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N-(2,3-Dimercaptopropyl)phthalamidic acid (DMPA) increases polonium-210 excretion

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Summary. Polonium-210 is one of the most hazardous radionuclides. As recently as 1988, there have been concerns regarding accidental exposures of humans to it. Yet, there have been no studies on the effectiveness of the newer dithiol chelating agents in increasing the excretion of this radioactive heavy metal. In order to accomplish this, a safe and effective method for determining the radioactivity of polonium-210, an α emitter, in the feces was developed. The excretion of polonium-210 was studied by giving male Sprague-Dawley rats ^{210}Po (3.33×10^7 cpm/kg, intraperitoneal); 1 h later they were given either 5% sodium bicarbonate, *N*-(2,3-dimercaptopropyl)phthalamidic acid (DMPA) or *meso*-dimercaptosuccinic acid (DMSA) (0.20 mmol/kg, subcutaneous). Treatment was repeated daily for 12 days. DMPA and DMSA increased the urinary excretion of ^{210}Po, as compared to control animals, 8-fold and 5-fold, respectively. DMPA increased the fecal excretion of ^{210}Po compared to the other treatments and also decreased the level of ^{210}Po in the spleen, a radiosensitive organ. DMPA (0.20 mmol/kg, intravenous) increased biliary levels of ^{210}Po 5-fold compared to controls. The results indicate that DMPA has greater specificity in chelating and increasing the excretion of ^{210}Po than DMSA.

Key words: Polonium-210 - DMPA - DMSA - Excretion - Chelating agent

Introduction

Polonium-210 is a high-energy, α-emitting radioisotope and is possibly the most hazardous of the radionuclides (Stara et al. 1971). It has been used in weapons production, in satellites as a source of thermal power and in antistatic devices. Accidental exposures of humans to it have been reported (Fink 1950; Beardsley 1983). Some of these exposures have resulted in hematopoietic depression as well as functional impairment of the liver, kidneys and sex glands (Fink 1950). Ionizing guns containing this radioisotope are used to remove static electricity and dust from bottles, cans and other packaging. Losses of ^{210}Po at packaging plants in the United States have occurred as recently as 1988 (NY Times 1988). Kidneys, spleen and gonads of animals exposed to this radioactive isotope undergo degenerative effects associated with its radiation (Fink 1950). Concentrations of ^{210}Po in these organs, along with the liver and lymph nodes, are the highest in most species. The metabolism and biological effects of polonium-210 have been reviewed (Moroz and Parfenov 1972). ^{210}Po in the body consists of an aggregated or insoluble fraction and an ionic or soluble fraction which are in equilibrium but have different clearance rates (Moroz and Parfenov 1972).

Our laboratory has been examining agents which protect against ^{210}Po. We have focused on the water-soluble dithiol chelating agents (Fig. 1) such as *meso*-dimercaptosuccinic acid (DMSA), 2,3-dimercapto-1-propane-sulfonic acid (DMPS) and *N*-(2,3-dimercapto-

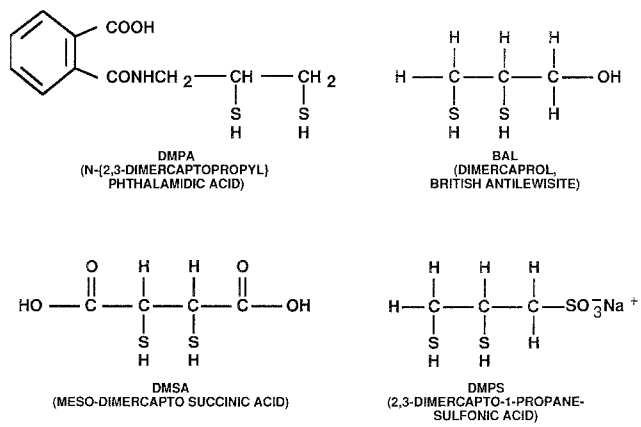

Fig. 1. Dithiol chelating agents

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propyl)phthalamidic acid (DMPA). British Antilewisite (BAL) has been the major treatment for exposures to ^{210}Po (Hursh 1952a, 1952b) but as a drug it has many disadvantages. BAL administration is limited by its side effects which include tachycardia, headache, nausea, vomiting and abdominal pain (Klaassen 1980). DMPA, DMSA and DMPS, chemical analogs of BAL, have been shown to increase median survival time of rats given ^{210}Po (Aposhian et al. 1987). DMPA also decreased tissue concentrations of ^{210}Po (Aposhian et al. 1987). ^{210}Po has an affinity for intracellular sulfhydryl groups on cellular proteins (Lanzola et al. 1973). Thus, DMPA appears to compete with these intracellular thiol groups for ^{210}Po.

The goal of this study was to determine whether DMPA would increase the excretion of ^{210}Po from the body. Although previous work entailed more detailed analysis of ^{210}Po levels in a wider variety of tissues after a longer period of DMPA treatment, it did not determine if excretion of ^{210}Po was actually enhanced. Therefore, we determined whether DMPA increases the urinary and/or fecal excretion of ^{210}Po. In order to do this, it was necessary to develop a safe and effective method of digesting feces in order to quantify ^{210}Po excreted via this route. The urine, feces and selected tissues of rats given ^{210}Po followed by either DMPA or DMSA were examined for ^{210}Po content. DMPA was found to increase the biliary, fecal and urinary excretion of ^{210}Po.

Materials and methods

Animals. Male Sprague-Dawley rats (180–210 g) were purchased from Harlan Sprague-Dawley, Inc. and acclimated for one week before use. Food (Wayne Lab-Blox) and water were provided *ad libitum*. Rats were acclimated in Nalgene metabolism cages for a 2-day period before the metabolic studies.

Chemicals. Carrier-free polonium-210 as nitrate in 3M nitric acid was purchased from Amersham Corporation (Arlington Heights, IL) and diluted to the desired activity with 0.9% saline. *meso*-DMSA was a gift from Johnson & Johnson Baby Products Co (Skillman, NJ). DMPA was prepared by Eisai Ltd (Tokyo, Japan). Both DMPA and DMSA were dissolved in 5% sodium bicarbonate in 0.9% saline solution.

Treatment. Three groups of four rats each were administered ^{210}Po (3.33×10^7 cpm/kg, intraperitoneal); 1 h later one group received DMPA (0.20 mmol/kg, subcutaneous), a second group received DMSA (0.20 mmol/kg, subcutaneous) and a third group was given 5% sodium bicarbonate (2 ml/kg, subcutaneous) to serve as controls. Treatment was repeated at the same hour daily for 12 days. Urine and feces were collected at 48-h intervals, digested and analyzed for ^{210}Po content with a Beckman Scintillation counter model LS 7800. A scintillation cocktail containing Triton X-100 was used (Yelton and Aposhian 1972).

Fecal digestion. A fecal slurry was made by adding to feces 0.1 M NaOH to a final volume of 45 ml and revolving samples overnight at low speed on a Cole-Palmer rotor at room temperature until a uniform suspension was obtained. To 1 ml of the slurry was added 5 ml concentrated nitric acid. The mixture was heated for 5 h at 70° C; 2 ml 30% H_2O_2 was added and heating was continued for 1 h. After cooling to room temperature, 1 ml of the fecal digest was placed in a scintillation vial and 15 ml scintillation cocktail was added. The vials were shaken vigorously by hand and then counted.

Urine digestion. Each 48-h urine sample was brought to 100 ml. A 1-ml aliquot was digested with 1 ml 30% H_2O_2/70% perchloric acid (1:1, by vol.). After 12 h, the digestion was heated for 5 h at 70° C and then cooled. Scintillation cocktail (15 ml) was added. The vials were vigorously shaken by hand and then counted.

Tissues. On day 14, liver, left kidney, heart, right testis and spleen were removed, weighed and ^{210}Po content determined as described previously (Aposhian et al. 1987). This digestion system was based on that of Seidel and Volf (1972) except heating time and scintillation fluid were changed to decrease random counts and those due to luminescence.

Bile experiments. Male Sprague-Dawley rats were divided into two groups of three and administered intraperitoneally 3.33×10^7 cpm ^{210}Po/kg. After 24 h they were anesthetized with 75% urethane (2 ml/kg, intraperitoneal) and infused intravenously with 0.9% saline via the jugular vein at the rate of 1.5 ml/h. Bile ducts were cannulated and bile collected in 30-min intervals over 3.5 h. After two 30-min collections of bile, rats were given either DMPA (0.20 mmol/kg, intravenous) or a comparable volume of saline (2 ml/kg, intravenous). Bile samples (0.10 ml) were then digested using the above procedure to digest urine samples and ^{210}Po content determined.

Results

Fecal analysis

The accuracy and reproducibility of the fecal digestion procedure to quantify ^{210}Po content was determined. A

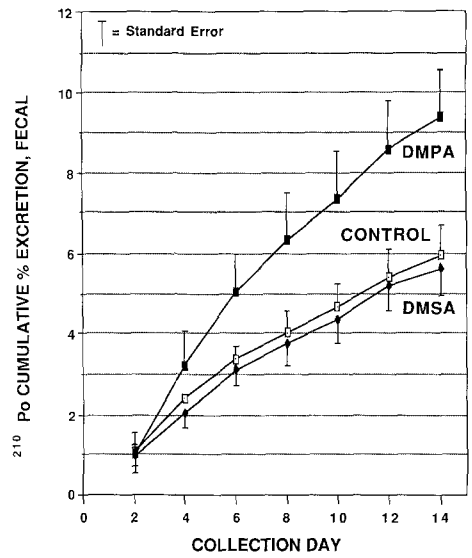

Fig. 2. DMPA increases the fecal excretion of polonium-210. Polonium-210 given at day 0 (3.33×10^7 cpm/kg, intraperitoneal) was followed 1 h later with one of three treatments: 5% sodium bicarbonate control (2 ml/kg, subcutaneous), DMPA or DMSA (0.20 mmol/kg, subcutaneous). Treatments were given once daily for 12 days. Feces were collected, digested and analyzed for polonium-210 content every 2 days. Each point represents the mean ($n = 4$) value. Two-way Anova statistical analysis indicated DMPA ($P < 0.032$) was significantly different from DMSA and control groups

known amount of ^{210}Po was added to feces and processed as described. The detection of ^{210}Po per digested sample was 95.8 ± 1.8% of the known amount added to the feces ($n = 4$). Rats receiving DMPA treatment had a 1.5-fold increase in the fecal excretion of ^{210}Po compared to controls (Fig. 2). DMSA showed no deviation from untreated control levels of ^{210}Po excretion via the feces (Fig. 2). By day 14 more than 9% of the ^{210}Po administered was accounted for in the feces of the DMPA-treated rats. The DMSA or control treatments resulted in less than 6%.

Urinary analysis

Cumulative urinary excretion of ^{210}Po for rats treated with DMPA and DMSA was 8-fold and 5-fold greater, respectively, than the control group (Fig. 3). DMSA initially produced higher urinary levels of ^{210}Po but was surpassed by DMPA at day 8. The levels of ^{210}Po excretion via the urine of controls was consistent with rates previously reported, 0.1%/day per rat (Fink 1950). By day 14 almost 10% of administered ^{210}Po was excreted via the urine by rats receiving DMPA. Rats receiving DMSA or sodium bicarbonate excreted in the urine slightly over 6% and 1%, respectively, of the administered ^{210}Po. It is pertinent to point out that the synthesis of DMPA is extremely difficult and its availability is very limited at this time. The excretion experiments were stopped at 14 days because of the extremely small supply of DMPA. We have no evidence that the DMPA dose used was optimal but suspect it was not.

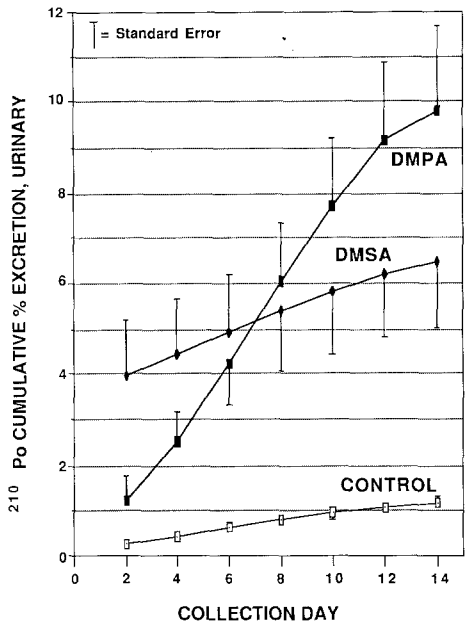

Fig. 3. DMPA increases the urinary excretion of polonium-210. Polonium-210 given at day 0 (3.33×10^7 cpm/kg, intraperitoneal) was followed 1 h later with one of three treatments: 5% sodium bicarbonate control (2 ml/kg, subcutaneous), DMPA or DMSA (0.20 mmol/kg, subcutaneous). Treatments were given once daily for 12 days. Urine was collected, digested and analyzed for polonium-210 content every 2 days. Each point represents the mean ($n = 4$) value. Two-way Anova statistical analysis indicated DMPA and DMSA differ from each other and control group ($P < 0.05$)

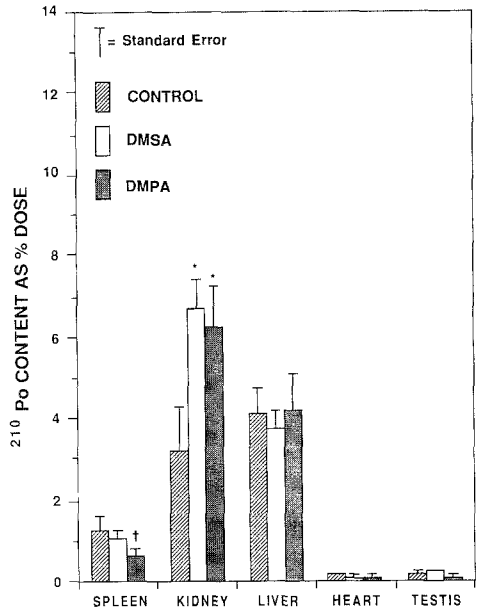

Fig. 4. Tissue distribution of polonium-210 after 5% sodium bicarbonate control, DMSA or DMPA. Polonium-210 given at day 0 (3.33×10^7 cpm/kg, intraperitoneal) was followed 1 h later with one of three treatments: 5% sodium bicarbonate (2 ml/kg, subcutaneous), DMPA or DMSA (0.20 mmol/kg, subcutaneous). Treatments were given once daily for 12 days. On day 14 tissues were removed, digested and analyzed for polonium-210 content. Each point represents the mean ($n = 4$) value. Two-way Anova statistical analysis indicated (†) spleen polonium-210 content of the DMPA group was significantly different from DMSA and control groups ($P < 0.05$). (*) Kidney polonium-210 content of DMPA and DMSA groups were significantly different from control group ($P < 0.05$)

Tissue analysis

DMPA treatment significantly decreased the ^{210}Po content of spleen compared to the other two groups (Fig. 4). DMPA and DMSA increased kidney levels of ^{210}Po compared to controls under these experimental conditions. Liver, testis and heart show no difference in ^{210}Po levels among the three treatments. On day 14 total amounts of ^{210}Po in tissues analyzed were approximately 9%, 12% and 11.5% of the administered ^{210}Po for control, DMSA and DMPA treated groups, respectively.

Biliary analysis

Upon injection of DMPA, biliary levels of ^{210}Po increased 5-fold within 30 min and then returned to control levels by 150–180 min (Fig. 5).

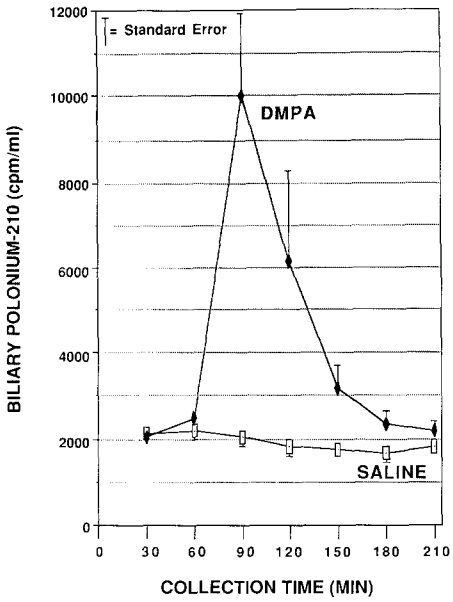

Fig. 5. DMPA increases biliary concentrations of polonium-210. Polonium-210 (3.33×10^7 cpm/kg, intraperitoneal) administered 24 h prior to bile cannulation. Either saline (2 ml/kg, intravenous) or DMPA (0.20 mmol/kg, intravenous) were administered 1 h after bile collection began. Bile was collected in 30-min intervals over 3.5 h. Each point represents the mean ($n=3$) value. Two-way Anova statistical analysis indicated DMPA curve differs from saline in level ($P=0.043$) and shape ($P=0.002$)

Discussion

The results of these experiments demonstrated that DMPA is an effective agent for increasing polonium-210 excretion. It increased the fecal and urinary excretion of ^{210}Po (Figs. 2 and 3). Total excretion of ^{210}Po via these routes was approximately 19% with DMPA treatment. DMSA or sodium bicarbonate resulted in only a 12% and 7% excretion of ^{210}Po, respectively, by these routes. It should be emphasized that no attempt has been made to determine the optimal dose, length or schedule of DMPA treatments because of the extremely limited supply of DMPA at the present time. The purpose of the present experiments was to determine whether DMPA increased the excretion of ^{210}Po from the body.

DMPA at the studied concentrations reduced levels of polonium-210 in radiosensitive tissue such as spleen (Fig. 4). Although the levels of ^{210}Po in kidney were elevated in animals receiving DMPA compared to controls, this is transient and due to its active excretion via urine. As was reported previously, twice-daily injections of DMPA for 21 days resulted in more reduced levels of ^{210}Po in various tissues (Aposhian et al. 1987). The accumulation of ^{210}Po in kidneys is not transient with DMSA treatment. Even after extended treatment with DMSA, kidney ^{210}Po levels remained elevated above controls (Aposhian et al. 1987). Also, DMSA is not as effective in reducing ^{210}Po in splenic tissue (Fig. 4).

DMPA increased the biliary excretion of polonium-210 (Fig. 5). This excretion route has been labeled the most important one for polonium-210 (Parfenov 1974). DMSA treatment (0.20 mmol/kg, intravenous) did not increase biliary ^{210}Po levels over the same experimental time course (Zheng and Aposhian unpublished). The increase in ^{210}Po in bile after DMPA but not after DMSA administration supports the difference seen in their excretion via feces. In the rat, bile is transported from the liver to the small intestines through the bile duct. There some bile is reabsorbed while a portion is incorporated into feces. Increasing biliary concentrations of ^{210}Po will therefore result in its increased fecal elimination as was found to be the case with DMPA administration (Figs. 2 and 5).

The difference between DMPA and DMSA fecal excretion also supports their proposed distributions. DMPA is believed to penetrate cell membranes (Zheng et al. 1990) and to compete with intracellular sulfhydryl groups for polonium-210. Since DMPA injection increased ^{210}Po in bile, it confirms that DMPA can pass through hepatocytes to enter bile. DMSA when administered to rats, was not detected in the bile (Zheng et al. 1990). Therefore DMSA would not be expected to increase biliary levels of ^{210}Po.

The results of these experiments demonstrate the effectiveness of DMPA in the elimination of ^{210}Po. Hursh (1952a) showed that BAL protected rats against ^{210}Po and increased the excretion of this radionuclide. BAL is administered intramuscularly in a peanut oil preparation and has many disadvantages, including instability (Klaassen 1980). Since DMPA is a crystalline preparation which is more stable than BAL, it can be administered orally and warrants further study as an antidote for polonium-210.

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