 21.06.2019, 76, p. S27.
5. Gęgotek A, Wójcik P, **Atalay S**, Wroński A, Łuczaj W, Žarkovic N, Skrzydlewska E. *Changes in lymphocytes redox balance and lipid metabolism in development of psoriasis*. The 44th FEBS Congress, From Molecules to Living Systems; Krakow, Poland, 06.07.2019 - 11.07.2019, P-01-054.

6. **Atalay S**, Gęgotek A, Jastrząb A, Skrzydlewska E. *Nrf2 signaling pathway in skin keratinocytes treated with cannabidiol*. The 44th FEBS Congress, From Molecules to Living Systems; Krakow, Poland, 06.07.2019 - 11.07.2019, P-06-035.
7. **Atalay S**, Jarocka-Karpowicz I, Biernacki M, Skrzydlewska E. *Cannabidiol as a protective factor for keratinocytes exposed to oxidative stress*. iCBD3 International Cannabinoid-Based Drug Discovery and Development Congress; Rotterdam, Netherlands, 04.03.2020 - 5.03.2020, C3.
8. Gęgotek A, **Atalay S**, Biernacki M, Skrzydlewska E. *Exogenous antioxidants in protection against UV-induced changes in the status of low molecular weight thiols in skin cell cultured in vitro*. Low molecular weight thiols: lessons learned and new perspectives, organized by Biochemical Society; online; 07.12.2020 - 09.12.2020.
9. **Atalay S**, Jastrzą A, Gęgotek A, Wójcik P, Skrzydlewska E. *Changes in glutathione/thioredoxin-dependent systems under psoriasis vulgaris and psoriatic arthritis*. Low molecular weight thiols: lessons learned and new perspectives, organized by Biochemical Society; online; 07.12.2020 - 09.12.2020.
10. Gęgotek A, **Atalay S**, Skrzydlewska E. *UV induced changes in profile and structure of protein in rats plasma are retained by topically applied cannabidiol*. SFRR-E 2021 Annual Meeting; online; 15.06.2021 - 18.06.2021.
11. **Atalay S**, Gęgotek A, Skrzydlewska E. *Cannabidiol treatment of keratinocytes, before and after hydrogen peroxide exposure, reduces protein adducts formation with lipid peroxidation products in membrane proteome*. SFRR-E 2021 Annual Meeting; online; 15.06.2021 - 18.06.2021.
12. **Atalay S**, Gęgotek A, Skrzydlewska E. *Cannabidiol reverses the hydrogen peroxide induced-changes in membrane proteome of skin keratinocytes*. 45th FEBS Congress, entitled "Molecules of Life: Towards New Horizons"; online; 03.07.2021 - 08.07.2021.

Chapter 13

Curriculum vitae

Personal Data

Name and surname:	Sinemyiz Atalay Ekiner
Date of birth:	09.03.1991
Place of birth:	Konak, Izmir, Turkey
Nationality:	Turkey

Education

2018 - 2022	Medical University of Bialystok, Poland International Interdisciplinary PhD Studies in Biomedical Research and Biostatistics (ImPRESS) *Funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 754432 and the Polish Ministry of Science and Higher Education, from financial resources for science in 2018-2023 granted for the implementation of an international co-financed project*
2014 - 2017	Ege University, Institute of Health Sciences, Turkey Department of Stem Cell *Thesis title: Determination of potential glycosylation profile for human telomerase's hTERT subunit in hepatocellular carcinoma and neuroblastoma cell line with bioinformatics and experimental methods*
2009 - 2014	Ege University, Faculty of Science, Turkey Department of Biology, Majored in Molecular Biology and Genetic

Experience

2018 - 2022	*Assistant* Medical University of Bialystok, Poland Department of Inorganic and Analytical Chemistry
2016 - 2018	*Clinical Research Site Study Coordinator* MEDEX Site Management Organization, Turkey

Publications

9 articles with total IF = 61.370

15 conference abstracts:

- oral presentations, 3
- poster presentations, as the first co-author, 6

International Internships and trainings

Internships

01.12.2019 - 13.12.2019	**University of Aveiro, Mass Spectrometry Centre, Aveiro, Portugal** *Two-weeks internship for proteomic examination, statistical analysis of proteomic data, determination of protein-protein interactions and analysis of biological pathways*
25.04.2019 - 09.05.2019	**University of Southern Denmark, Department of Biochemistry and Molecular Biology, Protein research group, Odense, Denmark** *Two weeks internship for proteomic analysis, proteomic examination using quantitative proteomics software MaxQuant and proteomic data analysis using Perseus and ComplexBrowser*

Trainings

20.06.2021 – 26.06.2021	**"Chemometric tools in omics analyses"** *49 hours online training by by Perlan Technologies*
15.03.2021 - 17.03.2021	"**R/Bioconductor for mass spectrometry and proteomics**" *18 hours online workshop by Physalia – Courses*
22.09.2019 - 28.09.2019	"**Modern Trends in the use of the separation techniques in combination with tandem mass spectrometry for metabolomics-lipidomic-proteomic studies**" *One-week workshop by Perlan Technologies, Gdynia, Poland*
04.09.2019 - 06.09.2019	**R as programming, Bialystok, Poland** *An extracurricular course organized by Medical University of Bialystok*

Projects conducted as principal investigator

2020 - 2022	Determination of effects of cannabidiol treatment, in a concentration-dependent manner, on the apoptotic response of UVB irradiated keratinocytes *(MNS/2/H2/21/001/2202)*
2019 - 2020	Estimation of the keratinocyte proteome differences after cannabidiol treatment *(MNS/2/H2/20/001/2202)*
2018 - 2019	The response of keratinocytes membrane and intercellular signaling to cannabidiol, used as a protection against the influence of physical and chemical environmental factors *(MNS/2/H2/19/001/2202)*

Award

2021 START scholarship from The Foundation for Polish Science (FNP)