Administration of apple juice concentrate decreases perfluorooctane sulfonic acid (PFOS) absorption in C57BL/6J male mice

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STEEP Sources, Transport, Exposure & Effects of PFASs
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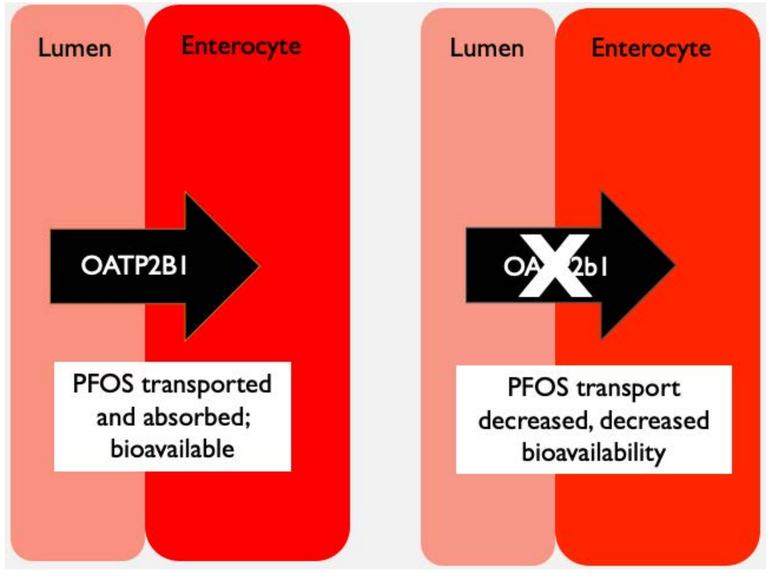
ABSTRACT

Perfluorooctane sulfonic acid (PFOS) is part of a class of synthetic chemical compounds known as perfluorinated alkyl substances (PFAS). PFOS exposure has been linked to increased risk for adverse health outcomes such as hypercholesterolemia and elevation of serum liver injury biomarkers. Rodent studies indicate that PFOS is highly bioavailable and well absorbed in the gastrointestinal tract (GIT). PFOS likely has low permeability in the GIT but is known to be a substrate for the Organic Anion Transporting Polypeptide 2B1 (OATP2B1) transporter. Polyphenols present in apple juice extract (AJE), have been shown to be inhibitors of OATP2B1.Thus, it was hypothesized that AJE can decrease PFOS absorption in mice, presumably through Oatp2b1 inhibition. Adult male mice (n=4/time point and treatment group) were pre-administered either a single dose of 4X AJE (10 ml/kg, pH 3) or phosphate-buffer saline (10 ml/kg, pH 3) via oral gavage. One hour later, mice received a single dose of PFOS (0.5 mg/kg) in 4X AJE or 1x PBS. Plasma and tissues were collected 2 or 5 hours after PFOS administration, snap frozen, homogenized, and processed for LC/MS analysis using QuECheERS. AJE administration reduced plasma and liver PFOS concentrations at 2 hours and 5 hours after dosing. Plasma PFOS concentrations were reduced to 56% and 65% of the PBS controls, respectively. PFOS tissue concentration measurements are ongoing. Overall, this study suggests AJE may block PFOS absorption via Oatp2b1 inhibition.

INTRODUCTION

PFAS are per- and polyfluoroalkyl substances. They are man-made chemicals compounds consisting of a hydrophobic alkyl chain which can be partially or fully fluorinated. PFOS is part of the PFAS chemical class. PFOS has been detected in the serum of 98% of a group representative of the United States population at concentrations ranging from <0.4µg/L to 435 µg/L. PFAS exposure has been linked to elevated serum liver enzymes, decreased glomerular filtration rate, and increased cardiovascular disease risk. PFAS are highly absorbed through the gastrointestinal system but can have low permeability in the GI tract due to pH. Intestinal transporters likely contribute to PFAS absorption. OATP2B1, located at the apical membrane of enterocytes and the sinusoidal membrane of hepatocytes, is involved in the sodium-independent uptake of endogenous compounds, clinically available drugs and PFOS. Oatp2b1 may be a transporter contributing to PFAS absorption. Plant polyphenols found in apple juice concentrate, such as hesperidin and naringenin have been identified as potent OATP2B1 inhibitors. We propose a study to evaluate the impact of apple juice concentrate on PFOS plasma and tissue concentrations in male mice at 2- and 5-hour time points.



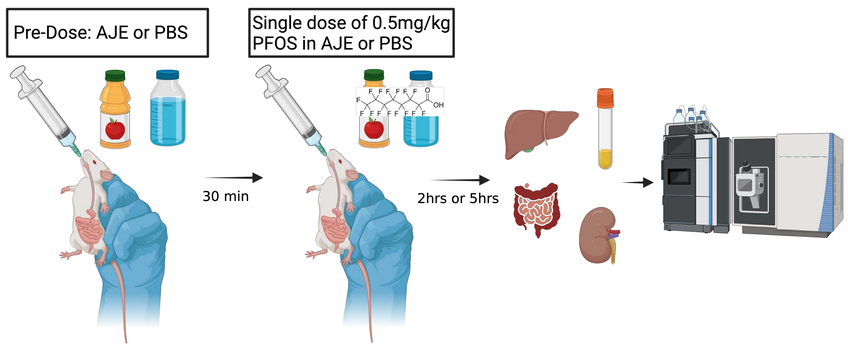
HYPOTHESIS

C57BL/6 male mice administered 10 ml/kg of a single pre-dose of 4X apple juice concentrate, followed by a single dose of 0.5mg/kg PFOS in 4X apple juice concentrate will have lower plasma and tissue PFOS concentrations at the 2-hour and 5-hour time points, compared to the C57BL/6 male mice that were administered 10 ml/kg of a single pre-dose of PBS, followed by a single dose of 0.5mg/kg PFOS in PBS.

MATERIALS and METHODS

Pre-Dosing Solutions:
- Phosphate- buffered saline, pH~4
- Stop and Shop frozen 100% Pure Apple Juice, pH ~4

Dosing Solutions:
- 0.5 mg/kg PFOS, in phosphate-buffered saline, pH ~4
- 0.5 mg/kg PFOS, in Stop and Shop frozen 100% Pure Apple Juice, pH ~4



Treatment Paradigm: C57BL/6J male mice, aged 10 weeks, were administered 10ml/kg of a single pre-dose of 4X apple juice concentrate(n=4) or PBS solution(n=4), via oral gavage. One hour later mice were administered a single dose of 0.5mg/kg PFOS in 4X apple juice concentrate or PBS. After a 2-hour time window(n=8) and a 5-hour time window(n=8), necropsy occurred. Mice were sedated using isoflurane and ~20uL of blood was collected, into heparinized tubes. Upon completion of the necropsy, the supernatant plasma was removed and stored at -20C to be analyzed later. Mice were euthanized and the liver, kidneys, intestines, brain and lungs were collected and snap frozen in liquid nitrogen.

LC/MS Sample Preparation: PFOS (Sigma Aldrich, St. Louis, MO) was extracted according to the RoQ TM Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) manufacturer protocol (Phenomenex, Torrance, CA). An input of 10uL of plasma and about ~20 mg of liver, kidney, intestine, brain, and lung were used in the sample preparations. All samples were spiked with isotope-labeled internal standard (M4PFOS) from Wellington Laboratories (Ontario, Canada). Samples were run on a Sciex 6500 QTRAP LC-MS/MS (Framingham, MA). Data was analyzed and presented using GraphPad Prism 10 (La Jolla, CA)

RESULTS

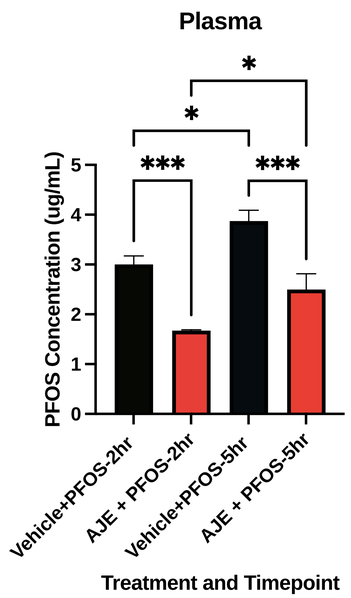


Figure 1. Average Plasma PFOS Concentrations (ng/mL) 2 and 5 hours after administration of a single dose of 0.5mg/kg PFOS in PBS (vehicle) or apple juice concentrate. Mice (n=4/treatment group/timepoint) were administered a single pre-dosed of PBS or 4X apple juice concentrate and 1 hour later were administered a single dose of 0.5mg/kg PFOS in PBS or 4X apple juice concentrate. Plasma and tissues were collected 2 and 5 hours post dosing. A significant difference was observed between Vehicle+PFOS and AJE+PFOS at both 2- and 5-hour timepoints, p value <0.0001.

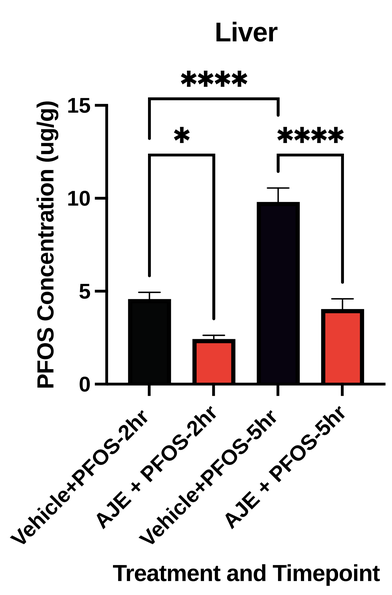


Figure 2. Average Liver PFOS Concentrations (ng/mL) 2 and 5 hours after administration of a single dose of 0.5mg/kg PFOS in PBS (vehicle) or apple juice concentrate. Mice (n=4/treatment group/timepoint) were administered a single pre-dosed of PBS or 4X apple juice concentrate and 1 hour later were administered a single dose of 0.5mg/kg PFOS in PBS or 4X apple juice concentrate. Plasma and tissues were collected 2 and 5 hours post dosing. A significant difference was observed between Vehicle+PFOS and AJE+PFOS at both 2- and 5-hour timepoints, p value <0.0001.

RESULTS: Continued

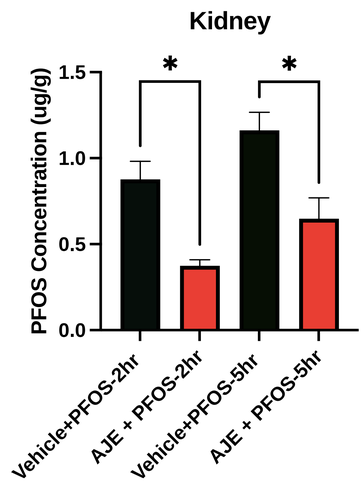


Figure 3. Average Kidney PFOS Concentrations (ng/mL) 2 and 5 hours after administration of a single dose of 0.5 mg/kg PFOS in PBS (vehicle) or apple juice concentrate. Mice (n=4/treatment group/timepoint) were administered a single pre-dosed of PBS or 4X apple juice concentrate and 1 hour later were administered a single dose of 0.5 mg/kg PFOS in PBS or 4X apple juice concentrate. Plasma and tissues were collected 2 and 5 hours post dosing. A significant difference was observed between Vehicle+PFOS and AJE+PFOS at both 2- and 5-hour timepoints, p value 0.0007.

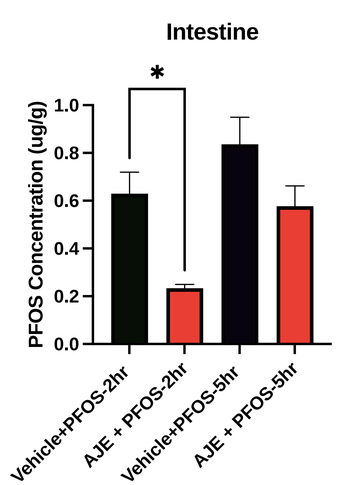


Figure 4. Average Intestine PFOS Concentrations (ng/mL) 2 and 5 hours after administration of a single dose of 0. 5mg/kg PFOS in PBS (vehicle) or apple juice concentrate. Mice (n=4/treatment group/timepoint) were administered a single pre-dosed of PBS or 4X apple juice concentrate and 1 hour later were administered a single dose of 0.5 mg/kg PFOS in PBS or 4X apple juice concentrate. Plasma and tissues were collected 2 and 5 hours post dosing. A significant difference was observed between Vehicle+PFOS and AJE+PFOS at the 2-hour timepoint, p value 0.004.

Results Summary:
- Apple juice administration reduced plasma PFOS concentrations at 2 hours and 5 hours after dosing to 56% and 65% of the PBS controls
- Apple juice administration reduced liver PFOS concentrations at 2 hours and 5 hours after dosing to 56% and 39% of the PBS controls
- Apple juice administration reduced kidney PFOS concentrations at 2 hours and 5 hours after dosing to 43% and 53% of the PBS controls
- Apple juice administration reduced intestine PFOS concentrations at 2 hours and 5 hours after dosing to 38% and 82% of the PBS controls

CONCLUSION

- Administration of 0.5 mg/kg PFOS to mice resulted detectable levels in plasma, liver, kidney, and intestine at 2 and 5 hours post administration. PFOS levels in plasma, liver, kidney and intestine were higher at 5 hours compared to 2 hours, which is previous observations.
- Administration of 4x AJE concentrate resulted in lower plasma, liver, kidney, and PFOS levels at 2 and 5 hrs post PFOS administration. 4x AJE concentrate administration lowered intestinal PFOS concentrations at 2 hr post administration.
- Overall, this study is demonstrating a potential utility of AJE to block PFOS absorption in mice and suggests that Oatp2b1 inhibition may be a critical mechanism to interfere with PFAS absorption via the gut.
- This study demonstrates the potential utility of blocking Oatp2b1 activity as a molecular mechanism to decrease PFAS absorption by the GIT.

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