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Impact of Kanamycin on Melanogenesis and Antioxidant Enzymes Activity in Melanocytes—An In Vitro Study

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ABSTRACT

Aminoglycosides, broad spectrum aminoglycoside antibiotics, are used in various infections therapy due to their good antimicrobial characteristics. However, their adverse effects such as nephrotoxicity and auditory ototoxicity, as well as some toxic effects directed to pigmented tissues, complicate the use of these agents. This study was undertaken to investigate the effect of aminoglycoside antibiotic—kanamycin on viability, melanogenesis and antioxidant enzymes activity in cultured human normal melanocytes (HEMa-LP). It has been demonstrated that kanamycin induces concentration-dependent loss in melanocytes viability. The value of EC_{50} was found to be ~6.0 mM. Kanamycin suppressed melanin biosynthesis: antibiotic was shown to inhibit cellular tyrosinase activity and to reduce melanin content in normal human melanocytes. Significant changes in the cellular antioxidant enzymes: SOD, CAT and GPx were stated in melanocytes exposed to kanamycin. Moreover, it was observed that kanamycin caused depletion of antioxidant defense sytem. It is concluded that the inhibitory effect of kanamycin on melanogenesis and not sufficient antioxidant defense mechanism in melanocytes in vitro may explain the potential mechanisms of undesirable side effects of this drug directed to pigmented tissues in vivo. J. Cell. Biochem. 114: 2746–2752, 2013.

KEY WORDS: KANAMYCIN; MELANIN; MELANIZATION; MELANOCYTES; ANTIOXIDANT ENZYMES

The aminoglycoside antibiotics represent a family of drugs that are used primarily in the treatment of infections caused by aerobic Gram-negative bacteria [Schroeder et al., 2000; Buszman et al., 2007a; Xie et al., 2011, and references therein]. They consist of one or more amino sugars joined to a six-carbon aminocyclitol moiety via glycosidic linkages [Forge and Schacht, 2000; Durante-Mangoni et al., 2009]. The aminoglycoside molecules exert their bactericidal activity by binding directly to the 16 S rRNA in the 30 S subunit of the ribosome and interfering with the translocation step of the translation process [Buszman et al., 2007a; Bindu and Reddy, 2008]. A number of factors contribute to these antibiotics successful and continued use including their rapid bactericidal concentration-dependent killing, a low prevalence of bacterial resistance, a post-antibiotic effect, and low cost. Nonetheless, side effects such as nephrotoxicity and auditory ototoxicity complicate therapy with these antibiotics. Nephrotoxicity is reversible, because proximal tubular cells are able to regenerate [Mingeot-Leclercq and Tulkens, 1999], but destruction of auditory hair cells is irreversible, resulting in permanent hearing loss [Buszman et al., 2007a; Bindu and Reddy, 2008]. Also, some serious adverse events caused by aminoglycosides in pigmented tissues, for example, toxic retinopathy [Penha et al., 2010], conjunctivitis [Robert and Adenis, 2001], and contact dermatitis [Padua et al., 2008], have been described.

Mammalian pigment cells produce melanin as the main pigment. Melanocytes, one of the two types of mammalian pigment cells, differentiate from the neural crest, and migrate to a variety of organs during development [Tolleson, 2005; Simon et al., 2009]. Melanocytes exist not only in the skin, eyes, and hair but also in other sites such as inner ear—in the vestibular organ for balance perception and in the cochlea for auditory perception [Tachibana, 1999; Uehara et al., 2009].

Melanins are broad class of functional macromolecules found throughout nature [Plonka et al., 2009]. Melanin is an antioxidant, a free radical scavenger and has an affinity for drugs, and other chemical substances. Because of these properties, melanin efficiently filters toxic substances and protects tissues from oxidative and chemical stress. However, with chronic exposure to toxic substances the properties of melanin change so that under severe oxidative stress and binding of excessive amount of toxins, melanin itself may induce damage to cells [Hu, 2008]. In addition, the melanin bound drug forms

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a depot that releases the drug over a long period and increases the level of noxious substances stored on melanin, what may cause degeneration in the melanin-containing cells (especially in the eye, ear, skin, and brain) and surrounding tissues [Larsson, 1993].

It is known that different clinical disorders are caused by excessive production of free radicals, especially reactive oxygen species (ROS). Free radicals are highly reactive molecules with a very short half life. 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 stuctures. 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 free radical formation and to overcome the limit of damaging effects. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the enzymatic defense systems of cells against oxygen radicals [Finaud et al., 2006; Marí et al., 2010].

Previously, we have documented that kanamycin [Wrześniok et al., 2005], amikacin, neomycin, tobramycin and netilmicin [Buszman et al., 2006, 2007b; Wrześniok et al., 2011, 2012] form stable complexes with model synthetic melanin. Our studies have also demonstrated that melanin modifies the inhibitory effect of gentamicin [Wrześniok et al., 2002], kanamycin [Wrześniok et al., 2005], and netilmicin [Buszman et al., 2006] on collagen biosynthesis in human skin fibroblasts.

Although there have been numerous studies in animals [e.g., Oum et al., 1992; Hancock et al., 2005], there have been no reports on normal melanocytes regarding the correlation between the amino-glycoside administration and melanization or antioxidant enzymes activities. Therefore, we studied the impact of aminoglycoside antibiotic—kanamycin on viability, melanogenesis and antioxidant defense system in human normal melanocytes HEMa-LP.

MATERIALS AND METHODS

MATERIALS

Kanamycin (sulphate salt), L-3,4-dihydroxyphenylalanine (L-DOPA), synthetic melanin, Triton X-100, and mushroom tyrosinase were 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). 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 S.A.(Poland).

CELL CULTURE

The normal human melanocytes (HEMa-LP, 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.

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 disulfonate) 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, 5,000 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 kanamycin solutions in a concentration range from 0.01 to 10 mM. After 21-h incubation, 10 μl of WST-1 were added to 100 μl of culture medium in each well, and the incubation was continued for 3 h. The absorbance of the samples was measured at 440 nm with a reference wavelenght of 650 nm, against the controls (the same cells but not treated with kanamycin) 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.

MEASUREMENT OF MELANIN CONTENT

The melanocytes were seeded in a 35 mm dish at a density of 1×10^5 cells per dish. Kanamycin treatment in concentration range from 0.06 to 6.0 mM, began 48 h after seeding. After 24 h of incubation, the cells were detached with trypsin/EDTA. 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 [Ozeki et al., 1996]. A standard synthetic melanin curve (0–400 μg/ml) was performed in triplicate for each experiment. Melanin content in kanamycin treated cells was expressed as the percentage of the controls (untreated melanocytes).

TYROSINASE ACTIVITY ASSAY

Tyrosinase activity in HEMa-LP cells was determined by measuring the rate of oxidation of L-DOPA to dopachrome according to the method described by Kim et al. [2005] and Busca et al. [1996], with a slight modification. The cells were cultured at a density of 1×10^5 cells in a 35 mm dish for 48 h. After 24-h incubation with kanamycin (concentration range from 0.06 to 6.0 mM] cells were lysed with phosphate buffer (pH 6.8) containing 0.1% Triton X-100, and lysates were clarified by centrifugation at 10,000*g* for 5 min. The protein content in each cell lysate was determined according to the Lowry method [Lowry et al., 1951]. A tyrosinase substrate L-DOPA (2 mg/ml) was prepared in the same lysis phosphate buffer (without Triton). Hundred microliter 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. Absorbance of dopachrome was measured every 10 min for at least 1 h at 475 nm using a microplate reader. Tyrosinase activity was expressed in μmol/min/mg protein.

A cell-free assay system was used to test for direct effects on tyrosinase activity. 130 μl of phosphate buffer containing kanamycin in a concentration range from 0.06 to 10.0 mM, were mixed with 20 μl of mushroom tyrosinase (1,000 units), and 100 μl of L-DOPA solution (2 mg/ml) was added to each well. The assay mixtures were incubated at 37°C for 20 min, and absorbance of dopachrome was measured at 475 nm in a microplate reader. The mushroom tyrosinase

activities were calculated in the relation to the controls (samples without kanamycin). The value IC_{50} (the concentration of a drug that inhibits a standard response by 50%) was calculated on the basis of a dose-dependent inhibition curve, as described by Chung et al. [2009].

SUPEROXIDE DISMUTASE ASSAY

Superoxide dismutase (SOD) 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 in U/mg protein.

CATALASE ASSAY

Catalase (CAT) 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 in nmol/min/mg protein.

GLUTATHIONE PEROXIDASE ASSAY

Glutathione peroxidase (GPx) 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 in nmol/min/mg protein.

STATISTICAL ANALYSIS

In all experiments, mean values of at least three separate experiments ($n = 3$) performed in triplicate ± standard error of the mean (SEM) were calculated. The results were analyzed statistically with the Student's *t*-test using GraphPad Prism 6.01 Software.

RESULTS

IMPACT OF KANAMYCIN ON CELL VIABILITY

Melanocytes were treated with kanamycin in a range of concentration from 0.01 mM to 10 mM for 24 h (Fig. 1). It has been demonstrated that at a relative low antibiotic concentration (0.01 mM) the loss in cell viability is not observed. Treatment of cells with 0.1, 1, 2.5, 7.5, and 10 mM of kanamycin for 24 h has led to the loss of about 7.8%, 10.1%, 13.0%, 63.6%, and 91.8% in the cell viability, respectively. The value of EC_{50} (the concentration of a drug that produces loss in cell viability by 50%) was ~6.0 mM.

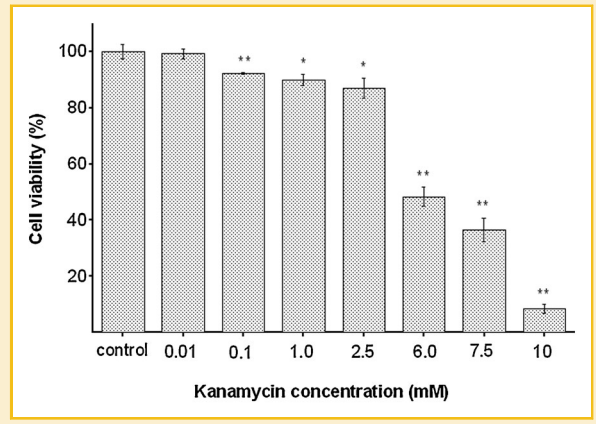

Fig. 1. The effect of kanamycin on viability of melanocytes. Cells were treated with various doses of kanamycin (0.01—10 mM) and examined by WST-1 assay. Data are expressed as % of cell viability. Mean values ± SEM from three independent experiments performed in triplicate are presented. $^{*}P < 0.05$ versus the control samples; $^{**}P < 0.005$ versus the control samples.

IMPACT OF KANAMYCIN ON MELANIZATION PROCESS IN MELANOCYTES

The effectiveness of melanization process was estimated by measuring the melanin content and cellular tyrosinase activity in melanocytes treated with kanamycin in concentration EC_{50} as well as 10-fold and 100-fold lower, for 24 h. After performing a calibration curve, the melanin content per cell was determined as 26.3 to 35.0 pg/cell for melanocytes treated with antibiotic and 37.1 ± 3.4 pg/cell for a control sample. The obtained results, recalculated for culture (1×10^5 cells), were finally expressed as a percentage of the controls (Fig. 2). Kanamycin in concentration 0.06 mM had no effect on

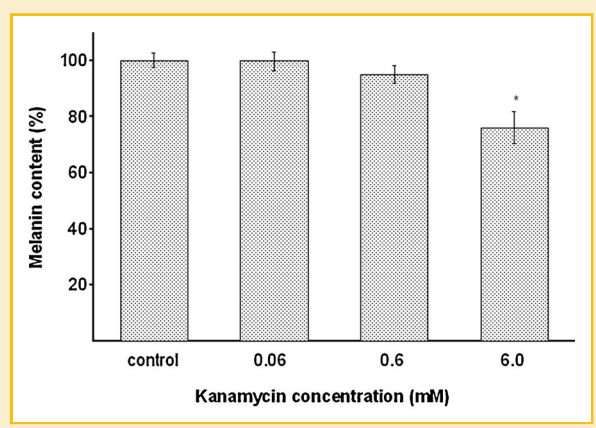

Fig. 2. The effect of kanamycin on melanin content in melanocytes. Cells were cultured with 0.06, 0.6, or 6.0 mM of kanamycin for 24 h and melanin content was measured as described in Materials and Methods Section. Results are expressed as percentages of the controls. Data are mean ± SEM of at least three independent experiments performed in triplicate. $^{*}P < 0.05$ versus the control samples.

melanin content. Treatment of cells with 0.6 and 6.0 mM of kanamycin for 24 h reduced melanin production by about 5% and 24%, respectively.

Tyrosinase activity in HEMa-LP cells treated with kanamycin also decreased in a manner correlating well with the inhibitory effect on melanin production (Fig. 3). After 24-h incubation with antibiotic, tyrosinase activity was suppressed to 74% at 0.6 mM and to 62% at 6.0 mM of kanamycin, as compared with control. Kanamycin in concentration 0.06 mM had no effect on the cellular tyrosinase activity.

The analyzed drug significantly decreased mushroom tyrosinase activity (Table I) in a concentration-dependent manner. The concentration of kanamycin required for 50% inhibition of mushroom tyrosinase activity (IC_{50}) was 7.06 mM.

IMPACT OF KANAMYCIN ON ANTIOXIDANT ENZYMES ACTIVITY

To understand the mechanism underlying the effect of the tested antibiotic on ROS metabolism, the activities of the antioxidant enzymes were characterized. Human melanocytes HEMa-LP were exposed to kanamycin in concentration 0.6 mM or 6.0 mM (EC_{50}) for 24 h. The first enzyme measured was the SOD, which catalyzes the formation of hydrogen peroxide from superoxide anion. Kanamycin enhanced SOD activity (Fig. 4). The treatment of cells with 0.6 and 6.0 mM of kanamycin, increased the SOD activity by 35% and 62%, respectively, as compared with controls. CAT and GPx work in concert to catalyze the breakdown of hydrogen peroxide, produced by SOD, to water. The intracellular CAT activity (Fig. 5) was significantly increased by 34% for cells treated with kanamycin at EC_{50} concentration (6.0 mM) and by 63% for cells exposed to kanamycin in concentration 0.6 mM. In contrast to SOD and CAT, there was no significant differences in melanocytes GPx activity after treatment with 0.6 mM of kanamycin, in comparison to control cells. However, the activity of GPx was significantly decreased (by 30%) for cells treated with kanamycin at EC_{50} concentration (Fig. 6).

TABLE I. Inhibitory Effect of Kanamycin on Mushroom Tyrosinase Activity

Analyzed drug	Concentration (mM)	Inhibition ± SEM[a] (%)	IC_{50}[b] (mM)
Kanamycin	0.060	23.89 ± 1.04	7.06
	0.60	33.97 ± 3.46	
	6.0	46.32 ± 2.71	
	10.0	58.06 ± 0.51	

[a]Samples contained phosphate buffer with different kanamycin concentrations, mushroom tyrosinase (1,000 units) and L-DOPA solution (2 mg/ml). Tyrosinase activities were measured as decribed in Materials and Methods Section.
[b]50% Inhibitory concentration.

DISCUSSION

The aminoglycoside antibiotics comprise a large group of naturally occurring or semisynthetic polycationic compounds. A wide range of adverse effects can occur following the administration of these drugs, ototoxicity, and nephrotoxicity being the most important. However, some toxic effects directed to pigmented tissues have also been stated [Robert and Adenis, 2001; Padua et al., 2008; Penha et al., 2010].

In the present study the effect of kanamycin on melanocytes viability was analyzed. We have found that kanamycin in concentration from 0.1 to 10.0 mM decreased the cell viability in a dose-dependent manner (Fig. 1). This finding may explain the damage of pigmented cells caused by kanamycin in vivo.

Pigmentation is a consequence of enzymatically controlled synthesis of the pigmentation substance melanin [reviewed in Plonka et al., 2009]. Melanin occurs in two forms: eumelanin—a dark-black insoluble polymer and pheomelanin—which is a light red-yellow sulphur containing polymer. Both are indole derivatives of 3,4-

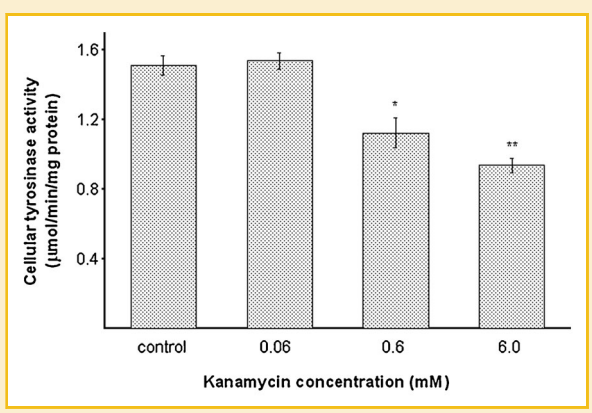

Fig. 3. The effect of kanamycin on tyrosinase activity in melanocytes. Cells were cultured with 0.06, 0.6, or 6.0 mM of kanamycin for 24 h and tyrosinase activity was measured as described in Materials and Methods Section. Data are mean ± SEM of at least three independent experiments performed in triplicate. $^{*}P < 0.05$ versus the control samples; $^{**}P < 0.005$ versus the control samples.

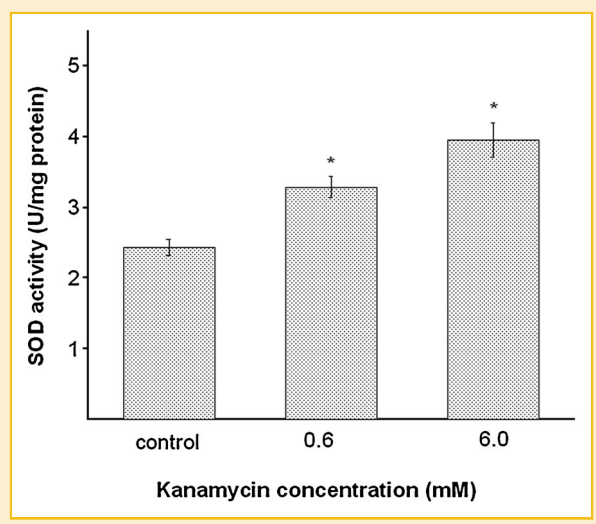

Fig. 4. Superoxide dismutase (SOD) activity in HEMa-LP cells after 24-h incubation with 0.6 or 6.0 mM of kanamycin. Data are mean ± SEM of at least three independent experiments performed in triplicate. $^{*}P < 0.05$ versus the control samples.

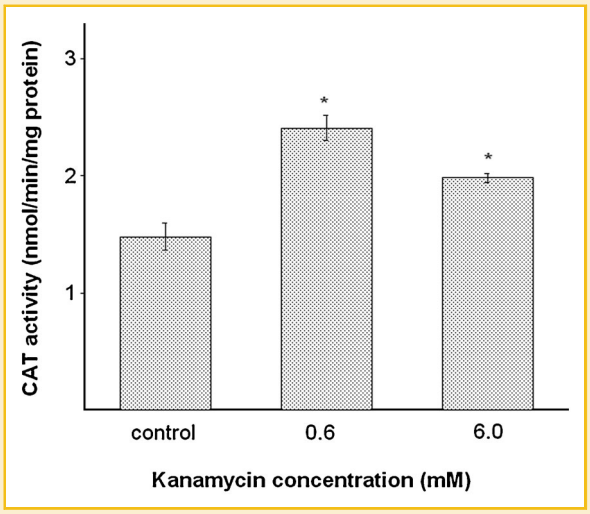

Fig. 5. Catalase (CAT) activity in HEMa-LP cells after 24-h incubation with 0.6 or 6.0 mM of kanamycin. Data are mean ± SEM of at least three independent experiments performed in triplicate. $^{*}P < 0.05$ versus the control samples.

dihydroxyphenylalanine (DOPA) [Yamaguchi and Hearing, 2009; Scherer and Kumar, 2010]. The enzyme tyrosinase (copper dependent monophenol monooxygenase) in a critical rate limiting step catalyzes the oxidation of tyrosine to L-DOPA and dopaquinone [Benedito et al., 1997; Scherer and Kumar, 2010]. The subsequent metabolism of dopaquinone by various melanocyte-specific enzymes, including tyrosinase-related proteins: TRP1 and TRP2, results in synthesis of eumelanin. The synthesis of pheomelanin involves production of cysteinyldopa through conjugation of dopaquinone by thiol-containing cysteine or glutathione [Scherer and Kumar, 2010; Otręba et al., 2012].

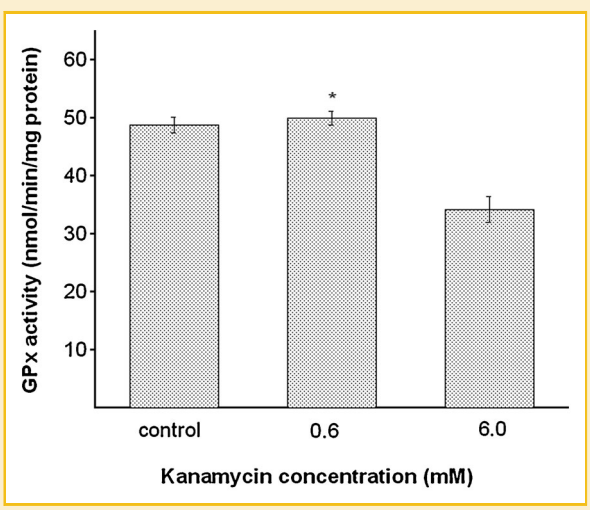

Fig. 6. Glutathione peroxidase (GPx) activity in HEMa-LP cells after 24-h incubation with 0.6 or 6.0 mM of kanamycin. Data are mean ± SEM of at least three independent experiments performed in triplicate. $^{*}P < 0.05$ versus the control samples.

Since tyrosinase is a major regulator of melanin synthesis, we were interested in examining the direct inhibitory effect of kanamycin on the activity of tyrosinase in melanocytes. Kanamycin in concentration of 0.6 and 6.0 mM decreased the tyrosinase activity in melanocytes by 25% and 38%, respectively (Fig. 3). Simultaneously, the cellular tyrosinase activity was not changed at the lowest analyzed kanamycin concentration (0.06 mM).

We repeated the experiment with mushroom tyrosinase, and we obtained similar results (Table I). Our results indicate that an inhibitory effect of kanamycin on melanogenesis is probably due to its direct inhibition of tyrosinase activity. The possible mechanism of kanamycin-dependent inhibition of tyrosinase activity may be explained by the capacity of this drug to bind copper (II) ions [Szczepanik et al., 2004], which are essential for tyrosinase catalytic activity [Otręba et al., 2012].

To prove the effect of kanamycin on the effectiveness of melanogenesis in normal human melanocytes, melanin content in cells cultured in the presence or absence of a drug was measured. When kanamycin concentration was 0.06 mM, melanin production was similar to the production by untreated control cells, whereas kanamycin concentration of 0.6 and 6.0 mM decreased melanin content by 5% and 24%, respectively (Fig. 2). For kanamycin concentration EC_{50} the melanin content in melanocytes does not correlate with the number of living cells, but the melanin content was determined for all cells in the analyzed culture.

Free radicals, including ROS, are generated as by-products of biochemical reactions within cells and, hence, considered as inherent intermediates of many physiologic processes. However, when produced in large amounts or in an uncontrolled fashion, free radicals inflict tissue damage, and are implicated in many pathologic processes. The understanding of the fine balance between the physiologic and pathologic effects of free radicals is an important driving force in this field of research that may have an impact on physiology, cell biology as well as clinical medicine [Marí et al., 2010].

Melanocytes have evolved sophisticated mechanisms to combat the potential deleterious effects of ROS, resulting from various metabolic processes, in the form of a defense system consisting of enzymes such as SOD, CAT and GPx [Pintea et al., 2009]. According to the hypothesis of ROS involvement in aminoglycosides toxicity [Kawamoto et al., 2004; Xie et al., 2011], we observed for the first time that kanamycin causes a significant alteration in the activities of the antioxidant enzymes, SOD, CAT and GPx in melanocytes. SOD and CAT are very important enzymes against the toxic effects of oxygen metabolism. In melanocytes, CAT is the main enzyme responsible for degrading hydrogen peroxide wich is one of the most cytotoxic radical species [Meresca et al., 2010]. Although SOD is an antioxidant enzyme, some studies have suggested that its overexpression is harmful to cells [Gardner et al., 2002]. The presented increase in SOD activity after exposure of melanocytes to kanamycin is associated with overproduction of the superoxide anion and subsequent formation of H_2O_2, what leads to the increase in CAT activity. Both generated superoxide anion and H_2O_2 may cause oxidative damages of mitochondrial components resulting in mitochondrial dysfuntion

and apoptosis or necrosis of cells. As exposure to H_2O_2 is connected with depletion of antioxidant defense system, we measured the activity of CAT and GPx in melanocytes. Treatment of cells with 0.6 mM of kanamycin has led to higher increase in CAT activity in regard to the drug in concentration of 6.0 mM. The observed changes may be a reason of the induction of oxidative stress inside the melanocytes. In addition, kanamycin in concentration of 6.0 mM decreases GPx level. Thus, it may be assumed that alterations in CAT and GPx activity play a critical role in aminoglycosides toxicity as a result of redundant H_2O_2 level that cannot be eliminated.

It should be taken into consideration, that not only SOD, CAT, and GPx are involved in ROS detoxification, but also glutathione-S-transferase (GST) and GR. GST constitutes a superfamily of xenobiotic metabolizing enzymes and catalyzes the nucleophilic addition of glutathione to electrophilic compounds. It has also been shown that GST plays an important role in melanin formation catalyzing the synthesis of 5-S-glutathione-3,4-dihydroxyphenylalanine, an intermediate in pheomelanin biosynthesis [Moral et al., 1997]. GPx is not directly involved in melanogenesis pathway, however, the coexistence of GPx and GST has been recognized in some pigmented organs [Fujimura et al., 2008] and it is natural to suppose that the distribution of these enzymes may be related to pigmentation. Thus, the obsereved inhibitory effect of kanamycin in concentration of 6.0 mM on GPx activity may be related to the decrease of melanin synthesis after the antibiotic treatment in the same concentration.

The kanamycin concentrations found to have an inhibitory effect on melanogenesis and antioxidant defense system are about 15-fold and 150-fold higher than the concentration normally observed in vivo [Doluisio et al., 1973]. However, we have previously demonstrated that kanamycin undergoes a specific interaction with melanin, what may lead to the accumulation of this drug in melanin reach tissues. Slow release of kanamycin from bonds may bild up high and long-lasting level of this drug stored on melanin and lead to the prolonged exposure of melanin containing cells and surrounding tissues to the toxicity of the tested aminoglycoside antibiotic [Wrześniok et al., 2005]. Thus, it is possible that in vivo kanamycin concentration in melanocytes may be significantly higher than that in serum and therefore the reduction of melanin content, the inhibition of tyrosinase activity as well as the depletion of antioxidant status in the presence of this drug could be observed.

This work strengthens our previous statements concerning the correlation between kanamycin binding to melanin biopolymer and this drug toxicity [Wrześniok et al., 2005]. We have demonstrated that kanamycin reduces melanocytes viability and decreases cellular tyrosinase activity, in parellel with decreased melanin content. This indicates that the tested drug inhibits melanogenesis through the decrease of tyrosinase activity. The observed changes in antioxidant enzymes activity (increase of SOD and CAT and decrease of GPx level) caused by kanamycin are probably responsible for the imbalance of antioxidant defense system in melanocytes. Thus, the demonstrated in vitro effect of kanamycin on melanocytes viability, melanogenesis, and antioxidant enzymes actitivity may explain the potential mechanisms of undesirable side effects of this drug directed to pigmented tissues in vivo.

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