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Vitamins A, C, and E may reduce intestinal Po-210 levels after ingestion

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Abstract

Damage to the gut mucosa is a probable contributory cause of death from ingested ²¹⁰Po. Therefore, medical products are needed that can prevent, mitigate, and/or repair gastrointestinal (GI) damage caused by high-LET radiation emitted by ²¹⁰Po. The present studies investigated the capacity of a diet highly enriched with vitamins A, C, and E (vitamin ACE) to protect against intestinal mucosal damage indicated by functional reductions in nutrient transport caused by orally ingested ²¹⁰Po. Mice were gavaged with 0 or 18.5 kBq ²¹⁰Po-citrate and fed a control or vitamin ACE-enriched diet (the latter beginning either 96 h before or immediately after gavage). Mouse intestines significantly retained ²¹⁰Po on day 8 post-gavage. The concentration of ²¹⁰Po in intestinal tissues was significantly ($p < 0.05$) lower in all vitamin ACE groups compared to control. There were borderline significant ²¹⁰Po-induced reductions in intestinal absorption of D-fructose. The combination of vitamins A, C, and E may reduce ²¹⁰Po incorporation in the intestines when given before, or enhance decorporation when provided after, ²¹⁰Po gavage.

Keywords

polonium; alpha particle; radioprotector; decorporation; intestine; nutrient transport

Introduction

Access to substantial and concentrated amounts of ²¹⁰Po is possible by chemically separating it from metal substrates such as those used in industrial static eliminators (Azure

and Howell 1994). This radionuclide is highly toxic and ingestion of small quantities is lethal (Harrison et al. 2007). Casarett identified damage to the gut mucosa as a probable contributory cause of death in rats given ^{210}Po orally (Casarett 1964). Alexander Litvinenko, who was lethally poisoned with ^{210}Po in 2006, presented to a hospital with acute, severe, progressive gastrointestinal symptoms (McFee and Leikin 2008). Thus, gastrointestinal (GI) injury in casualties of terrorism with ^{210}Po can be anticipated, although this is only one of several major organ dysfunctions that lead to death. Higher absorbed doses are received by the spleen, kidneys, liver and marrow (ICRP 1993). Therefore, there is interest in developing medical products that can prevent, mitigate, and/or repair organ damage caused by ^{210}Po .

There are several possible ways to reduce biological damage caused by ingested ^{210}Po , among them are to: 1) block incorporation into tissues, 2) enhance clearance of internalized radioactivity by decorporation, 3) prevent damage with radioprotectors, and 4) ameliorate damage with radiomitigators. In the latter two cases, one of the challenging requirements is to provide protection against damage caused by chronic α -irradiation of tissue. This challenge points to radioprotectors with low chemotoxicity even when administered over days to weeks (Weiss and Landauer 2000). Naturally occurring antioxidants may not be the most efficient radioprotectors but they may provide a longer window of protection against effects caused by low-dose rate exposures (Weiss and Landauer 2000). Vitamin A substantially mitigates the deleterious effects of chronic exposure to ^{210}Po in mouse testes (Harapanhalli et al. 1994). The combination of vitamin A and soybean oil yielded a dose modification factor (DMF) of 2 against high-LET α -particles emitted by ^{210}Po (Harapanhalli et al. 1994). Also of interest is vitamin C whose radioprotective properties have been studied in the context of protecting against radiation emitted by incorporated radionuclides in testes (Narra et al. 1993, Narra et al. 1994). Vitamin E has also been shown to confer protection of intestines against ionizing radiation (Empey et al. 1992, Srinivasan and Weiss 1992, Felemovicius et al. 1995, Weiss et al. 1995). Mixtures of radioprotectors offer a means to both improve DMF values and increase coverage with respect to the types of radiation damage that can be protected against. A combination of vitamins A, C and E reduced bone marrow toxicity caused by radioimmunotherapy with ^{131}I labeled antibodies (Blumenthal et al. 2000) and prevented reductions in intestinal nutrient transport caused by whole-body acute and chronic irradiation (Roche et al. 2011, Roche et al. 2015).

The preliminary study in this brief Note was designed initially to test whether a diet highly enriched with vitamins A, C and E can protect against reductions in intestinal nutrient transport caused by ingested ^{210}Po . We attempted to test the vitamin enriched diet's ability to protect intestinal capacity to transport two sugars, D-glucose and D-fructose. These sugars are absorbed by two different systems, one active and ATP-dependent, the other, facilitative. Nutrient uptakes were compared among mice provided a control, or a vitamin A, C and E (vitamin ACE) enriched diet before and after exposure to alpha particle radiation from ingested ^{210}Po . However, the activity of ^{210}Po gavaged was insufficient to cause a significant reduction in intestinal nutrient transport. Nevertheless, a perhaps more interesting finding emerged, namely the capacity of the vitamin ACE enriched diet to reduce uptake of and/or enhance decorporation of intestinal ^{210}Po .

Materials and Methods

Chemicals and Radiochemicals

D-glucose was purchased from Sigma (St Louis, MO), and D-fructose was obtained from Mallinckrodt (St Louis, MO). D-[^{14}C] glucose (31 MBq mL $^{-1}$) and L-[^3H] glucose (37 MBq mL $^{-1}$) were purchased from Sigma (St Louis, MO). D-[^{14}C] fructose (19 MBq mL $^{-1}$) and inulin [carboxyl- ^{14}C] (37 MBq mL $^{-1}$) were obtained from Perkin-Elmer (Waltham, MA), and $^{210}\text{PoCl}_4$ (370 MBq mL $^{-1}$) was purchased from Eckert & Ziegler (Valencia, CA).

Animal Husbandry and Study Design

The experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the former University of Medicine and Dentistry of New Jersey (Newark, NJ). Mice were housed in a sterile, filtered, positive air-flow cage system in a room that has a temperature and humidity stable environment on a 12 h light/dark cycle. Thirty Swiss-Webster male mice, 7-8 weeks of age, were obtained from Taconic Farms (Germantown, NY) and provided a purified AIN76A diet (Research Diets, New Brunswick, NJ). Mice were randomized by weight into five groups for the study.

The groups included control diet with gavage of 0 kBq or 18.5 kBq ^{210}Po ; vitamin ACE supplemented diet substituted for control diet 96 hours prior to gavage with 0 kBq or 18.5 kBq ^{210}Po (Pre 0 kBq or Pre 18.5 kBq); and vitamin ACE supplemented diet substituted for control diet immediately following gavage with 18.5 kBq ^{210}Po (Post 18.5 kBq). Details regarding the vitamin ACE supplemented diet were provided in earlier studies (Roche et al. 2011, Roche et al. 2015). Briefly, the vitamin ACE supplemented diet was the purified AIN76A diet supplemented with 400 IU g $^{-1}$ retinyl acetate (100 $\times$ control), 28.6 mg g $^{-1}$ ascorbic acid phosphate (35% active), and 12.5 IU g $^{-1}$ alpha-tocopherol acetate (250 $\times$ control) (Research Diets, New Brunswick, NJ). Water and feed were provided *ad libitum* throughout the study. Diet consumption and mouse body weights were recorded at daily intervals during the study.

^{210}Po -citrate Preparation and Administration

The LD $_{50/30}$ for oral gavage in rats is ~ 1100 kBq/kg (Dellarosa and Stannard 1964). To prepare the radionuclide for gavage, an aliquot of $^{210}\text{PoCl}_4$ in 2 M HCl was diluted with 1 M sodium citrate. The activity of this solution was determined and then further diluted with 10 mM, pH 6.0, sodium citrate buffer to an activity of 18.5 kBq ^{210}Po in 0.2 mL buffer. This corresponds to a gavage of ~ 450 kBq/kg, a little less than half of the LD $_{50/30}$. Mice were restrained and the ^{210}Po -citrate solution administered through a 20 g gavage needle. Mice in the unirradiated group were administered the citrate buffer solution alone. The postirradiation vitamin supplemented diet group was provided the substituted diet immediately following gavage (Fig. 1A). The preirradiation vitamin supplemented diet group was given the diet 96 hours prior to gavage with ^{210}Po (Fig. 1B). Mice were killed on day 8 after initiating irradiation (i.e. gavage) following the approach of earlier work (Roche et al. 2010, Roche et al. 2011, Roche et al. 2015), and tissue harvested for assessment of each nutrient uptake and residual polonium levels.

Nutrient Uptake

To study the transport of the selected nutrients, the small intestine of the deeply anesthetized mouse was perfused with a cold KRb solution and excised. Uptake rates were determined in excised intestine as described in detail earlier (Roche et al. 2010, Roche et al. 2011). Briefly, the proximal jejunum was cut into 1.5-cm sections and everted onto stainless steel rods with 3-mm outer diameter. Sections were secured with 000 surgical silk at 1-cm intervals and immersed into a 37.5°C KRb nonradioactive incubation bath for 5 min. Each section was then placed into an appropriate radiolabeled bath containing an excess of the cold sugar molecule (50 mM D-glucose or D-fructose) and a tracer nutrient radiolabelled molecule (D-[^{14}C] glucose or D-[^{14}C] fructose). The nonmetabolizable L-[^3H] glucose was used to test for tissue integrity and to correct for ^{14}C glucose or ^{14}C fructose in the adherent fluid. Incubation time was dependent on the nutrient studied. Rods were removed from the D-glucose or fructose bath and immersed for 20 s in a cold KRb bath; each section was cut at the 1-cm grooves and removed. A similar procedure without cold bath immersion was used to determine uptake rates of L-glucose. Tritiated L-glucose was combined with [^{14}C] inulin and used to estimate overall cellular integrity and molecular adhesion. Cut tissue sections were placed into tared 20-mL glass scintillation vials and homogenized by immersion in 1 mL of Solvable (PerkinElmer, Inc., Waltham, MA) for 18 h at 37°C. For radionuclide counting, 10 mL of Ecolume (MP Biomedical, Solon, OH) was added to each vial, vortexed and allowed to stand for 24 h prior to counting.

Measurement of Radionuclide Activities

A Beckman LS-6500 scintillation counter, equipped with the alpha-beta discrimination option, was used to simultaneously quantify the activities of ^3H , ^{14}C , and ^{210}Po in samples containing mixtures of the three radionuclides. A triple label quench correction curve was created to correct for the overlap in the liquid scintillation spectra of the three radionuclides. A stock solution of Solvable with dissolved sections of small intestine (200 mg mL $^{-1}$) from unirradiated mice was prepared. Four sets of 10 vials of standards were spiked with a 50 μL volume of known activity for each radionuclide (^3H , ^{14}C , ^{210}Po) or the combination of all three radionuclides. Ecolume (10 mL) was added to each vial and vortexed before counting. Vials with counts outside the required level of statistical certainty were rejected and not used in construction of the curve. Each set of standards was then spiked with increasing tissue mass by the addition of proportional volumes of tissue stock solution ranging from 0 to 150 mg. A complementary volume of Solvable was added to maintain a consistent 1 mL Solvable per sample. The radionuclide standard sets were recounted using the quench curve correction software of the LS-6500. The quench correction software of the LS-6500 allows for the recombination of the signature energies for each radionuclide into a single correction curve. The triple label standard set provided confirmation of the suitability of the curve for radionuclide activity estimates when the combination of ^3H , ^{14}C and ^{210}Po was present.

Results

Mouse Body Weight, Diet Consumption, and Intestinal Tissue Masses

Dietary intake was similar in all study groups. There were also no significant changes in body weights. There were no significant differences in intestinal tissue masses among the

five groups (Table 1). Thus, by the end of the short study period (designed initially to study nutrient transport), the ^{210}Po treatment and/or supplemented diet did not appear to influence the overall health of the animals, as measured by dietary intake, animal weight, intestinal uptake, tissue weight, and survival.

Intestinal Absorption of ^{210}Po

The ^{210}Po activity per gram in each of three sequential intestinal tissue sections used to analyze nutrient uptakes were highly correlated (0.741 to 0.965; $p < 0.0001$) indicating that the ^{210}Po was evenly distributed in the small intestine. Mice had measurable intestinal retention of ^{210}Po 8 d postgavage. Mean intestinal ^{210}Po concentration (Bq g^{-1}) was reduced by almost half ($p < 0.04$) in both groups of mice receiving the vitamin ACE before and after the gavage (Fig. 2).

Nutrient Uptakes

Neither gavaging $18.5 \text{ kBq } ^{210}\text{Po}$ -citrate nor supplementing the diet with vitamins ACE significantly influenced the uptake of D-glucose per mg or per cm (Fig. 3 A-B). There was a significantly borderline reduction in fructose uptake rate when the vitamin ACE diet was provided before ($p = 0.07$) or immediately following gavage ($p = 0.09$) (Fig. 3 C-D). The similarity in L-glucose uptakes for all groups suggests that cellular permeability was not compromised by radiation and vitamin ACE treatments (Fig. 3 E-F).

Discussion

A number of agents have been tested to decorporate ^{210}Po . 2,3-dimercaptopropanol (BAL) was likely the first ligand recommended for decorporating ^{210}Po from the body (Hursh 1951). BAL doubles the amount of activity eliminated from the body over 10 d, but also redistributes ^{210}Po to muscle, kidney, and intestines. Another thiol compound N-(2,3-dimercaptopropyl)phthalamidic acid (DMPA) reduced ^{210}Po activity in the spleen to 25% of the control (Aposhian et al. 1987). Derivatives of dithiocarbamate were somewhat effective with tissue reductions of 25-60% (Rencova et al. 1994, Rencova et al. 1995). However, some chemotoxicity was observed in a metabolic study. The most effective agent has been HOEtTTC (N,N'-dihydroxyethyldiethylenediamine-N,N'-bis-dithiocarbamate) which reduced ^{210}Po content in kidneys and bone by 75-80% of untreated controls (Rencova et al. 1994). Cuprimine® (contains active ingredient D-penicillamine) and Syprine® (N,N'-bis-(2-aminoethyl)-1,2-ethanediamine dihydrochloride) reduced ^{210}Po activity in the spleen (Levitskaia et al. 2010). Little information is available on the capacity of these agents to remove ^{210}Po from intestinal tissues. Thus, our remarkable finding that vitamins A, C, and E reduces the amount of ^{210}Po in intestinal tissues is novel. A cocktail of vitamin A, C, and E clearly reduces the intestinal incorporation when the diet is provided 96 hours prior to gavage and clearly increases intestinal decorporation when given immediately after.

Further studies are needed to determine: i) if intestinal ^{210}Po activity is reduced when the vitamins are provided 24 hours or longer after ingestion of ^{210}Po , ii) whether ^{210}Po activity is also reduced in other organ systems, and iii) whether intestinal activity was reduced as a consequence of redistribution to other organs or increased excretion. In addition, studies are

also needed to determine why gavaging 18.5 kBq of ^{210}Po (~ half of the $\text{LD}_{50/30}$) had no significant effect on intestinal transport of glucose and fructose in animals receiving the control diet while, surprisingly, the vitamin ACE diet appeared to reduce transport of these sugars in the presence of ^{210}Po . It is possible that the vitamin ACE diet not only reduced the amount of ^{210}Po in the intestines but also redistributed it to a more sensitive location within the tissue.

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Figure 1.

A. Protocol for gavaging mice with ^{210}Po -citrate followed by feeding them a diet highly enriched with vitamins A, C, and E. **B.** Protocol for providing the vitamin ACE diet prior to gavage of ^{210}Po -citrate.

Figure 2.

Bars are mean activity of ^{210}Po per unit mass (Bq g $^{-1}$) in adjacent tissue sections used to determine nutrient uptakes on day 8 post-gavage of ^{210}Po -citrate. Error bars are SE. Group means testing for 18.5 kBq ^{210}Po gavaged mice was significant for diet at p<0.05. Means not sharing the same letter are significantly different (p<0.05).

Figure 3.

Bars are means of nutrient uptake in $\text{nmoles min}^{-1} \text{cm}^{-1}$ (panel A, C, E) or $\text{nmoles min}^{-1} \text{mg}^{-1}$ (panel B, D, F), $N=5-7$, on day 8 post-gavage of ^{210}Po -citrate. Unshaded bars represent the control diet group and the shaded bars represent the vitamin ACE supplemented group. Error bars are SE. Shaded circles represent ^{210}Po (Bq cm^{-1}) (panel A, C, E) or ^{210}Po (Bq g^{-1}) (panel B, D, F). Group means testing by GLM was not significant for diet, dose, time to gavage or their interaction at $p<0.05$ (Panels A-F).

Table 1
Mass of one cm uptake sections of mouse proximal jejunum 8 days post gavage

Section	Diet	Activity kBq	Feed pre/post Gavage	N	Min Section Wgt (mg)	Max Section Wgt (mg)	$\bar{x}$ Section Wgt (mg)	SD Section Wgt (mg)
Fructose	Control	0		6	44.0	66.0	57.2	± 7.9
Fructose	Vit ACE	0	Pre	5	49.0	70.0	55.4	± 8.5
Fructose	Control	18.5		6	44.0	85.0	61.7	± 14.6
Fructose	Vit ACE	18.5	Pre	7	40.0	80.0	60.7	± 15.7
Fructose	Vit ACE	18.5	Post	6	39.0	83.0	59.3	± 18.3
D-glucose	Control	0		6	40.0	67.0	56.3	± 9.7
D-glucose	Vit ACE	0	Pre	5	46.0	66.0	58.6	± 8.3
D-glucose	Control	18.5		6	47.0	66.0	56.8	± 6.9
D-glucose	Vit ACE	18.5	Pre	7	48.0	73.0	59.1	± 10.4
D-glucose	Vit ACE	18.5	Post	6	47.0	72.0	63.8	± 9.2
L-glucose	Control	0		6	51.0	67.0	56.7	± 5.6
L-glucose	Vit ACE	0	Pre	6	46.0	67.0	55.2	± 8.5
L-glucose	Control	18.5		6	42.0	72.0	55.3	± 9.9
L-glucose	Vit ACE	18.5	Pre	6	35.0	64.0	49.9	± 13.1
L-glucose	Vit ACE	18.5	Post	6	42.0	74.0	55.7	± 12.3

Ad-libitum access of the Control Diet was provided to the Control diet group during the study period. "Pre" Feed is the substitution with supplemented ACE diet for the Control Diet prior to gavage and "Post" Feed is the substitution with supplemented ACE diet for the Control Diet immediately after gavage. Values are Means $\pm$ SD, N = 5-7.