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The effect of endo- and exogenous melanin on Zn^{2+} and Co^{2+} elimination and distribution in mice*

Paweł Nogaj, Ewa Buszman, Longina Świątkowska and Tadeusz Wilczok

Department of Biochemistry and Biophysics, Silesian Medical Academy, Narcyzów 1, 41–200 Sosnowiec, Poland

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The content of endogenous melanin in white Balb c and black C-57 Bl. mice affected Zn^{2+} and Co^{2+} accumulation, elimination and distribution following intraperitoneal administration of these ions. Elimination of Co^{2+} ions was significantly greater in white mice than in black mice. Accumulation of Zn^{2+} was higher than that of Co^{2+} and did not depend on melanin content in mice. The same response was observed upon administration of exogenous melanin. Both ions accumulated mainly in spleen and heart irrespective of melanin content.

It is well known that melanin biopolymers show high capacity to accumulate metal ions *in vivo* and to bind them *in vitro* [1–4]. Both the model complexes of synthetic melanins and the melanins isolated from natural sources using divalent and trivalent metal ions are relatively stable.

It has been demonstrated that melanin present in B-16 or Harding-Passey melanomas implanted to black mice modifies elimination velocity and organ retention of ^{59}Fe and ^{65}Zn radionuclides [5, 6]. In our experiments although concentration of ^{59}Fe was higher in black mice it was eliminated at higher rate than in white Balb c mice [7]. Administration of exogenous melanin led to different accumulation of ^{59}Fe in mice organs [7].

The aim of this work was to extend our studies on the effect of endogenous and exogenous melanin on accumulation, elimination and distribution of Zn^{2+} and Co^{2+} ions in mice.

MATERIALS AND METHODS

Melanin was obtained by oxidative polymerization of 3,4-dihydroxyphenylalanine (L-DOPA) in 0.067 M phosphate buffer at pH 8.0, according to Binns *et al.* [8]. For injection, melanin was suspended in 0.9% NaCl containing 0.1% Tween 80, to the final concentration of 4 mg melanin per 1 ml. Experiments were carried out using male mice (groups of ten), strains Balb c (white) and C-57 Bl. (black) of 25 ± 3 g of body weight. All mice were injected intraperitoneally with 0.5 ml of the metal ion solution in the following doses:

zinc	0.05, 0.5 and 2.0 mg Zn^{2+}/kg body weight (i.e. 1.25, 12.5 and 50.0 μg Zn^{2+}/mouse)
cobalt	0.08, 0.8 and 8.0 mg Co^{2+}/kg body weight (i.e. 2.0, 20.0 and 200.0 μg Co^{2+}/mouse).

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[1]Abbreviations: b.w., body weight; mel. melanin.

The maximal doses corresponded to 1/4th of the LD_{50} values of each ion [9]. Aliquots (0.5 ml) of melanin suspension were administered intraperitoneally in the dose of 80 mg/kg of body weight (i.e. 2.0 mg/mouse). Each day Zn^{2+} and Co^{2+} content in mice was monitored radiochemically using ^{65}Zn and ^{60}Co isotopes, added in trace amounts (about 3 kBq/mouse) to the ions solutions just before injections. After 24 days mice were decapitated and fragments of haired skin, liver, heart, lungs, spleen and kidneys were weighed and subjected to radioactivity measurements. The results were expressed as µg of metal ion/gram of organ.

Melanin localization was visualized by histochemical examination by staining with hematoxylin.

For statistic evaluation regression coefficients homogeneity, Cochran-Cox [10] and Student's *t*-test [11] were used.

The curves illustrating elimination of the ions out of the organism are described by the function $y = 1/(ax + b)$.

RESULTS AND DISCUSSION

Two classes of independent binding sites of high and low affinity were identified [4, 12] during interaction of Zn^{2+} and Co^{2+} ions with either synthetic melanin or with that isolated from various natural sources. The following strong (n_1) and weak (n_2) binding sites represented by association constants K_1 and K_2 for metal ions — DOPA-melanin complexes were found [12]:

Mel–Zn^{2+}	$K_1 = 5.9 \times 10^5\ M^{-1}$, $n_1 = 0.048$ µmole Zn/mg mel., $K_2 = 4.9 \times 10^3\ M^{-1}$, $n_2 = 0.257$ µmole Zn/mg mel.,
Mel–Co^{2+}	$K_1 = 2.5 \times 10^5\ M^{-1}$, $n_1 = 0.090$ µmole Co/mg mel., $K_2 = 6.5 \times 10^9\ M^{-1}$, $n_2 = 0.182$ µmole Co/mg mel.,

It is however of interest that despite similar affinities of both ions to melanin the binding capacity for Zn^{2+} and Co^{2+} ions in the organism appeared to be different.

As can be seen from Fig. 1, when Zn^{2+} and Co^{2+} were given to mice at the maximal ion concentration (2.0 mg Zn^{2+}/kg of b.w. and 8.0 mg Co^{2+}/kg of b.w.). Co^{2+} was eliminated

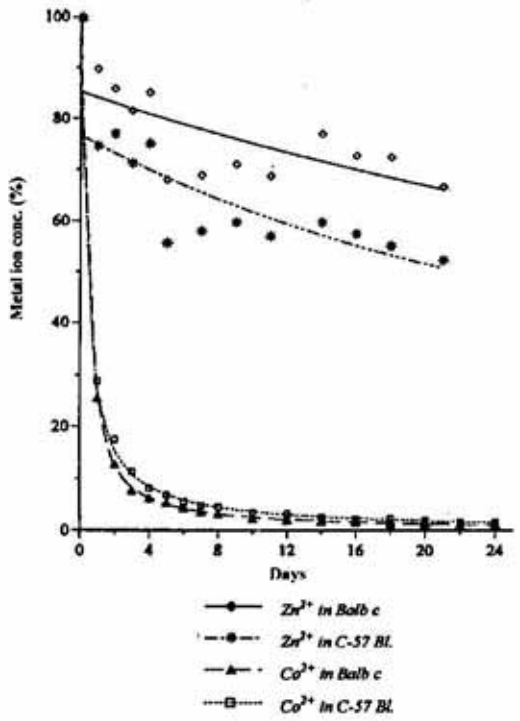

Fig. 1. Time course of Zn^{2+} and Co^{2+} elimination in Balb c and C-57 Bl. mice following intraperitoneal administration of the ions.
The initial metal ions concentration: 2.0 mg Zn^{2+}/kg of body weight or 8.0 mg Co^{2+}/kg of body weight.

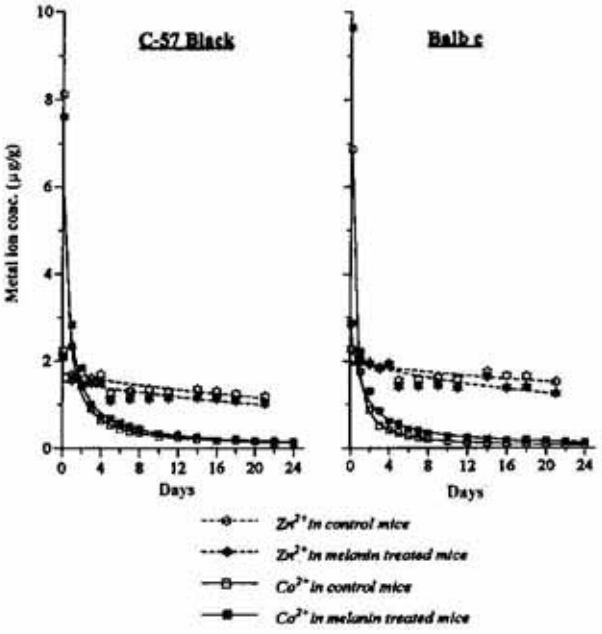

Fig. 2. Elimination of Zn^{2+} and Co^{2+} in treated by melanin and control groups of mice.
The initial metal ions concentration: 2.0 mg Zn^{2+} or 8.0 mg Co^{2+}/kg of body weight.

Table 1

Statistic evaluation (test for regression coefficients homogeneity) of the effect of endogenous melanin on Zn^{2+} and Co^{2+} elimination in black and white mice

Initial Zn^{2+} concentration	0.05 (mg/kg b.w.*)		0.5 (mg/kg b.w.)		2.0 (mg/kg b.w.)	
Mice strain	Balb c	C-57 Bl.	Balb c	C-57 Bl.	Balb c	C-57 Bl.
Test statistic value F	F = 0.004		F = 19.675		F = 3.516	
Critical value F_t	F_t (95, 1, 22) = 4.301					
Initial Co^{2+} concentration	0.08 (mg/kg b.w.)		0.8 (mg/kg b.w.)		8.0 (mg/kg b.w.)	
Mice strain	Balb c	C-57 Bl.	Balb c	C-57 Bl.	Balb c	C-57 Bl.
Test statistic value F	F = 104.303		F = 57.866		F = 157.319	
Critical value F_t	F_t (95, 1, 30) = 4.171					

*b.w., body weight.

more efficiently than Zn^{2+}: after 4 days following ion administration about 10% of Co^{2+} and as much as 60% of Zn^{2+} remained in mice. Elimination of Co^{2+} was slightly higher in white Balb c mice than in black C-57 Bl. mice and differences in Co^{2+} elimination were significant (Table 1): F values were 14 to 40 times higher than the critical F_t value. At the same time the differences between Zn^{2+} elimination in black and white mice were insignificant.

Data on distribution of Zn^{2+} and Co^{2+} in mice organs demonstrate organ differentiation in accumulation of these ions (Table 2).

After 24 day exposure, the highest Zn^{2+} concentration was found in spleen and the lowest in liver. Also in each organ accumulation of Zn^{2+} was always higher than that of Co^{2+}, concentration of both ions in particular organs being the higher the higher was the dose. However, as can be seen from Fig. 2, administration of exogenous melanin significantly affected Co^{2+} elimination velocity in either mice strain. The F values were 6 to 53 times higher than the critical F_t values (Table 3), but also as in the case with endogenous melanin the interstrain dif-

Table 2

Distribution of Zn^{2+} and Co^{2+} in organs of black and white mice

Metal ions	Initial metal ion conc.	Mice strain	Metal ion content (mg/g of organ) ± S.D.					
			Skin	Liver	Heart	Lungs	Spleen	Kidneys
Zn^{2+}	0.05 (mg/kg b.w.*)	Balb c	0.037±0.008	0.035±0.015	0.117±0.009	0.067±0.004	0.139±0.037	0.051±0.006
		C-57 Bl	0.025±0.005[b]	0.018±0.005[c]	0.092±0.013[a]	0.061±0.011	0.126±0.011	0.049±0.007
	0.5 (mg/kg b.w.)	Balb c	0.419±0.068	0.423±0.143	1.467±0.125	0.808±0.073	1.497±0.326	0.656±0.105
		C-57 Bl	0.274±0.048[a]	0.170±0.043[a]	1.183±0.124[a]	0.658±0.059[b]	1.203±0.184[f]	0.432±0.054[a]
	2.0 (mg/kg b.w.)	Balb c	1.454±0.211	0.887±0.111	4.434±0.709	2.693±0.264	4.094±0.459	1.790±0.199
		C-57 Bl	1.700±0.232[d]	0.961±0.150	5.267±0.548[d]	3.153±0.242[b]	4.891±0.821[e]	2.199±0.210[b]
Co^{2+}	0.8 (mg/kg b.w.)	Balb c	0.019±0.008	0.030±0.010	0.030±0.014	0.046±0.025	0.062±0.026	0.043±0.017
		C-57 Bl	0.023±0.013	0.040±0.017	0.064±0.029[f]	0.042±0.017	0.036±0.018	0.063±0.019
	8.0 (mg/kg b.w.)	Balb c	0.224±0.116	0.181±0.062	0.367±0.154	0.373±0.110	0.894±0.381	0.290±0.144
		C-57 Bl	0.261±0.118	0.748±0.325[a]	0.571±0.344	0.428±0.142	0.859±0.392	0.701±0.194[b]

*b.w., body weight; a = $P < 0.0001$, b = $P < 0.001$, c = $P < 0.005$, d = $P < 0.01$, e = $P < 0.02$, f = $P < 0.03$.

ferences between Zn^{2+} elimination measured in the whole animal were insignificant.

Data on distribution of Zn^{2+} and Co^{2+} in mice organs of melanin treated and control groups (Fig. 3 and Fig. 4) confirm the differences in organ distribution of Co^{2+} in both black and white mice, which were even more pronounced in the latter. It has been demonstrated that exogenous melanin has no effect on Zn^{2+} content in the analyzed mice organs, except for skin and liver of Balb c strain. The analysis of Co^{2+} distribution in mice organs has shown that statistically significant differences between the mice treated with melanin and controls can be demonstrated for liver, heart and kidneys of Balb c strain.

Histopathological evaluation of slides obtained from mice organs has proved that intraperitoneal injection of exogenous melanin to mice causes accumulation of this biopolymer in liver and spleen of both C-57 Bl. and Balb c strains in the form of melanin "capsules". The presence of exogenous melanin in hair and hair follicles was demonstrated only in C-57 Bl. mice.

The effect of exogenous melanin on distribution, elimination and accumulation of the metal ions in mice and localization of exogenous melanin in the form of capsules on the liver and spleen surfaces might suggest that application of melanin may be used for detoxification of metal ions in mice.

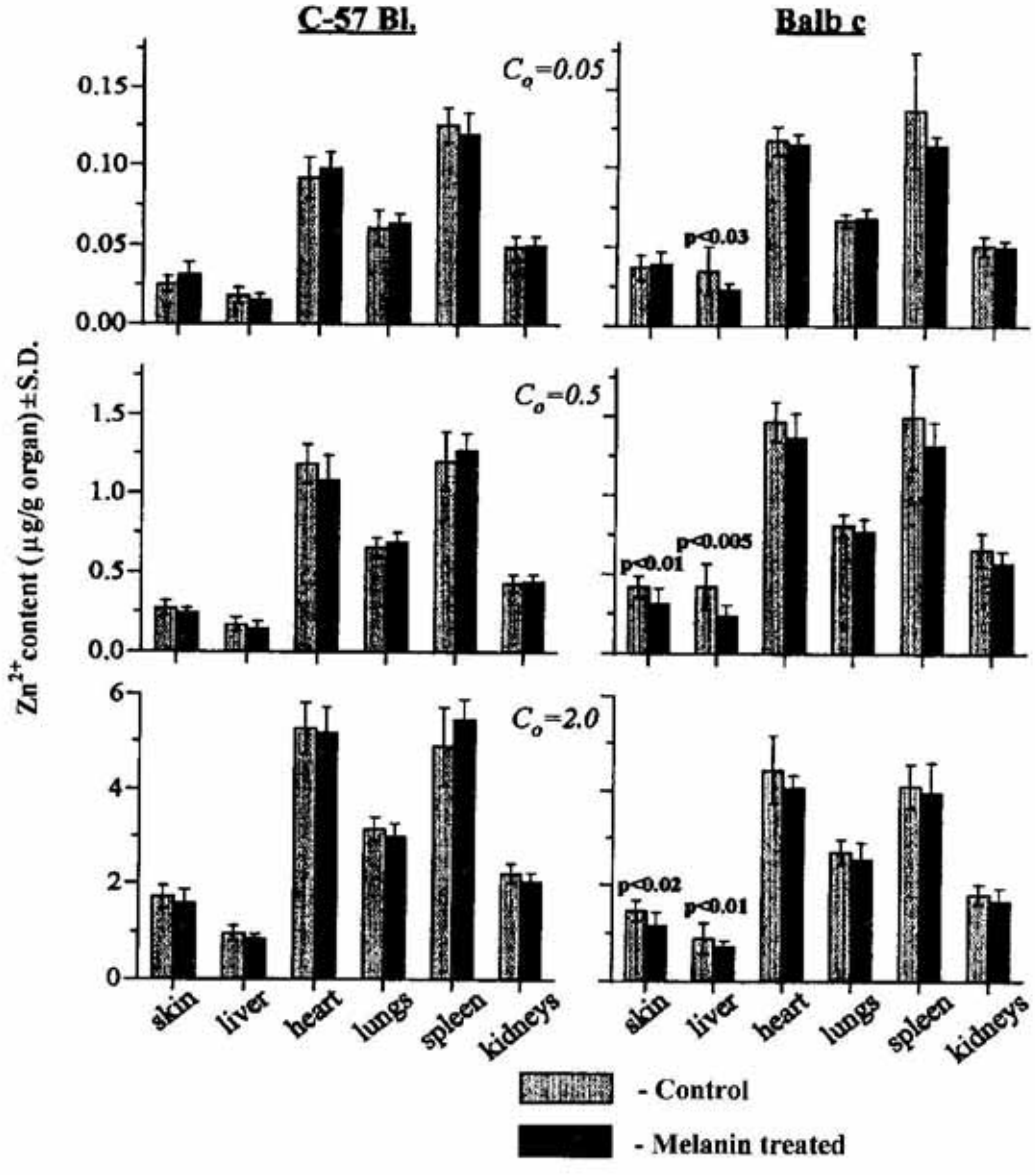

Fig. 3. Zn^{2+} distribution in mice organs of melanin treated and control groups. C_o, initial Zn^{2+} doses: 0.05, 0.5 and 2.0 mg Zn^{2+}/kg of body weight.

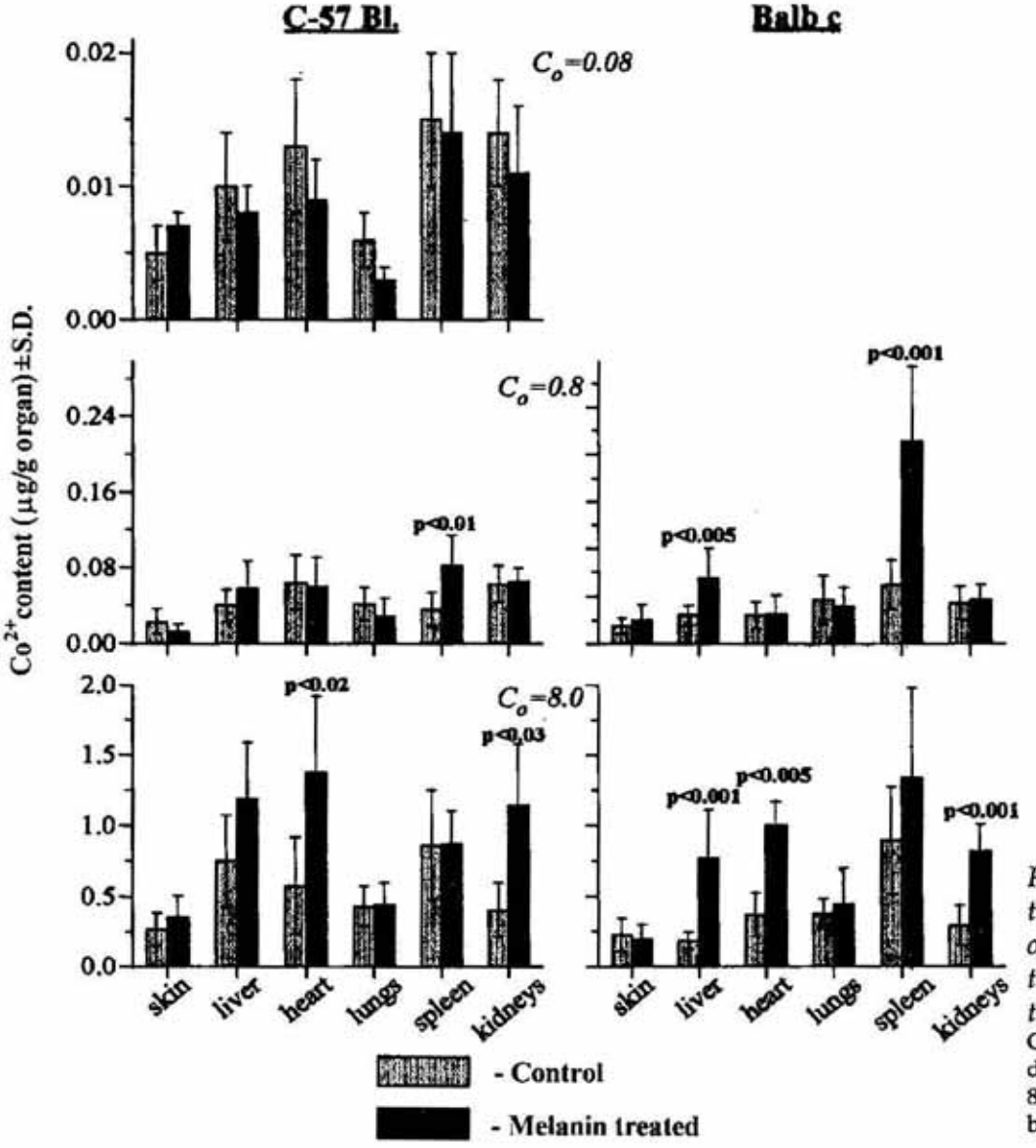

Fig. 4. Co^{2+} distribution in mice organs of melanin treated and control groups. C_o, initial Co^{2+} doses: 0.08, 0.8 and 8.0 mg Co^{2+}/kg of body weight.

Table 3

Statistic evaluation (test for regression coefficients homogeneity) of the effect of exogenous melanin on Zn^{2+} and Co^{2+} elimination in mice

Initial Zn^{2+} concentration	0.05 (mg/kg b.w.*)		0.5 (mg/kg b.w.)		2.0 (mg/kg b.w.)	
Mice strain	Balb c	C-57 Bl.	Balb c	C-57 Bl.	Balb c	C-57 Bl.
Test statistic value F	0.542	6.021	4.473	1.392	3.664	0.510
Critical value F_t	F_t (95, 1, 22) = 4.301					
Initial Co^{2+} concentration	0.08 (mg/kg b.w.)		0.8 (mg/kg b.w.)		8.0 (mg/kg b.w.)	
Mice strain	Balb c	C-57 Bl.	Balb c	C-57 Bl.	Balb c	C-57 Bl.
Test statistic value F	188.177	107.996	85.411	71.009	212.286	23.609
Critical value F_t	F_t (95, 1, 30) = 4.171					

*b.w., body weight.

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