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The effect of olestra on the absorption, excretion and storage of 2,2′,5,5′ tetrachlorobiphenyl; 3,3′,4,4′ tetrachlorobiphenyl; and perfluorooctanoic acid

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ABSTRACT

Mice were gavaged with either ^{14}C-labeled 2,2′5,5′ tetrachlorobiphenyl; 3,3′,4,4′ tetrachlorobiphenyl; or perfluorooctanoic acid. Absorption of these compounds was determined by assay of feces collected for 48 h after the gavage. Part of the animals received test diets containing olestra during this 48-hour period to determine its effect on absorption of the compounds. Mice that received the diet without olestra during this period were divided into groups that either continued the diet without olestra or changed to a diet containing olestra. These diets were continued for 7 days, and a second 48-hour fecal collection was made to measure the effect of olestra on enterohepatic circulation of the compounds and their metabolites. The animals were sacrificed, and blood, fat, and liver concentrations of ^{14}C were measured. Olestra decreased the absorption of 2,2′,5,5′ tetrachlorobiphenyl. It also reduced tissue and blood concentrations of this compound. Olestra also decreased the absorption of 3,3′,4,4′ tetrachlorobiphenyl, but it did not alter enterohepatic circulation or tissue concentrations. Olestra significantly increased the excretion of perfluorooctanoic acid in the second 48-hour collection, suggesting an effect on enterohepatic circulation. It did not, however, alter tissue concentrations of perfluorooctanoic acid. These data are consistent with previously observed effects of olestra on the absorption and storage of lipophilic compounds.

1. Introduction

The intentional and inadvertent production of organohalide compounds has resulted in their entry into the biosphere, including human tissues. These compounds are frequently characterized by their lipophilicity and their chemical stability. These properties often result in their storage in adipose tissue with long half lives. Many of these compounds are considered to be toxic and/or carcinogenic.

Although there are major efforts under way to limit the entry of organohalides into the environment, their resistance to chemical degradation ensures that potentially harmful levels will remain in many organisms for centuries. Another approach to the elimination of organohalides from humans is the use of nutritional interactions to reduce their absorption from the diet and interrupt their intestinal re-absorption during enterohepatic circulation (Hennig et al., 2007).

One nutritional approach is the use of non-absorbable dietary lipid. Dietary sucrose polyesters (e.g., olestra) and the inhibition of pancreatic lipase with orlistat result in an intestinal oil phase into which lipophilic compounds partition (Jandacek and Tso, 2007) These compounds can be carried into the feces without absorption, and their storage in the body can be reduced by interruption of enterohepatic absorption (Mutter et al., 1988; Redgrave et al., 2005). This observed effect on enterohepatic circulation may include sequestration in the non-absorbable lipid, enhancement of non-biliary excretion (movement of lipophiles from the enterocytes into the intestinal lumen), or alteration of other components of the intestinal milieu that might affect reabsorption.

These studies have focused on interruption of enterohepatic circulation of lipophilic compounds. It is clear, however, that an equally important approach to a reduction of body burdens of lipophilic compounds is decreasing their bioavailability from the diet. Based on long-term accumulation studies in humans, the rate of intake of organochlorine compounds exceeds their excretion rate (Costabeber and Emanuelli, 2003; Duarte-Davidson and Jones, 1994). Therefore intervention by effectively decreasing intake from the diet is a viable strategy for reducing body burdens. Volpenhein et al. (1980) reported that the absorption of DDT from the diet could be markedly reduced by the presence of non-absorbable lipid.

It is clear from the studies cited above that non-absorbable dietary lipid has marked effects on highly lipophilic compounds. Less studied, however, have been the effects on amphiphilic compounds or compounds of intermediate lipophilicity. We therefore have extended the studies of non-absorbable lipid to lipophilic compounds that form more polar metabolites (2,2′,5,5′ tetrachlorobiphenyl; 3,3′,4,4′ tetrachlorobiphenyl), and an amphiphilic compound (perfluorooctanoic acid). Although one might predict less interaction of non-absorbable lipid with the more polar species, there is the possibility that interfacial adsorption (with the unabsorbed lipid) of compounds with lipophilic moieties would affect their rate of removal. This effect was reported by Grundy et al. (1986), who reported an increase in the excretion of bile acids in patients fed sucrose polyester (olestra). Since bile acids are amphiphilic, it is possible that halogenated organic

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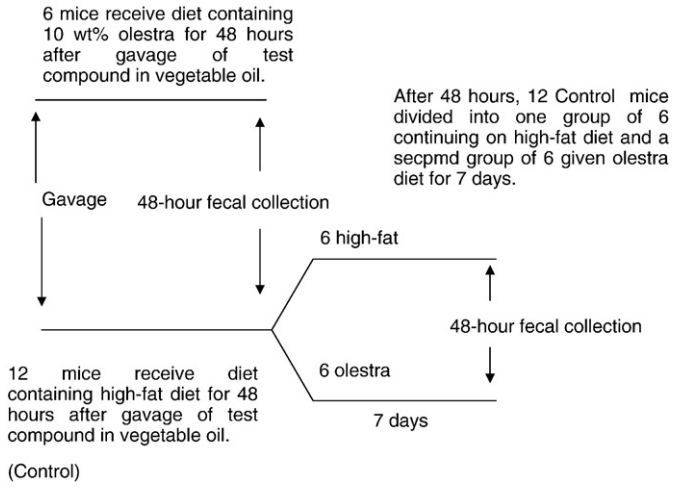

Fig. 1. A schematic diagram of the protocol used for each test material. Dietary absorption was determined from the first 48-hour fecal collection, and potential interruption of enterohepatic circulation was measured from the second collection.

compounds with hydrophilic moieties might also be affected by unabsorbed lipid. Moreover, the effect of non-absorbable lipid on the absorption of these compounds from the diet has not been studied, and as noted above, decreasing bioavailability can be an important approach to reducing body burdens.

2. Methods

The protocol was designed to determine the effects of dietary olestra on the absorption of compounds from the diet as well as on the reabsorption of parent compounds and their metabolites involved in enterohepatic circulation. Absorption was measured from the assay of dietary and fecal ^{14}C-labeled test compounds. Animals were gavaged with the test compounds in safflower oil, and feces were collected for 48 h immediately after the gavage to measure initial dietary absorption, and, for 48 h 7 days after the gavage to determine the interruption of enterohepatic circulation. After the second fecal collection, the animals were sacrificed and tissues were taken for analysis. A schematic diagram of the protocol is shown in Fig. 1.

2.1. Materials

2,2′,5,5′ tetrachlorobiphenyl (uniformly labeled ^{14}C, 7.2 mCi/mmol) and 3,3′,4,4′ tetrachlorobiphenyl (uniformly labeled ^{14}C, 12.5 mCi/mmol) were purchased from Sigma-Aldrich (St. Louis, MO). Perfluorooctanoic acid (1-^{14}C, 55 mCi/mmol) was synthesized by American Radiochemicals (St. Louis, MO).

The high-fat diet was prepared by Dyets, Inc. (Bethlehem, PA), and the olestra diet was prepared by Research Diets (New Brunswick, NJ). The high-fat diet was based on the AIN-93M diet, and comprised by weight 16.4% casein, 30.3% cornstarch, 9.0% sucrose, 1.0% soybean oil, and 19.0% butter fat. The olestra diet contained the same macronutrients, but also included 10 g of olestra (a gift from Procter & Gamble, Cincinnati, OH) added to each 100 g of diet. The olestra diet also included 5 times the recommended requirements for vitamins A, D, and E, and the recommended requirement of vitamin K.

2.2. Animals

Male, C57 BL/6 mice, 8–12 weeks of age, were purchased from Jackson Laboratories (Bar Harbor, ME). Each compound was gavaged in safflower oil into 18 mice. Twelve received a high-fat diet, and 6 received a diet containing olestra. Animals were assigned to groups to have similar weight distributions for each group. All animals were singly housed throughout the study.

Mice were gavaged with approximately 1.0 μCi of radioactivity in 0.1 mL of safflower oil. After the initial absorption measurement, the 12 control animals were divided into two groups of 6 that then received either the high-fat diet or the diet containing olestra. After 7 days, a second 48-hour fecal collection was made. The animals were then sacrificed, and blood, epididymal fat pad, and liver were removed for later analysis.

Table 1
The absorption, excretion, and tissue concentrations of 2,2′,5,5′ tetrachlorobiphenyl in mice given a bolus dose and receiving either a high-fat diet or a diet containing olestra ($n=6$; mean ± SEM; *$p<0.05$ olestra diet differs from high-fat diet).

Measurement	High-fat diet	Olestra diet
Initial absorption (% of dose in 48 h)	81.0 ± 1.4	55.2 ± 4.3*
Excretion 7 days after dose (% of dose in 48 h)	5.1 ± 0.32	5.3 ± 0.39
Adipose concentration (% of dose/g)	10.4 ± 0.53	8.2 ± 0.83*
Plasma concentration (% of dose/mL)	0.41 ± 0.049	0.13 ± 0.013*
Liver concentration (% of dose/g)	0.85 ± 0.034	0.52 ± 0.027*

2.3. Analyses

Complete 48-hour fecal analyses were obtained from each animal, and each collection was homogenized with 0.2 mL water. Tissues and feces were analyzed by oxidation and scintillation counting. Oxidation and conversion to carbon dioxide were carried out with the Harvey Biological Oxidizer OX700 (R.J. Harvey, Hillsdale, NJ). The tissues were burned at 900 °C, and the radioisotope was captured as $^{14}CO_2$ in ^{14}C scintillation cocktail (R. J. Harvey, Hillsdale, NJ). Two weighed aliquots were oxidized for each tissue collected from each animal. Plasma was dissolved directly in scintillation fluid (without oxidation). ^{14}C activity in each vial was counted with a scintillation counter and recorded as dpm. Red blood cells were measured by scintillation counting after combustion with the oxidizer.

Plasma samples were pooled from each group and separated by ultracentrifugation in solutions of known density of potassium bromide to determine distribution in density corresponding to lipoproteins: <1.020 (triglyceride-rich), and into 1.020–1.063 (LDL), and >1.063 (HDL and other proteins). One mL of the solution was evaporated on filter paper, combusted in the oxidizer, and counted by scintillation counting.

Comparisons of high-fat and olestra groups were made by *t*-test (Sigmastat). Significant differences were accepted when $p<0.05$. Results are reported as means and standard errors.

3. Results

3.1. 2,2′,5,5′ Tetrachlorobiphenyl

The results of the absorption, excretion, and tissue measurements are presented in Table 1. As discussed above, the total 48-hour excretion 7 days after the gavage was used to

Table 2
The absorption, excretion, and tissue concentrations of 3,3′,4,4′ tetrachlorobiphenyl in mice given a bolus dose and receiving either a high-fat diet or a diet containing olestra ($n=6$; mean ± SEM; *$p<0.05$ olestra diet differs from high-fat diet).

Measurement	High-fat diet (mean ± SEM, $n=6$)	Olestra diet (mean ± SEM, $n=6$)
Initial absorption (% of dose in 48 h)	43.7 ± 2.0	16.5 ± 4.7*
Excretion 7 days after dose (% of dose in 48 h)	1.5 ± 0.20	1.1 ± 0.15
Adipose concentration (% of dose/g)	0.37 ± 0.048	0.42 ± 0.048
Plasma concentration (% of dose/mL)	0.33 ± 0.087	0.23 ± 0.019
Liver concentration (% of dose/g)	0.40 ± 0.010	0.36 ± 0.044