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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 ¹⁴C-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 hours 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 ¹⁴C 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.

I. 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

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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. reported that the absorption of DDT from the diet could be markedly reduced by the presence of non-absorbable lipid(Volpenhein et al., 1980).

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., who reported an increase in the excretion of bile acids in patients fed sucrose polyester (olestra)(Grundy et al., 1986). Since bile acids are amphiphilic, it is possible that halogenated organic 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 hours immediately after the gavage to measure initial dietary absorption, and, for 48 hours 7 days after the gavage to determine the interruption of enterohepatic circulation. After the second fecal collection, the animals were sacrificed and tissues taken for analysis. A schematic diagram of the protocol is shown in Figure 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 ($1\text{-}^{14}\text{C}$, 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 microcurie 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.

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 $^{14}\text{CO}_2$ 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 determine the effect of olestra on enterohepatic circulation. No differences were seen between groups.

The concentration in the epididymal fat pad was significantly reduced in the group that ate olestra relative to the high-fat group. The concentration of ^{14}C in the livers of the animals in the olestra group was significantly less than that in the high-fat group.

The concentration of ^{14}C was determined in the red blood cells. The concentration in the cells of the olestra group was 0.057 ± 0.010 (% dose/mL), significantly less than that of the high-fat group, 0.124 ± 0.010 . The ^{14}C was predominantly carried in the plasma components, as shown by the ratios of plasma concentration (% dose/mL) to RBC concentration (% of dose/g). These ratios for the high-fat group and the olestra group did not differ (3.52 ± 0.59 ; 2.73 ± 0.54 , respectively).

Essentially all radioactivity was found in the fraction in the solution with >1.063 density. The concentration in this fraction for the pooled sample from the high-fat group was 1769 dpm/mL, compared with 523 dpm/mL for the olestra group.

3.2. 3,3',4,4' Tetrachlorobiphenyl

Absorption, excretion, and tissue concentrations of this congener are presented in Table 2. The total excretion 7 days after the gavage was used to determine the effect of olestra on enterohepatic circulation. There was no difference between groups. There was no difference between groups in the plasma concentration of ^{14}C 7 days after gavage of the 3,3',4,4' tetrachlorobiphenyl. In addition the concentrations in the fat pads and livers did not differ between groups..

The concentration of ^{14}C in the red blood cells of the olestra group was 0.060 ± 0.011 (% dose/g), not different from that of the high-fat group, 0.085 ± 0.066 . The ^{14}C was predominantly carried in the plasma components, as shown by the ratios of plasma concentration (% dose/mL) to RBC concentration (% of dose/g). These ratios for the high-fat group and the olestra group did not differ (4.14 ± 1.11 ; 4.28 ± 0.56 , respectively).

Essentially all radioactivity was found in the fraction in the solution with >1.063 density. The concentration in this fraction for the pooled sample from the high-fat group was 335 dpm/mL, compared with 217 dpm/mL for the olestra group.

3.3. Perfluorooctanoic Acid

The absorption, excretion, and tissue concentrations of perfluorooctanoic acid are presented in Table 3. The absorption, based on the measurement of excretion 48 hours after the gavage of the test compound, was essentially complete for both groups.

The excretion of ^{14}C 7 days after the gavage of the dose was different between the high-fat and olestra groups. Although the excretion rates were low, the rate of excretion in the olestra group was more than twice that of the high-fat group. The concentrations in plasma, livers, and fat pads did not differ between groups.

There was no difference between groups in the concentrations of ^{14}C in red blood cells, ratio of plasma to red blood cells, and lipoprotein fraction. The concentrations of ^{14}C in total plasma were 3.71 ± 0.29 and $4.25 \pm 0.48\%$ of dose/mL for the high-fat and olestra groups, respectively. Concentrations in red blood cells were 1.01 ± 19 and 1.15 ± 0.28 % dose/g for the high-fat and olestra groups, respectively. The ratios of concentrations in plasma (% dose/mL) to red cells (% dose/g) were 4.06 ± 0.58 and 4.31 ± 1.07 , for the high-fat and olestra groups, respectively. Essentially all radioactivity in plasma was found in the >1.063 fraction. The concentration in the plasma of the high-fat group was 12379 dpm/mL, and that in the olestra group was 12542 dpm/mL.

4. Discussion

4.1 2,2',5,5' Tetrachlorobiphenyl

This PCB congener was well absorbed from a high-fat diet, and olestra significantly interfered with the absorption of the parent compound, based on the initial 48-hour fecal collection. After 7 days, the excretion rates were similar between the two groups, however the remaining isotope in the plasma, fat, and liver were less in the olestra group relative to the high-fat group. This pattern is consistent with a depletion of the body burden in the olestra group during the 7-days before the fecal collection. Although the excretion rate at 7 days in terms of percent of administered dose is the same between groups, it is probably higher in the olestra group in terms of the dose remaining in the body. The data therefore suggest that olestra interacts with the parent compound in the diet and remaining parent compound and metabolites in enterohepatic circulation.

The ratio of concentration in the adipose relative to plasma is markedly less than those reported by Clevenger et al. during 7 days after exposure of mice to this congener (Clevenger et al., 1989). However, the results in that study were obtained after 8 daily doses of 100 mg/kg body weight, which were far greater than the single bolus of approximately 2 mg/kg in our study. This difference in dose translated into adipose levels in our study that were nearly 40-fold less than the lowest in the Clevenger study. It is therefore difficult to compare our results for the partitioning between adipose and blood with those of Clevenger.

4.2. 3,3',4,4' Tetrachlorobiphenyl

The results for this congener clearly demonstrated an effect of olestra on the absorption of the parent compound. It should be noted that this congener is not well absorbed, even from the high-fat diet, and olestra decreased absorption by more than 50%. This low absorption might be predicted from the high melting point (176 °C) of this coplanar congener, which reduces its solubility in and bioavailability from dietary fat (Clevenger and et al., 1989). Lower absorption of this congener relative to that of the 2,2',5,5' congener can be inferred from previously reported adipose tissue concentrations (Clevenger et al., 1989). There is no evidence that olestra had any further effect on removal of this congener or its metabolites from the body. Levels in tissues were not different between the groups. These observations suggest a formation of polar metabolites that were sparingly soluble in olestra, so that it did not prevent enterohepatic circulation and enhance their excretion in feces.

As in the case of the 2,2',5,5' congener, the ratio of concentration in the adipose relative to plasma was less than those reported by Clevenger et al. during 7 days after exposure. In this case the results in that study were obtained after 10 daily doses of 8 mg/kg body weight, greater than our single bolus of approximately 2 mg/kg (Clevenger et al., 1989).

The rate of excretion 7 days after the bolus dose was less than that seen for the 2,2',5,5' congener. This observation is consistent with the lower absorption of the bolus dose of the 3,3',4,4' congener and with its shorter half life (Clevenger et al., 1989). Therefore after 7 days, the retained dose was less than for the 2,2',5,5' congener, and the daily excretion reflected the lower retention.

4.3. Perfluorooctanoic Acid

Perfluorooctanoic acid was readily absorbed from the gavage formulation. There was a statistically significant reduction in absorption when dietary olestra followed the gavage, but this decrease would not be expected to have biological significance. Tissue levels of perfluorooctanoic acid were not different between the two dietary groups. There was a marked, significant increase in ^{14}C in the feces in the olestra group relative to the high-fat diet group.

The absolute difference between the groups was small and was not reflected in the tissue levels. The effect of olestra may reflect the excretion of a non-polar form of the compound. It may also reflect the partitioning of a portion of the parent compound into the olestra with interruption of enterohepatic circulation. Although the observed effect is small, it is possible that it may be physiologically significant if it causes a meaningful decrease in the retention of a slowly removed pool of perfluorooctanoic acid.

5. Conclusions

The body burden of organohalide compounds reflects the rates of input and output of the compounds and their metabolites. Two intervention sites are possible for the reduction of a human body burden of organohalide compounds, diet sources and enterohepatic circulation. Minimizing or eliminating dietary absorption of organohalides reduces the input, and interference with absorption of enterohepatic circulation increases output via fecal excretion.

It is clear from the data presented above that dietary non-absorbable lipid reduces the intake from the diet of the studied PCB congeners. Although a statistically significant reduction in the absorption of perfluorooctanoic acid was observed, the magnitude of this reduction is not meaningful in terms of reduction of body burden of this compound.

There was no apparent effect of non-absorbable lipid on the enterohepatic circulation of 2,2', 5,5' tetrachlorobiphenyl based on fecal excretion in terms of administered dose. However, there were significant reductions in the concentrations of the ^{14}C in plasma, fat, and liver 7 days after gavage. Excretion may have been more rapid during the first part of the 7 day period, resulting in reduction in storage of this congener. It is also likely that as metabolism to the more polar hydroxylated metabolites occurred, the effect of the non-absorbable lipid decreased.

Contrary to the effect on dietary absorption, there were apparently no effects of non-absorbable lipid on the excretion of stored 3,3',4,4' tetrachlorobiphenyl. This observation is consistent with the rapid formation of polar metabolites that were rapidly eliminated. The concentration in the fat pad is approximately 3% of that seen for the 2,2',5,5' congener.

It is possible that non-absorbable lipid will have larger effects on non-polar PCB congeners and their metabolites that remain in adipose tissue with long half lives. The case study of a patient exposed to a mixture of PCB congeners responded to dietary non-absorbable lipid with a marked reduction in PCB body burden (Redgrave et al., 2005).

The effect of non-absorbable lipid on perfluorooctanoic acid excretion is interesting. It is known that the excretion of perfluorooctanoic acid differs by sex and species, and that excretion takes place by both urinary and fecal routes. There is little metabolism of the parent compound (Kudo and Kawachima, 2003). Interruption of enterohepatic circulation of perfluorooctanoic acid was seen with dietary cholestyramine in rats, with a nearly 10-fold increase in the rate of excretion (Johnson et al., 1984).

Olestra doubled the rate of fecal elimination, however that increase was not reflected in decreases in levels of ^{14}C in fat, liver, or plasma. The half-life of perfluorooctanoic acid in humans is estimated to be 4 years, suggesting a slowly released pool of the stored compound (Kudo and Kawachima, 2003). If the rate of fecal excretion dominates the urinary excretion in this slow pool, it is possible that doubling the fecal excretion rate could effectively reduce the half life of the stored compound. Given the reported differences in species and gender on the rate of elimination, it is impossible to say with certainty that non-absorbable lipid will have a clinically meaningful effect on the body burden of perfluorooctanoic acid.

It is well documented that olestra can both reduce absorption of and enhance excretion of very lipophilic organochlorine compounds such as DDT, hexachlorobenzene, and mixed congeners of PCBs (Jandacek and Tso, 2001; Redgrave et al., 2005). In the studies presented here we sought to extend the scope of understanding about the kinds of compounds that might be affected by non-absorbable fat in the diet. We have found marked reduction of the absorption of lipophilic PCB parent congeners, but we conclude that there is minimal effect of olestra on the excretion of polar metabolites. We found no biologically significant effect on the absorption of perfluorooctanoic acid. This result is consistent with the hydrophilicity of the compound in its ionic form. We did observe an increase in the slow excretion rate of this compound. It is not clear as to whether or not this increase will significantly reduce the half life of perfluorooctanoic acid retained in the body.

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Figure 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.

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

Measurement	High-Fat Diet	Olestra Diet
Initial Absorption (% of Dose in 48 Hours)	81.0±1.4	55.2±4.3 *
Excretion 7 Days after Dose (% of Dose in 48 Hours)	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 *

(n=6; mean ± SEM)

* p < 0.05 olestra diet differs from high-fat diet).

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

Measurement	High-Fat Diet (Mean $\pm$ SEM, n=6)	Olestra Diet (Mean $\pm$ SEM, n=6)
Initial Absorption (% of Dose in 48 Hours)	43.7 $\pm$ 2.0	16.5 $\pm$ 4.7*
Excretion 7 Days after Dose (% of Dose in 48 Hours)	1.5 $\pm$ 0.20	1.1 $\pm$ 0.15
Adipose Concentration (% of Dose/g)	0.37 $\pm$ 0.048	0.42 $\pm$ 0.048
Plasma Concentration (% of Dose/mL)	0.33 $\pm$ 0.087	0.23 $\pm$ 0.019
Liver Concentration (% of Dose/g)	0.40 $\pm$ 0.010	0.36 $\pm$ 0.044

(n=6; mean $\pm$ SEM)

* p < 0.05 olestra diet differs from high-fat diet).

Table 3

The absorption, excretion, and tissue concentrations of perfluorooctanoic acid in mice given a bolus dose and receiving either a high-fat diet or a diet containing olestra

Measurement	High-Fat Diet (Mean $\pm$ SEM, n=6)	Olestra Diet (Mean $\pm$ SEM, n=6)
Initial Absorption (% of Dose in 48 Hours)	98.8 $\pm$ 0.17	98.0 $\pm$ 0.26*
Excretion 7 Days after Dose (% of Dose in 48 Hours)	0.12 $\pm$ 0.01	0.28 $\pm$ 0.03*
Adipose Concentration (% of Dose/g)	0.34 $\pm$ 0.064	0.34 $\pm$ 0.037
Plasma Concentration (% of Dose/mL)	3.71 $\pm$ 0.29	4.25 $\pm$ 0.48
Liver Concentration (% of Dose/g)	19.3 $\pm$ 0.2	19.5 $\pm$ 1.1

(n=6; mean $\pm$ SEM)

* p < 0.05 olestra diet differs from high-fat diet).