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DMPS Metall

Influence of heavy metals upon the retention and mobilization of polonium-210 in rats

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Abstract.
Purpose: To provide information about the tissue retention and mobilization of the alpha-emitting radionuclide, polonium-210 (^{210}Po), in rats under combined exposure to heavy metal ions and the chelating agent, 2, 3-dimercaptopropane-1-sulfonate (DMPS).
Materials and methods: Rats were pre-exposed intraperitoneally to either $CdCl_2$ or $Pb(CH_3COO)_2$. 9 or 15 h later they received ^{210}Po nitrate intravenously. The retention and excretion of ^{210}Po via the urine and faeces of pre-exposed rats, as well as in pre-exposed rats treated with DMPS, were followed. The radioactivity due to ^{210}Po in a broad spectrum of body tissues and excreta was measured by the liquid scintillation counting after sample digestion in a mixture of perchloric acid and hydrogen peroxide. The immunohistochemical localization of metallothioneins (MT) was studied using a mixture of murine monoclonal antibodies directed against MT I+II.
Results: The present study revealed different tissue distributions of polonium-210 in the rats pre-exposed to lead or cadmium ions when compared with that in ^{210}Po only controls. Under combined exposure to Pb or Cd, the spontaneous excretion of ^{210}Po was enhanced and could be further enhanced by treatment with DMPS. Treatment with this chelator was efficient even when its start was postponed until 24h after internal contamination of the body with ^{210}Po.
Conclusions: Polonium-210 is bound *in vivo* to binding sites on various biomolecules, among them erythrocytic enzymes and MT. This phenomenon explains the different affinity and overall distribution of ^{210}Po in control body tissues. When the appropriate binding sites are occupied by lead or cadmium, enhanced natural excretion of polonium-210 occurs.

1. Introduction

Human health is affected by many chemical and physical factors. The human body takes in toxic heavy metals and radionuclides from pollution in workplaces as well as from the environment. Soluble metal compounds follow the metabolic pathways of the compounds of essential elements to which they are chemically similar and become incorporated into organs and tissues. Toxic elements interfere with the normal functions of biological systems via their interaction with thiol and histidyl groups of enzymes and membrane proteins. The toxicity of some metal ions may be modified by binding to metallothioneins (MT) *in vivo* (Cherian and Goyer 1978, Klaassen 1999, Park *et al.* 2001). Metallothioneins are inducible proteins normally synthesized at low basal levels. Exposure to a wide variety of chemicals, physiological and physical factors may increase their biosynthesis (Klaassen 1999, Oh *et al.* 1978, Koropatnick *et al.* 1989).

There is no doubt that a combination of factors may affect physiological processes differently as compared to each factor alone. To improve risk assessment from the intake of noxious species, it is desirable to investigate their biokinetics in combination. The aim of the present study was to provide information about: (a) the tissue retention of the alpha-emitting radionuclide polonium-210 in rats pre-exposed to lead or cadmium which are ubiquitous environmental pollutants (WHO 1995, 1992) and compare it with that of ^{210}Po alone; (b) the possibility of reducing the radiation risk from incorporated ^{210}Po under combined exposure to heavy metals by chelating agent treatment.

Polonium-210 and lead are members of the ^{238}U decay series. Polonium-210 is used industrially (see references in Bogdan and Aposhian 1990). Internal contamination with ^{210}Po (see references in Rencová *et al.* 1993) and heavy metals in humans may arise from environmental or industrial exposures. Polonium-210 reacts with thiol groups and its binding to MT in the liver has been proven (Aposhian and Bruce 1991). The retention of ^{210}Po with different affinities in a wide spectrum of organs and tissues (Stannard 1964, Rencová *et al.* 1993) can reflect the reaction of the individual organs and tissues to combined exposure.

2. Materials and methods

2.1. *Animals*

Specific pathogen free female Wistar rats (160–180g) were purchased from Bio Test s.r.o. (Konárovice, Czech Republic). They received standard

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feed pellets (St-1, Velaz, Czech Republic) and tap water *ad libitum*. The animals were sacrificed by bleeding under ether anaesthesia. The protocols used in experiments conformed to Czech Act No. 331/1997 Dig. on the Breeding and Using of Experimental Animals.

2.2. *Chemicals and treatment*

Rats (160–170 g) were pre-exposed intraperitoneally (ip) to solutions of $CdCl_2$ (Cd-rats) or $Pb(CH_3COO)_2$ (Pb-rats). The toxicities of $CdCl_2$ and $Pb(CH_3COO)_2$ injected ip are quite different, their LD_{50} being 3.55 and 286 mg kg^{-1} body weight, respectively (Kotsonis and Klaasen 1977, Pryor *et al.* 1983, respectively). Therefore, different amounts of Cd and Pb were used for the pre-exposure (1 mg Cd kg^{-1} and 5 mg Pb kg^{-1}).

9 or 15 h later, the pre-exposed rats received ^{210}Po nitrate (35 kBq kg^{-1} body weight) via a tail vein (iv). A stock solution of about 50 MBq ^{210}Po per ml 2M nitric acid was purchased from M.G.P.-AEA Technology (Zlín, Czech Republic). It was diluted to give an injection solution containing the appropriate amount of ^{210}Po in 0.1 ml of 0.05M HNO_3. The resulting, pH 1.3, ensures 99% filterability of the ^{210}Po from the unbuffered solution of simple electrolytes (Morrow *et al.* 1964). In the previous experiments, the same distribution pattern of ^{210}Po was found in rat tissues after iv injection in 0.1 M or 0.026 M nitric acid (Rencová et. al. 1993). The radioactivity of the injection solution was checked by counting known aliquots taken before, during and after injection of each experimental group.

Chelation treatment with sodium 2,3-dimercaptopropane-1-sulfonate (DMPS, Sigma, Germany, 1.0 mmol kg^{-1} body mass, 0.5 ml per subcutaneous injection) was repeated once daily for five consecutive days. The solution was prepared every day under an inert atmosphere (N_2) to avoid oxidation of the –SH groups.

2.3. *Determination of radioactivity*

Samples of the dissected tissues and excreta were first digested with a mixture of perchloric acid and hydrogen peroxide (1:1) (Seidel and Volf 1972, Rencová *et al.* 1995). The alpha radioactivity of the original as well as of the injected solutions was measured after addition of Instagel using TRI-CARB 2700 TR counter (Canberra-Packard, Praha, Czech Republic). The total radioactivity in the muscles, skin, blood and skeleton was calculated from that in the measured aliquots, which were multiplied by known factors (Rencová *et al.* 2000). It was assumed that muscle, skin and blood contributed 45%, 19% and 6.5% of the body mass, respectively. Total skeletal radioactivity was calculated to be 20 times higher than that of one femur (a factor derived from the distribution of bone-seeking alpha-emitters in rats). The sum of the ^{210}Po measured or estimated for individual organs was considered to be the total body ^{210}Po. The statistical significance of the differences ($p < 0.05$) between tissues of experimental groups was evaluated using the Student's *t*-test.

2.4. *Histological and immunochemical examination*

The liver, kidneys, lungs, spleen, thymus, heart, muscle, small and large intestine were examined. Formalin-fixed portions of rat tissues were embedded in paraffin. Sections were stained with hematoxylin-eosin and by the green trichrome Masson method. Histological evaluation of tissues was performed under light microscopy.

For the immunohistochemical localization of MT, a mixture of murine monoclonal antibodies directed against MT I+II (Dako-MT, E9, Milan, Italy) was used (Elias, 2003, Guesdon *et al*, 1979). Paraffin-embedded sections were deparaffinized with xylene and ethanol. Endogenous peroxidase activity was blocked by incubation in 3% H_2O_2. After washing in phosphate bruffered saline (PBS) buffer, sections were incubated for 50 min with anti-MT at 1:50 dilution in PBS buffer. After washing in PBS buffer, the secondary antibody conjugated with biotin (Dako LSAB System LINK, Milan, Italy) and the streptavidin-peroxidase complex (Dako, LSAB+HPR Streptavidin, Milan, Italy) were added and incubated for 20 min. The sections were developed with 3,3′-diaminobenzidine (Dako Liquid DAB, Milan, Italy) and then counterstained with hematoxylin. To assess MT overexpression, the intensity of the stain was classified as slight (±), weak (+), moderate (++), strong (+++) and very strong (++++) immunoreactivity.

3. Results

3.1. *Retention studies*

Groups of six rats were used and ^{210}Po was injected 9 or 15 h after pre-exposure to Pb^{2+} (Pb-rats) or Cd^{2+} (Cd-rats). The retention of ^{210}Po in the tissues of pre-exposed rats was compared with that in animals exposed to ^{210}Po alone (control groups). The ^{210}Po content in the blood, spleen, liver, kidneys, lungs, thymus, femur, heart, brain, muscle, skin, small and large intestine (cleared of content by flushing with physiological saline) and femoral bone marrow was measured 3 days (72 h) after injection of ^{210}Po.

In the Pb-rats (9-h interval, figure 1), the radioactivity decreased in the blood, liver and bone marrow to 15%, 35% and 65% of controls, respectively. However, ^{210}Po radioactivity increased in the small and large intestine, thymus, kidneys and skeleton to 135%, 137%, 127%, 118% and 112% of controls, respectively. In the case of a 15-h delay, a large decrease of ^{210}Po was found only in the blood and liver, to 18% and 42%, respectively. The content of ^{210}Po increased in a larger number of tissues than in the 9-h interval (also in the spleen, muscles and skin). The total retention of ^{210}Po in organs and tissues was reduced at these two time intervals, to 72% and 81% of that in controls, respectively.

In the Cd-rats, there was a small but significant change in the retention of ^{210}Po injected 9 h after Cd^{2+} in most of the investigated tissues when compared with controls (figure 2). Generally only a small decrease of ^{210}Po was found but in the femoral marrow and small intestine, the decrease was to 60% and 63% of controls, respectively. On the other hand, in the thymus and large intestine the ^{210}Po radioactivity increased to 128% and 136% of that in controls, respectively. The total retention of radioactivity in the tissues decreased to 85% of controls. The effect of the pre-treatment was less pronounced when ^{210}Po was injected 15 h after Cd^{2+}, but it was still statistically significant.

3.2. *Mobilization studies*

Groups of five rats were used and ^{210}Po was injected 15 h after Pb^{2+} and 9 h after Cd^{2+}. In the 7-day experiments, besides the retention of ^{210}Po in the tissues of the pre-exposed as well as of the control rats, the excretion of ^{210}Po via urine and faeces was also examined. Furthermore, decorporation studies of the enhancement of the excretion of ^{210}Po induced in the pre-exposed rats by chelation treatment with DMPS were conducted and compared with the data for control rats.

In the Pb-rats, the pattern of ^{210}Po retention in the tissues (table 1) was similar but less pronounced, to that previously described in the 3-day experiment. A large decrease of ^{210}Po retention was found in the blood and liver, to 44% and 50% of control tissues,

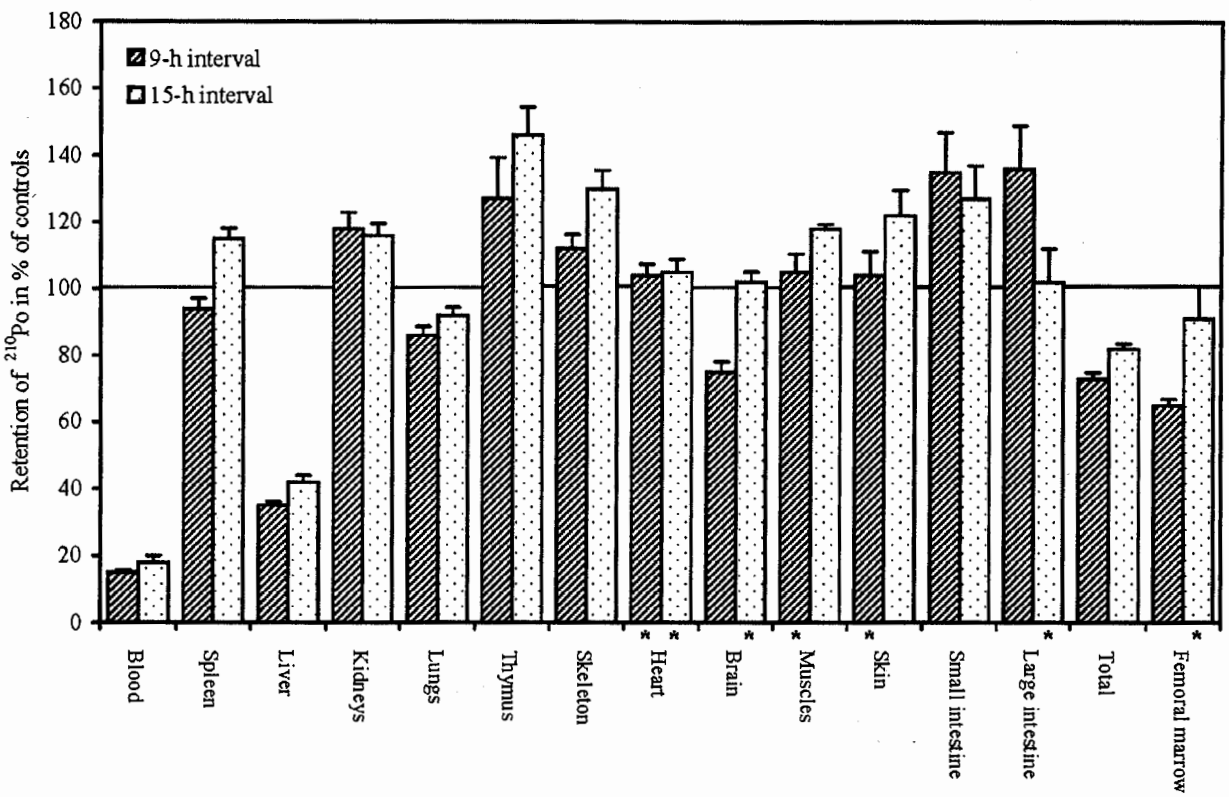

Figure 1. Retention of ^{210}Po in rat tissues pre-exposed to Pb^{2+}. Values represent the arithmetic mean ± SEM of six rats per group. * = Differences between the mean after pre-exposure and that in controls, which are not statistically significant. All other means are significantly different from respective controls ($p < 0.05$). Retention of ^{210}Po in control tissues in percent of injected radioactivity: blood, 15.2 ± 1.0; spleen, 2.9 ± 0.2; liver, 13.2 ± 0.9; kidneys, 6.3 ± 0.3; lungs, 1.9 ± 0.1; thymus, 0.4 ± 0.04; skeleton, 6.8 ± 0.4; heart, 0.4 ± 0.02; brain, 0.1 ± 0.002; muscles, 13.9 ± 0.6; skin, 6.4 ± 0.3; small intestine 0.5 ± 0.06; large intestine 0.3 ± 0.02; total, 67.6 ± 2.2; femoral bone marrow 0.05 ± 0.005.

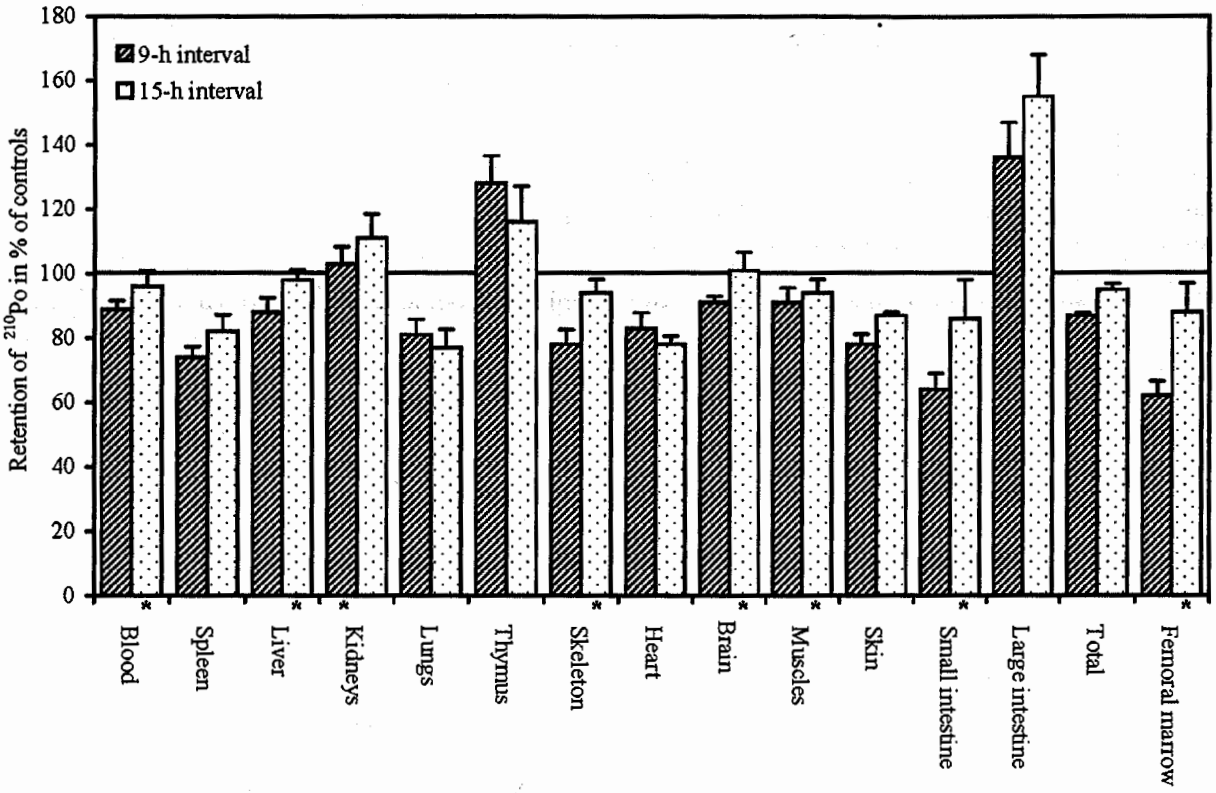

Figure 2. Retention of ^{210}Po in rat tissues pre-exposed to Cd^{2+}. Values represent the arithmetic mean ± SEM of six rats per group. * = Differences between the mean after pre-exposure and that in controls which are not statistically significant. All other means are significantly different from respective controls ($p < 0.05$). Retention of ^{210}Po in control tissues in percent of injected radioactivity: blood, 12.4 ± 0.3; spleen, 3.8 ± 0.3; liver, 12.6 ± 0.3; kidneys, 6.4 ± 0.2; lungs, 1.8 ± 0.06; thymus, 0.4 ± 0.04; skeleton, 8.6 ± 0.4; heart, 0.4 ± 0.02; brain, 0.1 ± 0.004; muscles, 12.6 ± 0.3; skin, 6.7 ± 0.3; small intestine 0.6 ± 0.06; large intestine 0.3 ± 0.02; total, 66.0 ± 0.8; femoral bone marrow 0.07 ± 0.006.

respectively. Excretion analyses revealed that over the experimental period the Pb-rats excreted 16.7% of the injected ^{210}Po via the faeces versus 11.1% in controls (figure 3). The total urinary excretion was less than 1 % of the injected radioactivity in both cases (figure 4).

In the Cd-rats, a different pattern of ^{210}Po distribution in tissues was observed (table 1), as compared to the 3-day experiment. Polonium-210 radioactivity in the brain, skin, thymus, kidneys and bone marrow increased. In the small and large intestine, a 20 and 3.4 fold content of ^{210}Po of control tissues was found, respectively (table 1). Total faecal excretion in the Cd-rats was 25.9% of the injected radioactivity versus 11.1% in control rats (figure 3). Total urinary excretion was the same as in the control group (figure 4).

In the decorporation study with the Pb-rats and the ^{210}Po only rats, treatment with DMPS was started 1 h after injection of ^{210}Po. A decrease in the retention of ^{210}Po was observed in most of the investigated tissues (table 1). However, a 2–2.5-fold increase in the ^{210}Po radioactivity in the kidneys was found in both DMPS-treated groups compared with the ^{210}Po only controls. DMPS also increased the ^{210}Po radioactivity in the liver, which was substantially reduced by the Pb pre-treatment. The total retention of radioactivity in tissues was the same in both, the Pb- and control decorporated rats: 43.1 ± 1.5 and 43.3 ± 1.5% of the injected radioactivity, respectively. Both DMPS-treated groups of rats excreted 19.4 and 16.5% of the injected radioactivity via the faeces and 14.7 and 16.0% via the urine (figures 3, 4). The total excretion was the same in both decorporated groups: 34.1 ± 1.6 and 32.5 ± 1.4% of the injected radioactivity, respectively.

The treatment of the Cd-rats with DMPS was started 24 h after injection of ^{210}Po. A substantial decrease of ^{210}Po was found in all investigated tissues except for the kidneys when compared with untreated Cd-rats (table 1). Treatment with DMPS further enhanced the increased content of ^{210}Po observed in the kidneys of Cd-rats. The total retention of ^{210}Po in tissues was 52.5 ± 1.7% of the injected amount. In

Table 1. Mobilization studies - Retention of ^{210}Po in rat tissues.

Group	Retention of ^{210}Po at 7 d in % of injected ^{210}Po/in % of controls[a]														
	Blood	Spleen	Skeleton	Liver	Kidneys	Brain	Muscles	Skin	Lungs	Heart	Thymus	Small intestine	Large intestine	Total	Femoral marrow
^{210}Po Controls	7.7±0.4	4.2±0.3	8.5±0.8	9.9±0.4	5.4±0.3	0.09±0.04	13.3±0.9	7.4±0.8	1.9±0.1	0.3±0.02	0.4±0.04	0.10±0.02	0.16±0.02	57.7±1.4	0.05±0.005
Pb+^{210}Po	3.4±0.1	4.0±0.3[b]	9.2±0.5[b]	5.0±0.4	6.1±0.3	0.09±0.01[b]	14.3±0.8[b]	7.0±0.2[b]	1.7±0.1[b]	0.3±0.02[b]	0.6±0.04	0.16±0.02	0.15±0.01[b]	51.2±1.1	0.05±0.003[b]
	44	95	108	50	113	100	107	94	89	100	150	155	97	89	100
Cd+^{210}Po	7.8±1.2[b]	4.4±0.2[b]	7.9±0.4[b]	11.3±0.4	9.4±0.7	0.12±0.01	14.3±0.5[b]	10.2±0.8	2.0±0.1[b]	0.3±0.03[b]	0.6±0.03	2.0±0.2	0.55±0.03	71.2±1.2	0.07±0.005
	101	105	93	114	174	133	107	138	105	100	150	2000	340	123	140
^{210}Po +DMPS	2.9±0.1	2.0±0.2	5.8±0.6	7.8±0.3	10.5±0.7	0.05±0.00	8.4±0.7	4.8±0.4	0.8±0.0	0.2±0.01	0.2±0.03	0.06±0.01	0.07±0.01	43.3±1.5	0.02±0.003
	38	48	68	79	194	55	63	65	42	67	50	61	48	75	40
Pb+^{210}Po +DMPS	3.0±0.1	2.2±0.2	4.7±0.4	9.9±0.5[b]	13.0±0.7	0.06±0.02	7.5±0.2	3.6±0.1	1.0±0.1	0.2±0.01	0.3±0.02	0.09±0.02	0.09±0.02	43.1±1.5	0.03±0.003
	39	52	55	100	241	67	56	49	53	67	75	86	60	75	60
Cd+^{210}Po +DMPS	3.5±0.2	2.5±0.1	3.7±0.2	9.9±0.4[b]	14.9±0.7	0.08±0.00	10.3±0.7	4.7±0.5	1.0±0.0	0.2±0.01	0.4±0.02[b]	0.9±0.07	0.25±0.03	52.5±1.7	0.03±0.004
	45	60	44	100	276	89	77	63	53	67	100	900	156	91	60

For the pre-exposure and treatment see paragraph 2.2.

[a]Values represent the arithmetic mean±SEM of five rats per group.

[b]Difference between the mean after pre-exposure and for treatment or that in controls is not statisticaly significant. All other means are significantly different from respective controls ($p<0.05$).

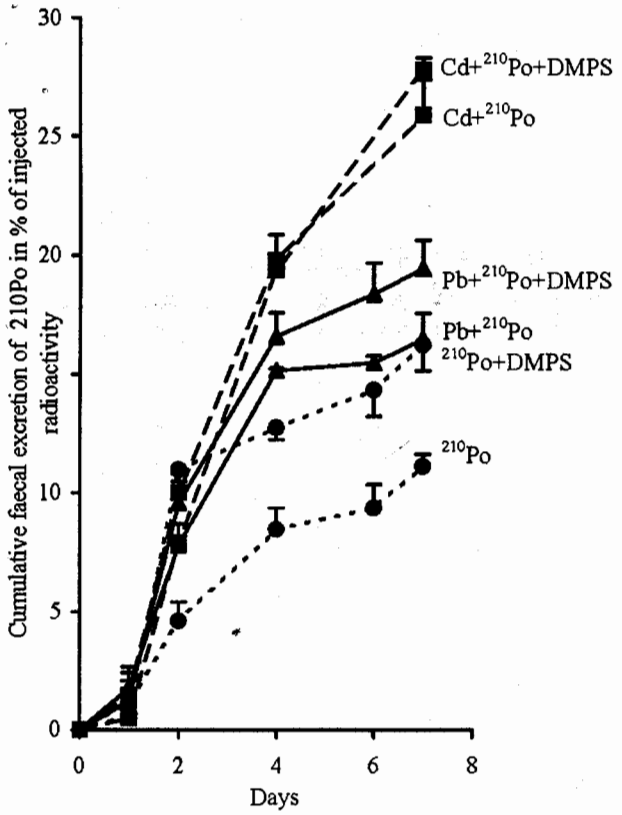

Figure 3. Cumulative faecal excretion of ^{210}Po in the control, pre-exposed and treated rats. For the pre-exposure and treatment with DMPS see paragraph 2.2.

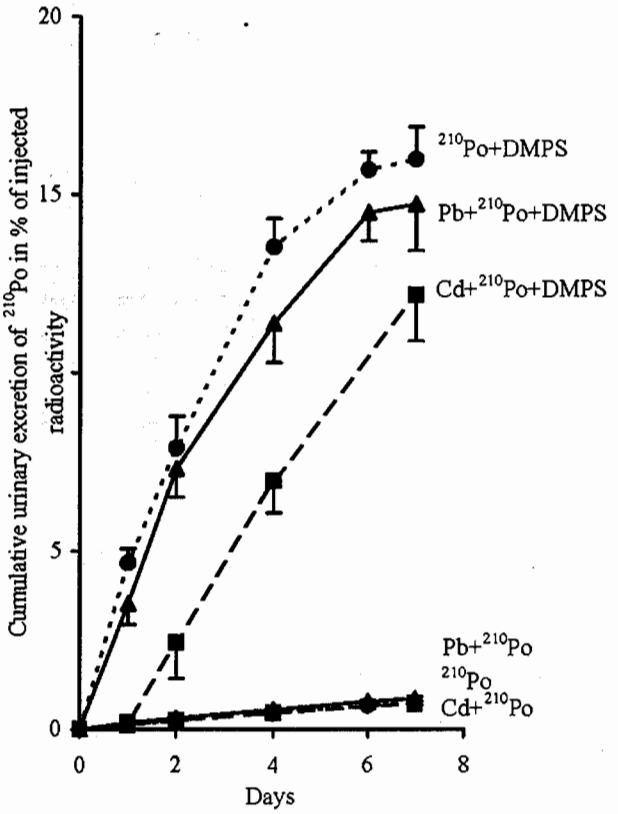

Figure 4. Cumulative urinary excretion of ^{210}Po in the control, pre-exposed and treated rats. For the pre-exposure and treatment with DMPS see paragraph 2.2.

the DMPS-treated group, faecal excretion was 27.7% and urinary excretion was 12.1% (figures 3, 4), yielding a total excretion of $39.9 \pm 1.2\%$ of the injected radioactivity.

3.3. *Histological and immunohistochemical evaluation*

Groups of three rats were sacrificed 9 and 15 h after the ip injection of a solution of Cd^{2+} or Pb^{2+} in order to estimate the health status of the animals and the induction of MT in the tissues prior to the injection of ^{210}Po.

Histological examination revealed no changes in the tissues of rats with heavy metal intoxication, when compared with the control group injected physiological saline solution.

The results of the immunohistochemical localization of MT demonstrated that MT in the liver and kidneys of control animals increased 9 h after ip injection of Cd^{2+}. In the liver, MT were found in the cytoplasm of a few single isolated hepatocytes (+ to + + +) in control rats, whereas the presence of MT in hepatocytes of exposed rats was diffuse (+ +). In the kidneys, MT were present in the proximal convoluted tubules of the cortex (+ + +) in control rats, whereas MT were found in the straight part of the proximal tubules leading far into the medulla and papilla in exposed rats.

Other investigated control tissues (spleen, muscle, lungs, heart, lungs, thymus) contained single isolated MT positive (+) cells. In these tissues of the Cd-rats, no induced MT were observed. Enterocytes and several Paneth cells in the small intestine of exposed as well as control rats showed uniformly high MT expression (+ + + and + + + +, respectively). Enterocytes and numerous Paneth cells in the large intestine of exposed rats, as well as control rats, showed MT expression too (+ +/+ + + and + + + +, respectively). It was not possible to distinguish the amounts of MT in the intestinal tract between the control and Cd-rats when using the immunohistochemical method. When exposed rats were sacrificed 15 h after ip injection of Cd, localization of MT in the liver and kidneys was less pronounced. No increased expression of MT in comparison with the control group was observed in any of the examined tissues of rats injected with Pb.

4. Discussion

In these experiments, the biokinetics of incorporated ^{210}Po has been studied under model conditions. The iv injection simulated mobilization of the radionuclide fraction which entered the blood from any other site of entry (inhalation, ingestion, contaminated wound). The whole amount of ^{210}Po immediately entered the blood of rats pre-exposed with heavy metal ions. The intervals of 9 and 15 h, in which ^{210}Po was injected after pre-exposure, were derived from the time course of MT induction by Cd (Koropatnick *et al.* 1989).

Our 3-day experiments have demonstrated that the pattern of the small decrease in the retention of ^{210}Po in the Cd-rats at both time intervals (9 and 15 h after Cd^{2+} injection) was similar but less pronounced when ^{210}Po was injected at the later interval (figure 2). Similarly, when exposed rats were sacrificed 15 h after ip injection of Cd, localization of MT in tissues was less pronounced than at the 9-h interval. Therefore ^{210}Po was injected 9 h after Cd in the 7-day experiments.

A quite different pattern of retention of ^{210}Po was found in the Pb-rats (figure 1). Polonium-210 in the blood dramatically decreased in both groups (9 and 15 h). Polonium-210 has a high affinity for red blood cells (Thomas 1964, Rencová *et al.* 1993). Both, Pb and ^{210}Po were reported to be bound to haemoglobin in red blood cells (WHO 1995, Thomas 1964). However, recently Bergdahl (1998) reported that the principal Pb-binding protein in the cytosol from red blood cells (erythrocytes) is δ-aminolevulinic acid dehydratase (ALAD), a zinc enzyme that takes part in the biosynthesis of haem. No haemoglobin-bound Pb was found with up-to-date bioinorganic analytical (LC-ICP-MS) and immunochemical methods. Bergdahl hypothesized that according to the molecular weight, further erythrocytic proteins binding Pb may be pyrimidine-5-nucleotidase and acyl-CoA-binding protein. This latter protein is also present in the kidneys. ALAD contains a unique catalytic zinc-binding site with three cysteine residues. Pb binds principally to this site more firmly than Zn (Godwin 2001). There are also several classes of proteins that contain such zinc-binding sites.

On the basis of the above literature on Pb binding and our results, it is possible to conclude that Pb and ^{210}Po in red blood cells are bound to the binding sites of the same biomolecules. The above-mentioned erythrocytic enzymes are the main proteins to which ^{210}Po is bound in red blood cells. It is probable that ^{210}Po could be also bound in tissues to the above-mentioned sites of other proteins.

Similarly, the substantial decrease of ^{210}Po in the liver in both groups (9 and 15 h) suggests that Pb and ^{210}Po are bound to the same binding sites in the liver. When binding sites are occupied by Pb, there is no place for ^{210}Po. It is obvious that the great decrease of ^{210}Po in the blood and liver caused partly higher retention of ^{210}Po in other tissues.

Besides translocation of ^{210}Po, the substantial decrease of the total radioactivity in the tissues in the 3-day experiment suggested increased excretion of ^{210}Po from the body of the Pb-rats (figure 3). Surprisingly, increased excretion was also found in the case of the Cd-rats (figure 3) where the decrease of the total balance in tissues was small. Only faecal excretion was enhanced, by 1.5- and 2.3-fold, in the Pb- and Cd-rats, respectively. Our immunohistopathological examinations confirmed published reports on the induction of MT (Klaasen 1999). The basal levels of MT present in the liver and kidneys of control rats were increased by injection of Cd but not of Pb. In the liver of the Cd-rats, only a small increase of ^{210}Po was found although induction of MT was high. The binding of ^{210}Po to the liver MT has been already reported (Aposhian and Bruce 1991). Spontaneous excretion via faeces was markedly enhanced. It seems that the binding sites on MT that were occupied by Cd were not free for ^{210}Po. The increased excretion of ^{210}Po via the faeces probably increased enormously the radioactivity of the intestine. Polonium-210 could be bound to MT if their level was high. However, in this case non-specific interaction of ^{210}Po with the intestinal wall could occur too. Increased content of MT in the kidneys could be a cause of increased ^{210}Po in this organ.

Spontaneous excretion was further increased by chelating treatment. Unlike other alpha-emitters, ^{210}Po reacts with thiol groups of proteins in tissues as well as with those of chelating agents, suitable for the decorporation of heavy metals. DMPS was chosen as a clinically acceptable chelator (Dimaval). However, it translocates ^{210}Po into kidneys (Volf *et al.* 1995). This chelator was already used in the past for the treatment of severe internal contamination of childern with ^{210}Po (Shantyr *et al.* 1969). In the Pb-rats treated with DMPS 1 h after injection of ^{210}Po, a small enhancement of excretion of ^{210}Po via the faeces was found when compared with the Pb-rats (figure 3) with the main part of ^{210}Po excreted in the urine (14.7 % of injected amount, figure 4). In the case of the Cd-rats where the treatment was delayed 24 h, enhanced ^{210}Po excretion occurred only via the urine (12.1 % of the injected amount, figure 4). The total balance of the excreted ^{210}Po in the Cd-rats treated with DMPS was the highest because the spontaneous excretion of ^{210}Po in Cd-rats was higher than that in Pb- and control rats. Both treatment

regimens (1- and 24-h delay) decreased substantially the content of ^{210}Po nearly in all the investigated tissues except the kidneys and liver (table 1). DMPS transferred ^{210}Po to the kidneys in all treated groups and to the liver of the Pb- rats in which ^{210}Po was substantially decreased when compared with controls. DMPS increased ^{210}Po to the level of control liver. It is known that DMPS also chelates Pb (Goyer *et al.* 1995). Probably it removed bound Pb and then free sites were occupied by ^{210}Po. However, DMPS decreased ^{210}Po in the liver of control and Cd-rats. The complex with the DMPS transferred the excretion route of ^{210}Po from the faeces to the urine. That could be the reason why the kidneys contained increased amounts of ^{210}Po in all treated groups.

It is important to note that retention of ^{210}Po in the bone marrow, thymus and spleen was substantially decreased by the chelating treatment in all cases (table 1). In our previous experiments on subacute lethal radiotoxicity (Rencová *et al.* 1997), severe damage from incorporated ^{210}Po was demonstrated in the femoral and humeral bone marrow, lymph nodes, thymus and spleen. This damage was prevented by another chelating agent.

Our findings that Pb and Cd ions influence the retention of ^{210}Po in tissues and increase its spontaneous excretion from the body in a different way call for further experimental studies. Experiments are planned on the excretion of ^{210}Po under combined pre-exposure to both Cd and Pb ions simultaneously.

Acknowledgements

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