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Impact of lomefloxacin on antioxidant enzymes activity in normal melanocytes HEMa-LP

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ABSTRACT

Lomefloxacin is a synthetic, broad-spectrum antibacterial antibiotic. The use of this drug in the treatment of various infections may be limited by inherent serious adverse effects on pigmented tissues. The exact mechanisms of lomefloxacin phototoxic side effects have not been well established yet. The aim of this work was to examine the effect of lomefloxacin on antioxidant enzymes activity in cultured human normal melanocytes (HEMa-LP). The demonstrated changes in the activity of antioxidant enzymes (SOD, CAT and GPx) in melanocytes in the presence of lomefloxacin indicate that the analyzed drug is responsible for generation of reactive oxygen species, mainly superoxide anion radical and hydrogen peroxide, what may be the reason of the reduction of melanocytes antioxidant status. Modulation of antioxidant defense system in melanocytes by lomefloxacin *in vitro* may explain a potential role of melanocytes and ROS in the mechanisms of lomefloxacin phototoxic effects *in vivo*.

Keywords: lomefloxacin, melanocytes, antioxidant enzymes

INTRODUCTION

Lomefloxacin, 1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (Fig. 1) is a bactericidal antibiotic that resembles the fluoroquinolone class. Like other fluoroquinolones, lomefloxacin acts by inhibiting DNA topoisomerases, of which DNA gyrase and topoisomerase IV are particularly important [10,17]. Fluoroquinolones have excellent antibacterial properties and are more and more largely prescribed for variety of gram-positive and gram-negative infections. However, the use of these drugs in the treatment of various infections is accompanied with serious adverse side-effects, like phototoxicity [10,11,15]. Phototoxicity is postulated to occur as a result of fluoroquinolones phototodegradation and the molecules ability to generate reactive oxygen species such as singlet oxygen, superoxide anion or hydroxyl radical. It has been proved that halogenation (chlorine, fluorine) of position 8 in concert with fluorination of position 6 (the so-called double-halogenated quinolones) demonstrates significant phototoxic potential [13]. Therefore, lomefloxacin and sparfloxacin have been reported to have relatively high phototoxic potential as compared with other fluoroquinolones like ciprofloxacin or norfloxacin [10,18]. UV-induced defluorination at the 8-position generates highly reactive aromatic carbene intermediates which in the presence of water and oxygen are converted to reactive quinone-imine and hydrogen peroxide with subsequent formation of hydroxyl radicals via Fenton reaction [1,18]. These oxidative radicals may attack cellular lipid membranes, initiating inflammatory processes, and cause mitochondria or DNA damage [10,13,18]. The study conducted by Marrot et al. [9] on human skin cells: normal human fibroblasts, keratinocytes and Caucasian melanocytes, confirmed the ability of lomefloxacin to induce DNA damage such as strand breaks and pyrimidine dimers. The activation of the p53 pathway was also demonstrated. An another study showed that fluoroquinolones, including lomefloxacin, absorb UV radiation that reaches the human lens epithelial cells [19]. Phototoxic damage leads to a loss of transparency of the human lens. Thus, fluoroquinolones

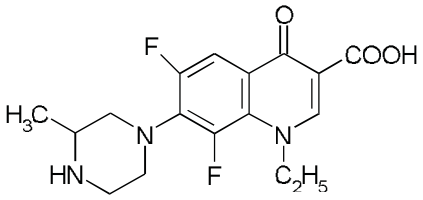

Fig. 1. The chemical structure of lomefloxacin

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taken systematically or injected intravitreally are potentially phototoxic to the eye and may contribute to early cataractogenesis. They may be also phototoxic to other melanin containing tissues, e.g. skin, causing allergy or toxic dermatitis [10,11]. The exact mechanism underlying the phototoxic effect has not been clarified up to now.

In this study we have used the culture of normal human epidermal melanocytes as an *in vitro* experimental model system. Human melanocytes develop from the neural crest, later becoming distributed in the epidermis, hair bulbs of the skin, the uveal tract, the retinal pigment epithelium, the inner ear, and the leptomeninges, which are collectively regarded as melanocyte organs [12,14].

Excessive production of reactive oxygen species (ROS) have been reported in wide variety of clinical disorders. ROS are highly reactive molecules. The balance between production and neutralization of ROS is maintained by concert action of enzymatic and non enzymatic defense system. ROS levels can increase dramatically, which may cause damage to cell structures and react with various biochemical reactions. When unbalanced, it may lead to oxidation of polyunsaturated fatty acids in lipids, amino acids in proteins and damage to DNA. Cells have their own set of antioxidant defense mechanisms to reduce ROS formation and to overcome the limit of damaging effects. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are the main antioxidant enzymes which protect cells against high levels of reactive oxygen species [2,5,8].

The aim of this work was to examine the effect of fluoroquinolone antibiotic lomefloxacin on antioxidant enzymes activity in cultured human normal melanocytes (HEMa-LP).

MATERIALS AND METHODS

Chemicals: Lomefloxacin hydrochloride was purchased from Sigma-Aldrich Inc.(USA). Penicillin was acquired from Polfa Tarchomin (Poland). Growth medium M-254, gentamicin, amphotericin B and human melanocyte growth supplement-2 (HMGS-2) were obtained from Cascade Biologics (UK). The remaining chemicals were produced by POCH S.A.(Poland).

Cell culture: The normal human melanocytes (HEMa-LP – human epidermal melanocytes, adult - light pigmented, Cascade Biologics) were grown according to the manufacturer's instruction. The cells were cultured in M-254 medium supplemented with HMGS-2, penicillin (100 U/ml), gentamicin (10 μg/ml) and amphotericin B (0.25 μg/ml) at 37°C in 5% CO_2. All experiments were performed using cells in the passages 5-7.

Superoxide dismutase (SOD) assay: Superoxide dismutase activity was measured using assay kit (Cayman, MI, USA) according to 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 as a percentage of control.

Catalase (CAT) assay: Catalase activity was measured using assay kit (Cayman, MI, USA) according to 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 per minute at 25°C. CAT activity was expressed as a percentage of control.

Glutathione peroxidase (GPx) assay: Glutathione peroxidase activity was measured using assay kit (Cayman, MI, USA) according to manufacturer's instruction. The measurement of GPx activity is based on the principle of a coupled reaction with glutathione reductase (GR). The oxidized glutathione (GSSG) formed after reduction of hydroperoxide by GPx is recycled to its reduced state by GR in the presence of NADPH. The oxidation of NADPH is accompanied by a decrease in absorbance at 340 nm. One unit of GPx was defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of NADPH per minute at 25°C. GPx activity was expressed as a percentage of control.

Statistical analysis: In all experiments, mean values of at least three separate experiments performed in triplicate ± standard deviations (S.D.) were calculated. The results were analyzed statistically with the Students *t*-test using GraphPad Prism 5.04 Software.

RESULTS

To understand the mechanism underlying the effect of lomefloxacin on reactive oxygen species metabolism, the activities of the antioxidative enzymes were analyzed. Human melanocytes HEMa-LP were exposed for 24 h to lomefloxacin in noncytotoxic concentration 0.075 mM and in concentration 0.75 mM that produces the loss in cell viability by 50% (EC_{50}) (unpublished data from our laboratory). The first enzyme measured was the SOD, which catalyzes the formation of hydrogen peroxide from superoxide anion. Lomefloxacin enhanced SOD activity (Fig. 2). The treatment of cells with 0.075 mM and 0.75mM of lomefloxacin, increased the SOD activity by 21% and 48%, respectively, as compared with controls (100%). CAT and GPx work in concert to catalyze the breakdown of hydrogen peroxide, produced by SOD, to water. The

intracellular CAT activity was significantly increased by 25% for cells treated with lomefloxacin at EC_{50} concentration (0.75 mM) and by 58% for cells exposed to lomefloxacin in concentration 0.075 mM (Fig.3). In contrast to SOD and CAT, the intracellular GPx activity was decreased by 15% for cells treated with lomefloxacin at EC_{50} concentration (0.75 mM) and increased by 12% for the antibiotic in noncytotoxic concentration (0.075mM) in comparison to control cells (Fig. 4).

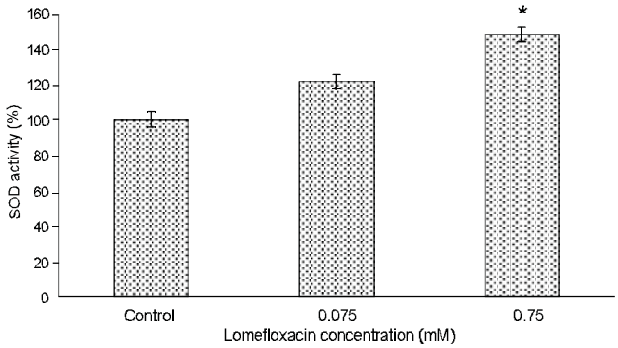

Fig 2. Superoxide dismutase (SOD) activity in HEMa-LP cells after 24-h incubation with 0.075 mM or 0.75 mM of lomefloxacin. Results are expressed as percentages of the controls. Data are mean ± SD of at least three independent experiments performed in triplicate. * p<0.05 *vs.* the control samples.

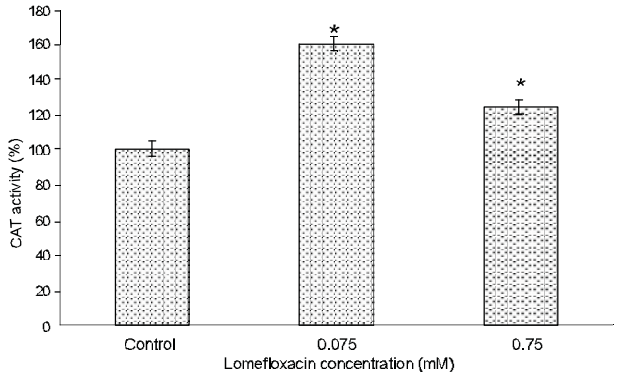

Fig. 3. Catalase (CAT) activity in HEMa-LP cells after 24-h incubation with 0.075 mM or 0.75 mM of lomefloxacin. Results are expressed as percentages of the controls. Data are mean ± SD of at least three independent experiments performed in triplicate. * p<0.05 *vs.* the control samples.

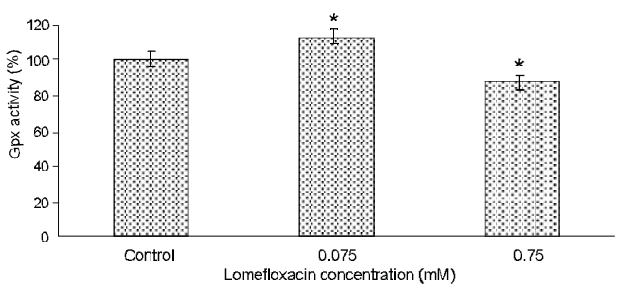

Fig. 4. Glutathione peroxidase (GPx) activity in HEMa-LP cells after 24-h incubation with 0.075 mM or 0.75 mM of lomefloxacin. Results are expressed as percentages of the controls. Data are mean ± SD of at least three independent experiments performed in triplicate. * p<0.05 *vs.* the control samples.

DISCUSSION

Fluoroquinolones exhibit varying abilities to cause phototoxicity, the exaggerated sunburn or severe burn that occur on skin exposed to ultraviolet light (mainly UVA). Phototoxicity is mostly influenced by fluorination of the 8-position in fluoroquinolone molecule, as evidenced by lomefloxacin or sparfloxacin which possess such a substituent and a relatively high phototoxic potential. In contrast, a methoxy group at this position confers photostability and a correspondingly reduced photocarcinogenic potential, as seen with the 8-methoxy fluoroquinolones gatifloxacin or moxifloxacin [18].

Oxygen radicals and other reactive species are generated in biological systems either as by-products of oxygen reduction or by xenobiotic catabolism. These ROS such as superoxide anion, hydroxyl radical and peroxy radicals are unstable and can attack biomolecules (lipids, proteins, nucleic acids), what may be the reason of different human disorders. Melanin is known to be a scavenger of free radicals and it has been suggested that it possesses superoxide dismutase activity [3]. Moreover, this biopolymer acts as a biochemical dustbin, mopping up potentially toxic agents [6]. Such properties may be important for protecting the pigment cells as well as surrounding tissues from the natural toxins, xenobiotics and reactive oxygen species (including free radicals) [16].

The extent of oxidative stress was assessed by measuring activities of cellular antioxidant enzymes: SOD, CAT and GPx. It has been observed that lomefloxacin causes significant changes in the activities of these enzymes in melanocytes. SOD and CAT are the most important enzymes against the toxic effects of oxygen metabolism. The presented increase in SOD activity after exposure of melanocytes to lomefloxacin (Fig. 2) might be the main reason for increased H_2O_2 formation. The enzyme responsible for degrading this high cytotoxic ROS is catalase. Treatment of cells with lomefloxacin in concentration 0.075 mM caused higher increase in CAT activity as compared with the drug concentration 0.75 mM (Fig. 3). At the same time lomefloxacin at higher concentration (EC_{50}) decreased GPx activity, whereas the use of the tested antibiotic in 10-fold diluted concentration (0.075 mM) increased GPx activity (Fig. 4). So, it may be assumed that the demonstrated alterations in cellular CAT and GPx activities are responsible for lomefloxacin phototoxicity, because these antioxidant enzymes cannot efficiently eliminate redundant H_2O_2. The study conducted by Li et al. [7] on rabbit chondrocytes confirmed the ability of one of the fluoroquinolones – ofloxacin to generate reactive oxygen species. It was observed that this drug induced a concentration-dependent increase of intracellular reactive oxygen species production and lipid peroxidation, which may be an early mediator of ofloxacin cytotoxicity. At the same time, the activity of antioxidant enzymes, such as

SOD, CAT and GPx, decreased. Furthermore, ofloxacin induced DNA damage which may be a reason for overproduction of ROS. Cells have a comprehensive array of antioxidant defense mechanisms to reduce free radical formation or limit their damaging effects. These mechanisms are not sufficient when the balance shifts to the side of free radicals generation [4,5], what was observed in our study. The obtained results indicate that the analyzed fluoroquinolone antibiotic is responsible for generation of reactive oxygen species, mainly superoxide anion and hydrogen peroxide, what may be the reason of the reduction of antioxidant status of cells.

CONCLUSION

The demonstrated modulation of antioxidant defense system in melanocytes by lomefloxacin *in vitro* may explain a potential role of melanocytes and ROS in the mechanisms of fluoroquinolones phototoxic effects *in vivo* during high-dose and/or long-term therapy.

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