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Effect of tetracycline and UV radiation on melanization and antioxidant status of melanocytes

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ABSTRACT

Tetracycline is a semisynthetic antibiotic and is used in several types of infections against both gram-positive and gram-negative bacteria. This therapy is often associated with phototoxic reactions that occur after exposure to UV radiation and lead to photo-onycholysis, pseudoporphyria, solar urticaria and the fixed drug eruption in the skin. The phototoxic reactions may be related to the melanin content which, on one side may bind drugs – leading to their accumulation, and on the other side, they have photoprotective and antioxidant properties.

In this study the effect of tetracycline and UVA irradiation on cell viability, biosynthesis of melanin and antioxidant defense system in cultured normal human epidermal melanocytes (HEMn-DP) was analyzed. The viability of the cells treated with tetracycline and exposed to UVA radiation decreased in a drug concentration-dependent manner. At the same time, the induction of the melanization process was observed. The significant alterations in antioxidant defense system, on the basis of changes in SOD, CAT and GPx activities, were stated. The obtained results may give explanation for the phototoxic effects of tetracycline therapy observed in skin cells exposed to UVA radiation.

1. Introduction

Tetracycline is one of the first generation tetracycline antibiotics, still in use today. The drug was synthesized by catalytic hydrogenation of chlortetracycline. The obtained C7-dechloro derivative had a better solubility profile and favorable pharmacological activity. This compound was approved by the FDA for clinical use in 1954, as the first novel tetracycline by modification of a natural product, and it was one of the first commercially successful semisynthetic antibiotics used in medicine [1].

Tetracyclines, despite the development of resistance by some bacterial species, are still effectively used against both gram-positive and gram-negative bacteria. These compounds are particularly useful in several types of infections, such as atypical pneumonias, community-acquired pneumonia, rickettsial and chlamydial infections, Lyme disease, cholera, syphilis and periodontal infections [2]. Tetracyclines bind primarily to the 30S subunit of bacterial ribosome where they inhibit protein synthesis by blocking the binding of aminoacylated tRNA (aa-tRNA) to the A site of the ribosome [3]. In addition to their antimicrobial activity, tetracyclines have a number of non-antibacterial effects, including the inhibition of inflammation, proteolysis and angiogenesis, as well as anticancer and antimetastatic activity [2,4].

It has been reported that tetracycline causes phototoxic reactions. Although tetracycline is less photoactive than chlortetracycline and doxycycline, it may cause among others photo-onycholysis, pseudoporphyria, solar urticaria and the fixed drug eruption [5–8]. In correlation with the clinical observations, it was found that tetracyclines cause singlet oxygen mediated oxidation, which leads to the damage of living cells [9].

Photosensitivity is an adverse cutaneous reaction that results when a certain chemical or drug is applied topically or taken systemically at the same time when a person is exposed to UV radiation or visible light [10]. This phenomenon has been recognized for hundreds of years. In the 13th century, Ibn El-Bitar – the Arab scholar noted that certain plant extracts could be applied in combination in exposure to sunlight to treat vitiligo. These herbal medicines were rediscovered in the 1940s and identified as furocoumarins (psoralens) – phototoxic substances, which have been used in modern photochemotherapy to treat chronic dermatoses, such as vitiligo, psoriasis and fungoides [5,11–13].

It was suggested, that phototoxic reactions may correspond to the binding of drug to melanin biopolymers and drug accumulation in pigmented tissues [14].

Melanins are produced by melanocytes – highly specialized cells residing primarily in the hair follicle, epidermis and eye.

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The melanogenesis process takes place in membrane-bound organelles called melanosomes. The active export of melanosomes from melanocytes to surrounding keratinocytes in the skin or to newly synthesized hair is the basis of skin and hair pigmentation [15].

The most well-known agent that enhances melanogenesis is UV radiation. UV-induced skin pigmentation plays a photoprotective role by preventing from DNA damages and mutations. The shielding effect of melanin, is achieved by its ability to serve as a physical barrier that scatters UV radiation, and as an absorbing filter that reduces the penetration of UV through the epidermis. Melanin, besides functioning as a broadband UV absorbent, has also antioxidant properties, acting as a free radical scavenger and having a superoxide dismutase properties [16].

Previously, we documented that aminoglycoside antibiotics: amikacin [17], kanamycin [18], netilmicin [19] and streptomycin [20] as well as fluoroquinolones: ciprofloxacin [21] and lomefloxacin [22], suppressed melanin biosynthesis and affected antioxidant enzymes activities in human light pigmented melanocytes. We also documented that nicotine modulated melanin biosynthesis and antioxidant enzymes activities in human dark pigmented melanocytes [23].

The purpose of this work was to estimate the effect of tetracycline and UV radiation on viability, melanogenesis and antioxidant defense system in cultured normal human epidermal melanocytes, dark pigmented (HEMn-DP).

2. Materials and methods

2.1. Materials

Tetracycline hydrochloride, amphotericin B solution (250 μg/ml), L-3,4-dihydroxyphenylalanine (L-DOPA) and phosphate buffered saline (PBS) were purchased from Sigma–Aldrich Inc. (USA). A growth medium M-254 and a human melanocyte growth supplement-2 (HMGS-2) were acquired from Cascade Biologics (UK). Neomycin sulfate was obtained from Amara (Poland). Penicillin was acquired from Polfa Tarchomin (Poland). Trypsin/EDTA was obtained from Cytogen (Poland). Cell Proliferation Reagent WST-1 was purchased from Roche GmbH (Germany). The remaining chemicals were produced by POCH SA (Poland).

2.2. Cell culture

Human epidermal melanocytes, neonatal, dark pigmented (HEMn-DP, Cascade Biologics) were grown according to the manufacturer's instruction. The cells were cultured in a M-254 medium supplemented with HMGS-2, penicillin (100 U/ml), neomycin (10 μg/ml) and amphotericin B (0.25 μg/ml) at 37 °C in 5% CO_2. All experiments were performed using cells from the passages 5 to 10.

2.3. UVA irradiation procedure

The ultraviolet light source used in this study was a filtered lamp BVL-8.LM (Vilber Lourmat, France). The intensity of UVA (λ_{max} = 365 nm) radiation was 720 mW/cm^2 at 15 cm. The cells, after 24-h incubation with a drug, were irradiated uncovered in petri dishes. Before irradiation the medium had been replaced by PBS. Time of UV exposure was 15 or 30 min. Simultaneously, the nonirradiated cell cultures (control samples) were kept in the dark at 37 °C and 5% CO_2. After irradiation PBS was removed from the cells and melanocytes were incubated in the growth medium for 24 h. Then the cells were lysed.

2.4. Cell viability assay

The viability of melanocytes was evaluated by the WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulphonate) colorimetric assay. WST-1 is a water-soluble tetrazolium salt, the rate of WST-1 cleavage by mitochondrial dehydrogenases correlates with the number of viable cells. In brief, 5000 cells per well were placed in a 96-well microplate in a supplemented M-254 growth medium and incubated at 37 °C and 5% CO_2 for 48 h. Then the medium was removed and cells were treated with tetracycline and exposed to UVA irradiation. After 21-h incubation since irradiation, 10 μl of WST-1 were added to 100 μl of culture medium in each well, and the incubation was continued for another 3 h. The absorbance of the samples was measured at 440 nm with a reference wavelength of 650 nm, against the controls (the same cells but not treated with a drug) using a microplate reader UVM 340 (Biogenet). The controls were normalized to 100% for each assay and treatments were expressed as the percentage of the controls.

2.5. Melanin content

Cell pellets were placed into Eppendorf tubes, dissolved in 100 μl of 1 M NaOH at 80 °C for 1 h, and then centrifuged for 20 min at 16,000*g*. The supernatants were placed into a 96-well microplate, and absorbance was measured using microplate reader at 405 nm – a wavelength at which melanin absorbs light [24]. Melanin content in tetracycline treated cells was expressed as the percentage of the controls (untreated melanocytes).

2.6. Tyrosinase activity

Tyrosinase activity in HEMn-DP cells was determined by measuring the rate of oxidation of L-DOPA to DOPAchrome according to the method described previously [25,26]. Cell lysates were clarified by centrifugation at 10,000*g* for 5 min. A tyrosinase substrate L-DOPA (2 mg/ml) was prepared in the phosphate buffer. 100 μl of each lysate were put in a 96-well plate, and the enzymatic assay was initiated by the addition of 40 μl of L-DOPA solution at 37 °C. Control wells contained 100 μl of lysis buffer and 40 μl of L-DOPA solution. Absorbance of dopachrome was measured every 10 min for at least 1.5 h at 475 nm using a microplate reader. Tyrosinase activity was expressed as the percentage of the controls.

2.7. Superoxide dismutase (SOD) assay

Superoxide dismutase (SOD) activity was measured using an assay kit (Cayman, MI, USA) according to the manufacturer's instruction. This kit utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical. SOD activity was expressed in U/mg protein.

2.8. Catalase (CAT) assay

Catalase (CAT) activity was measured using an assay kit (Cayman, MI, USA) according to the manufacturer's instruction. This kit utilizes the peroxidatic function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. One unit of CAT was defined as the amount of enzyme that causes the formation of 1.0 nmol of formaldehyde