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# Interaction of neomycin, tobramycin and amikacin with melanin *in vitro* in relation to aminoglycosides-induced ototoxicity

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The aim of this study was to examine *in vitro* the interaction between aminoglycoside antibiotics displaying adverse ototoxic effects and melanin which is a constituent of the inner ear. The binding of neomycin, tobramycin and amikacin to model synthetic melanin was studied. It has been demonstrated that all the investigated aminoglycosides form stable complexes with melanin biopolymer. The obtained results show that the amount of drug bound to melanin increases with the increase of initial drug concentration and the incubation time. An analysis of drugs binding to melanin by the use of Scatchard plots has shown that at least two classes of independent binding sites must be implicated in the studied aminoglycoside antibiotic-melanin complexes formation: strong binding sites ($n_1$) with the association constant $K_1 \sim 0.2–2.0 \cdot 10^5\ M^{-1}$ and weak binding sites ($n_2$) with $K_2 \sim 1.0–4.9 \cdot 10^3\ M^{-1}$. Based on the values of association constants the following order of drugs affinity to DOPA-melanin was found: tobramycin $>$ amikacin $\gg$ neomycin. The ability of the analyzed aminoglycoside antibiotics to form complexes with melanin *in vitro* may be one of the reasons for their ototoxicity *in vivo*, as a result of their accumulation in melanin in the inner ear.

## 1. Introduction

The aminoglycosides are a family of structurally diverse antibiotics that are effective against a broad spectrum of clinically important pathogenic organisms. This family of compounds, which includes the clinically relevant drugs tobramycin, kanamycin, gentamicin, neomycin and amikacin, consists of a central aminocyclitol ring with two or three substituted aminoglycan rings attached at different positions (Forge and Schacht 2000). Aminoglycosides are believed to exert their bactericidal effects by binding to the 16S rRNA of the 30S ribosomal fragment. The aminoglycoside antibiotics are known to affect renal tissues and sensory cells of the inner ear (O'Grady et al. 1997). The precise mechanism underlying the organ specificity of aminoglycoside-induced toxicity has not been fully established.

The melanin pigment is a high molecular mass polymer that occurs widely in living organisms and particularly in the skin, hair, eye, ear and brain (Ings 1984; Zucca et al. 2004; Wielgus and Sarna 2005, Liu et al. 2005). It is known that various drugs and other chemicals are bound (Ibrahim and Aubry 1995; Lowrey et al. 1997; Mårs and Larsson 1999) and retained for long periods in pigmented tissues due to melanin affinity. The accumulated compounds are very heterogeneous in structure: drugs of different categories – psychotropics, drugs for rheumatoid arthritis and malaria, local anaesthetics (Larsson 1993; Buszman et al. 2003; Buszman and Różańska 2003a), antiarrhythmic drugs (Buszman and Różańska 2003b), and also metal ions (Andrzejczyk and Buszman 1992; Liu et al. 2004; Hong et al. 2004), herbicides, dyes, alkaloids etc. (Larsson 1993). Among the drugs showing the greatest affinity to melanin pigment *in vitro* are policyclic amines, including chloroquine and aminoglycoside antibiotics (Wästerström 1984; Wrześniok et al. 2002; Pilawa et al. 2002; Wrześniok et al. 2005).

Since melanins are present in external and internal tissues, their capacity to bind to a wide number of drugs may result in various toxic effects due to lesions of pigmented tissues. This factor is of importance in the pathogenesis associated with long-term and/or high dose therapy with a number of drugs, including aminoglycoside antibiotics (Larsson 1993). It has been earlier suggested that the affinity of melanin pigment for aminoglycosides may cause these drugs to bind preferentially to the pigmented inner ear, producing greater ototoxicity than in the amelanotic albino cochlea (Wästerström 1984). The mechanism behind the development of lesions in the pigmented cells is probably a combination of selective retention, due to melanin binding, and toxicity, i.e., substances with low toxicity may scarcely induce lesions, in spite of high melanin affinity, while those with a more expressed or specific toxicity may induce the adverse effects (Larsson 1993). The evidence of greater ototoxicity in albinos has led to the hypothesis that melanin inhibits the toxicity of aminoglycoside antibiotics in the pigmented inner ear. On the other hand, ototoxicity in the pigmented animals may simply be delayed relative to albinos, only to become equal or even more severe with time (Conlee et al. 1995).

Under certain circumstances the possible protection mechanism may be a threat to the pigmented cells. Chronic exposure to certain toxic substances with melanin affinity ultimately causes adverse effects in the cells. These effects are mainly related to high dose, long-term exposure, and a prominent feature of the lesions is that the histologic changes are initially found in the pigmented cells, and successively in the adjacent tissues, such as receptor cells. The onset of the adverse effects may be delayed, and the entire manifestation of the lesions may occur even years after cessation of the offending substances. It is also possible that various toxic substances, which are retained in the melanin-containing tissues, are causing additive effects (Larsson 1993).

The physiological meaning and the mechanism of drug-melanin binding are still not fully understood. The aim of the presented studies was to examine *in vitro* the interaction between aminoglycoside antibiotics displaying adverse reactions in the inner ear, that is neomycin, tobramycin and amikacin, and melanin. For these studies synthetic DOPA-melanin was used because of its similarity to natural eumelanins.

## 2. Investigations and results

The binding capacity of three aminoglycoside antibiotics: neomycin, tobramycin and amikacin to synthetic DOPA-

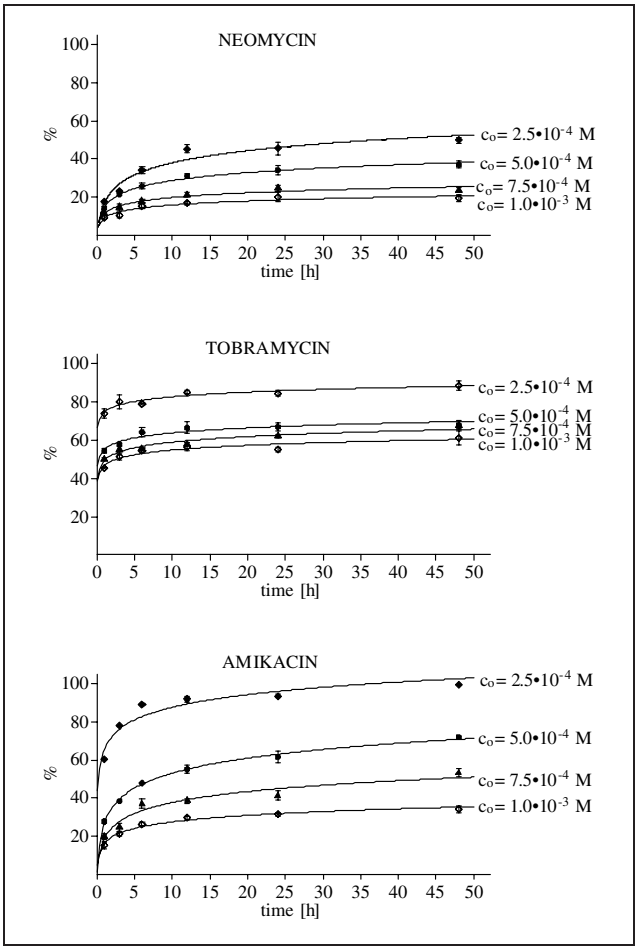


Fig. 1: Effect of incubation time and initial drug concentration ($c_0$) on the amount of neomycin, tobramycin and amikacin bound to DOPA-melanin (in %).
Mean values ± SD from three independent experiments are presented. Points without error bars indicate that SD was less than the size of the symbol

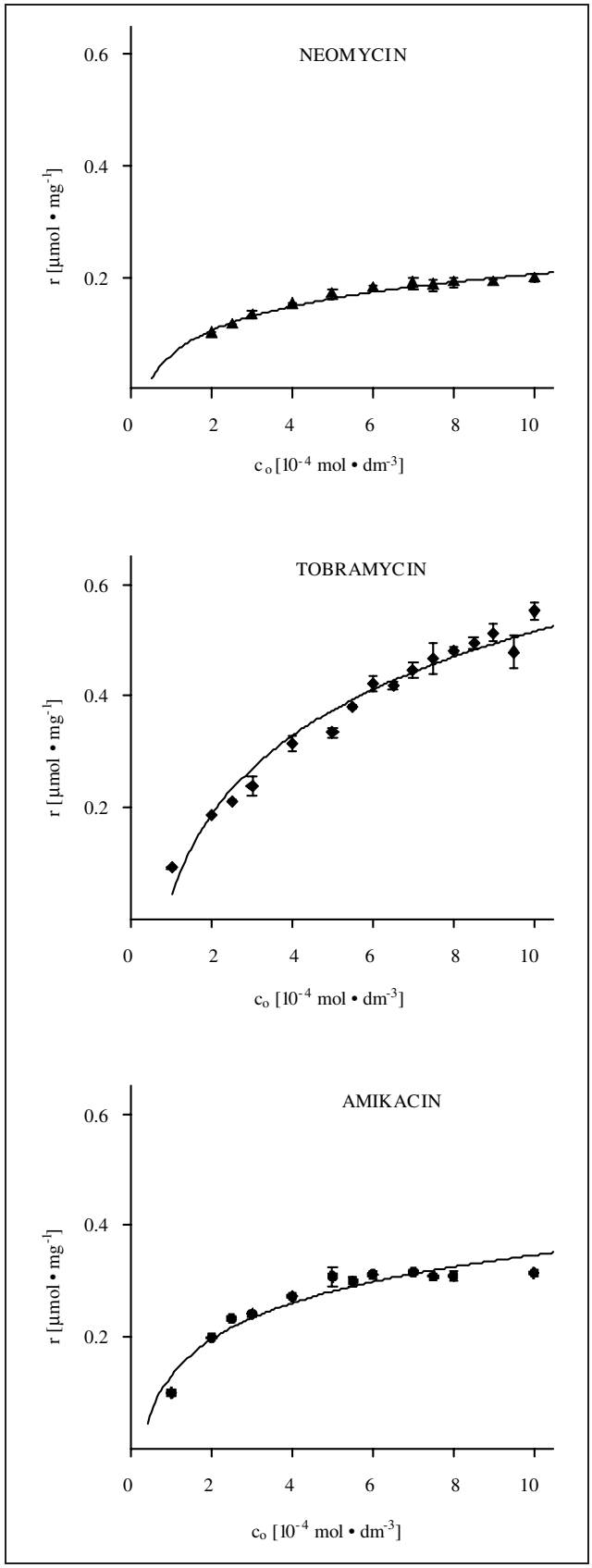


Fig. 2: Binding isotherms for neomycin-melanin, tobramycin-melanin and amikacin-melanin complexes. r – amount of drug bound to melanin; $c_o$ – initial drug concentration.
Mean values ± SD from three independent experiments are presented. Points without error bars indicate that SD was less than the size of the symbol

melanin was analyzed. The effect of the incubation time and initial drugs concentration on the amount of drugs bound to melanin is presented in Fig. 1. The results are given for four different initial aminoglycosides concentrations ($c_0$): $2.5 \cdot 10^{-4}$ M, $5 \cdot 10^{-4}$ M, $7.5 \cdot 10^{-4}$ M and $1 \cdot 10^{-3}$ M and for six different incubation times: 1, 3, 6, 12, 24, and 48 h. It can be seen that the amount of drug bound to melanin increases with the prolongation of incubation time and after 12–24 h it attains a plateau. It has been also shown that the amounts of antibiotics bound to melanin increase with increasing initial drug concentration. Simultaneously, the decrease of complex formation efficiency, expressed in % as the ratio of the amount of drug bound to melanin to the initial amount of drug added to melanin, was observed with the increase of the initial antibiotic concentration (Fig. 1.)

Dependence of the amount of aminoglycosides bound to melanin after 24 h of incubation as a function of the initial drug concentration is presented in Fig. 2 as binding isotherms. All the examined samples demonstrate an increase of bound antibiotics with the increase of the amount of added drug. It can be seen from binding isotherms that the amount of drugs bound to a constant amount of DOPA-melanin reaches a plateau at about 0.19 μmol neomycin per 1 mg melanin, which reflects the initial neomycin concentration $7 \cdot 10^{-4}$ M, about 0.47 μmol tobramycin/mg melanin for the initial tobramycin concentration $7.5 \cdot 10^{-4}$ M and about 0.31 μmol amikacin/mg melanin for the initial amikacin concentration $5 \cdot 10^{-4}$ M.

Dependencies of the amount of drugs bound to melanin (r) to the concentration of unbound drugs ($c_A$), i.e. $r/c_A$, versus r for neomycin, tobramycin and amikacin complexes with DOPA-melanin are presented in Fig. 3 as Scatchard plots.

The use of the Scatchard method can provide information about the number and nature of binding sites in the analyzed complexes. The analysis of aminoglycoside antibiotics binding to melanin shows that Scatchard plots are curvilinear with upward concavity in all cases, indicating that more than one binding class must be implicated in drug-melanin complex formation. The calculated binding parameters for the interaction of the analyzed drugs with DOPA-melanin are listed in the Table. Two classes of independent binding sites participate in the interaction of aminoglycoside antibiotics with melanin: strong binding sites ($n_1$) with the association constant $K_1$ about $0.2–2.0 \cdot 10^5$ $M^{-1}$ and weak binding sites ($n_2$) with the association constant $K_2$ about $1.0–4.9 \cdot 10^3$ $M^{-1}$.

The number of binding sites differs depending on the chemical nature of the analyzed drug. The highest number of strongly reacting sites ($n_1 = 0.29$ μmol/mg) for tobramycin-melanin and the lowest one ($n_1 = 0.14$ μmol/mg) for neomycin-melanin complexes were obtained. It can be noted that the total binding capacity ($n_1 + n_2$) of DOPA-

**Table: Binding parameters for neomycin, tobramycin and amikacin complexes with DOPA-melanin**

| Analyzed complex | Association constants K [$M^{-1}$] | Number of binding sites n [μmol drug/mg melanin) |
|---|---|---|
| Neomycin-melanin | $K_1 = 1.73 \cdot 10^4$ | $n_1 = 0.144$ |
| | $K_2 = 2.17 \cdot 10^3$ | $n_2 = 0.124$ |
| | | $\sum n = 0.268$ |
| Tobramycin-melanin | $K_1 = 2.08 \cdot 10^5$ | $n_1 = 0.288$ |
| | $K_2 = 4.94 \cdot 10^3$ | $n_2 = 0.341$ |
| | | $\sum n = 0.629$ |
| Amikacin-melanin | $K_1 = 1.04 \cdot 10^5$ | $n_1 = 0.264$ |
| | $K_2 = 1.01 \cdot 10^3$ | $n_2 = 0.236$ |
| | | $\sum n = 0.500$ |

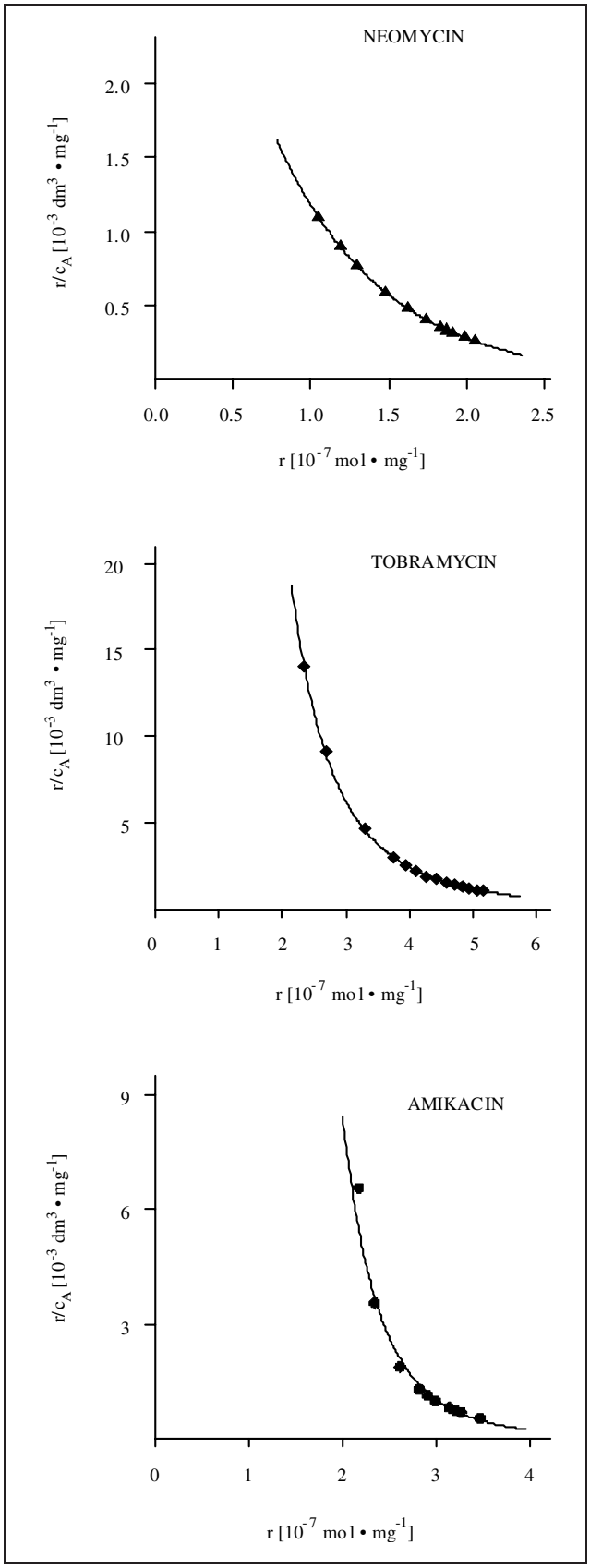


Fig. 3: Scatchard plots for neomycin-melanin, tobramycin-melanin and amikacin-melanin complexes. r – amount of drug bound to melanin; $c_A$ – concentration of unbound drug

melanin for the studied aminoglycoside antibiotics ranges from 0.268 μmol drug/mg melanin for neomycin-melanin to 0.629 μmol drug/mg for tobramycin-melanin complexes.

## 3. Discussion

Previous studies have shown a variety of substances, particularly aminoglycoside antibiotics, to be toxic to the inner ear (Wästerström 1984; Sullivan et al. 1987; Thomas et al. 1992; Roland 1994; Larsson 1995; Forge and Schacht 2000). Drug-induced ototoxicity can result in impaired auditory or vestibular functions. Symptoms of cochlear injury include tinnitus and hearing loss. Symptoms of vestibular apparatus injury include vertigo, nausea and vomiting. Aminoglycoside antibiotics induce ototoxic effects in the cochlea and in the vestibular system. Neomycin is the most cochleotoxic aminoglycoside. Tobramycin is cochleo- and vestibulotoxic, with incidences of 0.4% to 22%, and up to 4.6%, respectively. Amikacin is more cochleotoxic, with 3 to 24% incidences (Govaerts et al. 1990; Seligmann et al. 1996). Although the histopathologic effects of aminoglycoside antibiotics have been well demonstrated (Thomas et al. 1992), the mechanism by which these agents induce cell damage has remained unclear.

Ototoxic drugs have been shown to accumulate in the inner ear and this has been attributed to their affinity for melanin pigment which, in the cochlea, is found mainly in the stria vascularis. The implication that pigmented animals would accumulate ototoxic drugs in the inner ear to a greater extent than albino animals and would consequently be more likely to suffer hearing impairment has been investigated (Harpur and D'Arcy 1975). The electrophysiological, histological and electron microscopical findings presented by Wästerström et al. (Wästerström 1984, Wästerström and Bredberg 1986, Wästerström et al. 1986) showed that pigmented guinea pigs were more susceptible to inner ear damage caused by toxic doses of kanamycin than albino guinea pigs.

A fundamental distinction in the auditory system between albino and pigmented animals is a difference in pigmentation of the inner ear. Pigmented melanocytes are typically found in close association with the vasculature of the inner ear and notably the capillaries of the stria vascularis. In albinos, melanocytes have also been identified in the cochlea, but without the presence of melanin pigment (Conlee et al. 1989, 1995). The stria vascularis is involved in the production of endolymph, a fluid which is in contact with the receptor hair cells. Thus damage to the stria vascularis could affect the composition of the endolymph and the hair cells would frequently be very sensitive to such disruption of their immediate environment. It is possible that injury to the stria vascularis, associated with melanin-induced drug accumulation, could result in changes in endolymph composition and ultimate hair cell damage (Harpur and D'Arcy 1976). This hypothesis indicates that the aminoglycoside antibiotic-melanin interaction may be one of the reasons for ototoxic effects of these drugs.

On the other hand, it may be shown that the Fe(II) complexation and generation of hydroxyl radicals may be implicated in the mechanism of aminoglycosides toxicity. Studies suggesting that radical scavengers demonstrate variable efficacy in protecting cochlear hair cells against a number of ototoxicants indirectly implicate radical oxygen species in aminoglycoside ototoxicity (Garetz et al. 1994; Conlon et al. 1998; Forge and Schacht 2000). It has also been demonstrated that melanin acts as a biochemical dustbin, mopping up free radicals and other potentially toxic agents (Larsson 1993, 1995). Such properties would be important in protecting the pigment cells from the natural toxins, oxygen and reactive oxygen species (including free radicals), in addition to man-made toxins in the form of drugs and industrial chemicals (Eves et al. 1999). At present, many drugs are known to be markedly accumulated and retained for a considerable time by pigmented tissues of animals including humans and that the retention of these compounds is proportional to degree of melanin pigmentation (Larsson and Tjälve 1979). *In vitro* studies have demonstrated that toxic effects of aminoglycosides can be prevented and/or attenuated by the inner ear melanin. The melanin would protect tissue by keeping potentially harmful substances bound and slowly releasing the agents in low, non-toxic concentration (Larsson 1993, 1995).

Commonly shared risk factors associated with an increased incidence of aminoglycosides-induced ototoxicity include, among others, increased inner ear drug concentration and prolonged exposure of the inner ear to the drug (Govaerts et al. 1990; Seligmann et al. 1996). From the data presented by Wästerström (1984) it was clear that long term medication with a clinical dose of antibiotics did not result in ototoxic damage in the guinea pigs. However, studies using moderate to high doses of gentamicin have consistently shown greater ototoxicity in albino than in pigmented animals (Conlee et al. 1991). The smaller dose of gentamicin produced at least a moderate degree of hair cell degeneration toward the base of the albino cochlea, whereas hair cells in pigmented cochlea were not significantly affected (Conlee et al. 1995). Higher doses of antibiotics appear to compound the effects produced by a lower dose, with albino animals showing more severe changes in both the stria vascularis and the organ of Corti (Conlee et al. 1991). Larger doses also produce hair cell degeneration in the basal turn in pigmented animals (Conlee et al. 1989). Pigmented animals have shown significant ototoxicity when relatively high doses of antibiotics were given, however the degree of outer hair cell degeneration in albino animals was more than double that in pigmented animals (Conlee et al. 1989). Because of the known chemical affinity of melanin pigment for aminoglycoside antibiotics (Wästerström 1984; Larsson 1993), it has been hypothesized that these drugs bind to the inner ear pigment and cause, in high concentrations for long time therapy, lesions of pigmented tissues.

Melanin is a polymer composed basically of indole-5,6-quinone units, which can occur in different stages of oxidation (Ito 1986). Melanins contain carboxyl groups and o-semiquinones (Ito 2003; Ito and Wakamatsu 2003), which are negatively charged at physiological pH. The binding of chemicals to melanin is therefore mainly characterized by ionic interaction, which also may be strengthened by other forces (Larsson 1993).

Aminoglycoside antibiotics are highly polar organic bases, which exist as polycations at physiological pH (O'Grady et al. 1997; Delgado and Remers 1998) and therefore may interact with melanin polyanion. Van der Waals forces, charge-transfer reactions and hydrophobic interactions are also postulated for drug-melanin binding (Larsson and Tjälve 1979; Ings 1984; Buszman et al. 1984).

It has been shown in our studies that the amounts of neomycin, tobramycin and amikacin bound to melanin increase with the rise of initial drug concentration and the

incubation time. The obtained results also show that the tested aminoglycosides form stable complexes with DOPA-melanin. An analysis of drugs binding to melanin by the use of Scatchard plots has shown that more than one class of binding sites must be implicated in the studied aminoglycoside antibiotic-melanin complexes formation. Studies on the melanin affinity *in vitro* usually give accurate data of the intrinsic interaction, i.e., parameters values of the association constants and the binding capacity of melanin. The lowest association constant for strongly reacting sites for neomycin-melanin complexes was obtained: $K_1 = 1.73 \cdot 10^4\ M^{-1}$. It can be also noted that the total binding capacity ($n_1 + n_2$) of DOPA-melanin is the lowest one for neomycin (0.27 μmol/mg), too.

Based on the values of association constants $K_1$ and $K_2$ the following order of drugs affinity to DOPA-melanin has been found: tobramycin > amikacin ≫ neomycin. Simultaneously, the total number of binding sites ($n_1 + n_2$) for drug-melanin complexes decreases in the same order as well.

One of the most fundamental properties of melanin is its ability to act as a stable free radical scavenger (Larsson 1993) and it has been demonstrated that free radicals scavengers can protect the hair cells from aminoglycosides ototoxicity (Forge and Schacht 2000). The obtained results indicating that melanin forms stable complexes with neomycin, tobramycin and amikacin may support hypothesis that toxic effect of aminoglycosides may be attenuated by drug-inner ear melanin complexes formation. On the other hand, aminoglycoside antibiotic-melanin interaction, which may occur in the inner ear during the long-term and/or high-dose therapy of aminoglycosides, may be one of the factors in the etiology of lesions affecting pigmented tissues of the inner ear.

## 4. Experimental

### *4.1. Chemicals and apparatus*

L-3,4-Dihydroxyphnylalanine (L-DOPA) used in the studies was obtained from Sigma Chemical Co. The neomycin sulphate was obtained from Polfa Tarchomin S.A., Poland; the amikacin sulphate was obtained from Bristol-Myers Squibb, Italy; the tobramycin sulphate was obtained from Biogal, Hungary. The remaining chemicals were produced by POCH S.A., Poland. Spectrophotometric measurements were performed using a JASCO model V-530 UV-VIS spectrophotometer.

### *4.2. Melanin synthesis*

Model synthetic melanin was formed by oxidative polymerization of L-3,4-dihydroxyphenylalanine (L-DOPA) in 0.067 M phosphate buffer at pH 8.0 for 48 h according to the method described by Binns et al. (1970).

### *4.3. Drug – melanin complex formation*

Binding of neomycin, tobramycin and amikacin to melanin in 0.067 M phosphate buffer at pH 7.0 was studied. Melanin (5 mg) was placed in plastic test-tubes, where buffer solutions of antibiotics were added to a final volume of 5 ml. The initial concentration of drugs ranged from $2 \cdot 10^{-4}$ M to $1 \cdot 10^{-3}$ M for neomycin and from $1 \cdot 10^{-4}$ M to $1 \cdot 10^{-3}$ M for amikacin and tobramycin. Control samples contained 5 mg of melanin and 5 ml of buffer without drugs. All samples were incubated for 1–48 h at room temperature. The suspensions were filtered after incubation.

### *4.4. Determination of the amount of drugs bound to melanin*

The amount of drug in each filtrate with respect to the control sample was determined spectrophotometrically using chloranil as colour reagent (Rizk and Younis 1984) for the studied antibiotics. The concentrations of neomycin, tobramycin and amikacin remaining in each filtrate after incubation with melanin and the amounts of drugs bound to melanin were calculated, taking the molar absorption coefficients determined experimentally under the described conditions. The amount of drugs remaining in filtrates after complex removing represents the amount of neomycin, tobramycin or amikacin (μmol) unbound to 5 mg of melanin. For each sample the complex formation efficiency (in %) was determined as the ratio of drug (μmol) bound to melanin, to the total amount of drug (μmol) added to melanin, multiplied by 100. For the purpose of comparison the quantity of aminoglycoside antibiotics bound to DOPA-melanin for all the analysed complexes was calculated as the amount of bound drug (μmol) per 1 mg melanin.

### *4.5. Kinetics of drug-melanin complex formation*

Kinetics of formation of melanin complexes with neomycin, tobramycin and amikacin were evaluated on the basis of the relationship between the amount of drug bound to the polymer (μmol/mg) and the time for complex formation. In the studies the following initial drug concentrations were used: $2.5 \cdot 10^{-4}$, $5.0 \cdot 10^{-4}$, $7.5 \cdot 10^{-4}$ and $1 \cdot 10^{-3}$ M. Complex formation lasted for 1, 3, 6, 12, 24 and 48 h.

### *4.6. Binding parameters of drug-melanin complexes*

A qualitative analysis of drug – melanin interaction was performed using the Scatchard plots of the experimental data according to Kalblitzer and Stehlik (1979). Experimental binding isotherms were used to construct these plots. They show the relationship between the amount of the drug bound to melanin and its initial concentration after reaching equilibrium state, i.e. after 24 h. The initial drug concentration was $1 \cdot 10^{-4}$ M to $1 \cdot 10^{-3}$ M for all studied antibiotics. The number of binding sites (n) and the values of association constant (K) were calculated.

### *4.7. Statistical analysis*

In all experiments, the mean values for three experiments ± standard deviations (S.D.) were calculated, unless otherwise indicated.

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**References**

Andrzejczyk J, Buszman E (1992) Interaction of $Fe^{3+}$, $Cu^{2+}$ and $Zn^{2+}$ with melanin and melanoproteins from bovine eyes. Acta Biochim Polon 39: 85–88.

Binns F, Chapman RF, Robson NC, Swan GA, Waggott A (1970) Studies related to the chemistry of melanins. Part VIII. The pyrrolecarboxylic acids formed by oxidation or hydrolysis of melanins derived from 3,4-dihydroxyphenethylamine or (±)-3,4-dihydroxyphenylalanine. J Chem Soc C 1128–1134.

Buszman E, Kopera M, Wilczok T (1984) Electron spin resonance studies of chloroquine-melanin complexes. Biochem Pharmacol 33: 7–11.

Buszman E, Różańska R (2003a) Interaction of thioridazine with ocular melanin *in vitro*. Acta Pol Pharm 60: 257–261.

Buszman E, Różańska R (2003b) Interaction of quinidine, disopyramide and metoprolol with melanin *in vitro* in relation to drug-induced ocular toxicity. Pharmazie 58: 507–511.

Buszman E, Betlej B, Wrześniok D, Radwańska-Wala B (2003) Effect of metal ions on melanin-local anaesthetic drugs complexes. Bioinorg Chem Applicat 1: 113–122.

Conlee JW, Gill SS, McCandless PT, Creel DJ (1989) Differential susceptibility to gentamicin ototoxicity between albino and pigmented guinea pigs. Hear Res 41: 43–52.

Conlee JW, Jensen RP, Parks TN, Creel DJ (1991) Turn-specific and pigment-dependent differences in the stria vascularis of normal and gentamicin-treated albino and pigmented guinea pigs. Hear Res 55: 57–69.

Conlee JW, Bennett ML, Creel DJ (1995) Differential effects of gentamicin on the distribution of cochlear function in albino and pigmented guinea pigs. Acta Otolaryngol 115: 367–374.

Conlon BJ, Perry BP, Smith DW (1998) Attenuation of neomycin ototoxicity by iron chelation. Laryngoscope 108: 284–287.

Delgado JN, Remers WA (1998) Textbook of Organic Medicinal and Pharmaceutical Chemistry. Lippincott-Raven Publishers, Philadelphia, New York, p. 291–299.

Eves P, Smith-Thomas L, Hedley S, Wagner M, Balafa C, Mac Neil S (1999) A comparative study of the effect of pigment on drug toxicity in human choroidal melanocytes and retinal pigment epithelial cells. Pigment Cell Res 12: 22–35.

Forge A, Schacht J (2000) Aminoglycoside antibiotics. Audiol Neurootol 5: 3–22

Garetz SL, Altschuler RA, Schacht J (1994) Attenuation of gentamicin ototoxicity by glutathione in the guinea pig *in vivo*. Hear Res 77: 81–87.

Govaerts PJ, Clae, J, Van de Heyning PH, Jorens PG, Marquet J, De Broe ME (1990) Aminoglycoside-induced ototoxicity. Toxicol Lett 52: 227–251.

Harpur ES, D'Arcy PF (1975) The influence of pigmentation of rats and guinea pigs on the ototoxicity of kanamycin and neomycin. Experientia 31: 1323–1325.

Harpur ES, D'Arcy PF (1976) Comparison of the ototoxic effect of kanamycin on albino and pigmented rats, studied using an operant method. Experientia 32: 1562–1564.
Hong L, Liu Y, Simon JD (2004) Binding of metal ions to melanin and their effects on the aerobic reactivity. Photochem Photobiol 80: 477–481.
Ibrahim H, Aubry AF (1995) Development of a melanin-based high-performance liquid chromatography stationary phase and its use in the study of drug-melanin binding interactions. Anal Biochem 229: 272–277.
Ings RMJ (1984) The melanin binding of drugs and its implications. Drug Metab Rev 15: 1183–1212.
Ito S (1986) Reexamination of the structure of eumelanin. Biochim Biophys Acta 883: 155–161.
Ito S (2003) The IFPCS presidential lecture: a chemist's view of melanogenesis. Pigment Cell Res 16: 230–236.
Ito S, Wakamatsu K (2003) Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. Pigment Cell Res 16: 523–531.
Kalbitzer HR, Stehlik D (1979) On the analysis of competitive binding of various ligands to cooperative and independent binding sites of macromolecules. Z Naturforsch 34c: 757–769.
Larsson BS (1993) Interaction between chemicals and melanin. Pigment Cell Res 6: 127–133.
Larsson BS (1995) Accumulation of drugs on the melanin in the inner ear. In: Melanin: Its Role In Human Photoprotection, Valdenmar Publishing Co., Overland Park, KS, p. 215–219.
Larson BS, Tjälve H (1979) Studies on the mechanism of drug-binding to melanin. Biochem Pharmacol 28: 1181–1187.
Liu Y, Hong L, Kempf VR, Wakamatsu K, Ito S, Simon JD (2004) Ion-exchange and adsorption of Fe(III) by Sepia melanin. Pigment Cell Res 17: 262–269.
Liu Y, Hong L, Wakamatsu K, Ito S, Adhyaru BB, Cheng CY, Bowers CR, Simon JD (2005) Comparisons of the structural and chemical properties of melanosomes isolated from retinal pigment epithelium, iris and choroid of newborn and mature bovine eyes. Photochem Photobiol 81: 510–516.
Lowrey AH, Famini GR, Loumbev V, Wilson LY, Tosk JM (1997) Modeling drug-melanin interaction with theoretical linear solvation energy relationships. Pigment Cell Res 10: 251–256.
Mårs U, Larsson BS (1999) Pheomelanin as a binding site for drugs and chemicals. Pigment Cell Res 12: 266–274.
O'Grady F, Lambert HP, Finch RG, Greenwood D (1997) Antibiotic and Chemotherapy: Anti-infective Agents and Their Use in Therapy. Churchill Livingstone Inc., New York.
Pilawa B, Buszman E, Wrześniok D, Latocha M, Wilczok T (2002) Application of EPR spectroscopy to examination of gentamicin and kanamycin binding to DOPA-melanin. Appl Magn Reson 23: 181–192.
Rizk M, Younis F (1984) Spectrophotometric assay of certain aminoglycoside antibiotics using chloranil. Anal Lett 17: 1803–1809.
Roland PS (1994) Clinical ototoxicity of topical antibiotics drops. Otolaryngol Head Neck Surg 110: 598–602.
Seligmann H, Podoshin L, Ben-David J, Fradis M, Goldsher M (1996) Drug-induced tinnitus and other hearing disorders. Drug Safety 14: 198–212.
Sullivan MJ, Rarey KE, Conolly RB (1987) Comparative ototoxicity of gentamicin in the guinea pig and two strains of rats. Hear Res 31: 161–168.
Thomas J, Marion MS, Hinojosa R (1992) Neomycin ototoxicity. Am J Otolaryngol 13: 54–55.
Wästerström SA (1984) Accumulation of drugs on inner ear melanin – therapeutic and ototoxic mechanisms. Scand Audiol Suppl 23: 1–40.
Wästerström SA, Bredberg G (1986) Ototoxicity of kanamycin in albino and pigmented guinea pigs II. A scanning electron microscopic study. Am J Otol 7: 19–24.
Wästerström SA, Bredberg G, Linquist NG, Lyttkens L, Rask-Andersen H (1986) Ototoxicity of kanamycin in albino and pigmented guinea pigs I. A morphologic and electrophysiologic study. Am J Otol 7: 11–18.
Wielgus AR, Sarna T (2005) Melanin in human irides of different color and age of donors. Pigment Cell Res 18: 454–464.
Wrześniok D, Buszman E, Karna E, Nawrat P, Pałka J (2002) Melanin potentiates gentamicin-induced inhibition of collagen biosynthesis in human skin fibroblasts. Eur J Pharmacol 446: 7–13.
Wrześniok D, Buszman E, Karna E, Pałka J (2005) Melanin potentiates kanamycin-induced inhibition of collagen biosynthesis in human skin fibroblasts. Pharmazie 60: 439–443.
Zucca FA, Giaveri G, Gallorini M, Albertini A, Toscani M, Pezzoli G, Lucius R, Wilms H, Sulzer D, Ito S, Wakamatsu K, Zecca L (2004) The neuromelanin of human substantia nigra: physiological and pathogenic aspects. Pigment Cell Res 17: 610–617.