Regulatory Toxicology and Pharmacology 63 (2012) 505–508

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Letter to the Editor

Sucralose revisited: Rebuttal of two papers about Splenda safety

Two critiques were published in Regulatory Toxicology and Pharmacology (Grotz and Munro, 2009; Brusick et al., 2009) subsequent to our publication (Abou-Donia et al., 2008). In our controlled study, Splenda (comprised of the organochlorine sweetener, sucralose and the fillers maltodextrin and glucose) was administered at: 1.1, 3.3, 5.5 and 11 mg/kg/day sucralose; control: 0 mg/kg/day in water, over 12 weeks. These dosages were chosen based on doses approved by the FDA (US FDA, 1998) and the European Union (EU, 2004). Half of the animals were sacrificed at 12 weeks and half after 12 weeks of recovery without Splenda. We reported:

(a) Increased levels of the efflux transporter P-glycoprotein (P-gp) and of two cytochrome P450 isozymes (CYP3A4 and CYP2D1) that remained elevated after the 12-week recovery from the higher Splenda dosages.
(b) Decreased numbers of total anaerobes, bifidobacteria, lactobacilli, *Bacteroides*, clostridia, and total aerobic bacteria, but no significant effect on enterobacteria. Number of anaerobes was still depressed after 12-weeks recovery.
(c) Increased fecal pH during treatment that remained elevated after recovery from the three highest doses.
(d) Histopathological findings in gastrointestinal tract (GIT) including lymphocytic infiltrates into epithelium, epithelial scarring, mild depletion of goblet cells and glandular disorganization in the colon.

The magnitude of the concerted increase in expression of intestinal P-gp and cytochrome P450 (CYP) isozymes suggested that consumption of Splenda could reduce the bioavailability of therapeutic drugs and result in adverse drug interactions (Dürr et al., 2000; Benet, 2009).

Listed below are our responses to 14 claims and criticisms (labeled Critique 1–14) raised by Grotz and Munro and Brusick et al. Both articles were both authored by individuals with relationships to McNeil Nutritionals, the marketer of Splenda, and published consecutively in the same issue of Regulatory Toxicology and Pharmacology.

Critique 1: Sucralose "is stable in vivo" and "eliminated unchanged in the feces"

Our response: This claim is not supported by published thin layer chromatography (TLC) data. Previous studies found multiple peaks in TLC radiochromatograms from methanolic fecal extracts of ^{14}C sucralose-treated rats (Sims et al., 2000) and humans (Roberts et al., 2000). Fig. 1 is a photograph from Sims et al. that shows multiple closely eluting peaks of approximately equal height from a rat methanolic fecal extract after an oral dose of ^{14}C sucralose. The presence of multiple peaks in the trace indicates that at least two radioactive chemicals were extracted from the fecal material, i.e. sucralose is indeed metabolized in the GIT. While the chemical identity and toxicity of these metabolites of sucralose in mammals have not yet been established, previous reports have shown that metabolic byproducts of sucralose produced by microorganisms include 1,6-dichloro-l,6-dideoxy-D-fructose (a weak mutagen) and an unsaturated aldehyde of sucralose (Labare and Alexander, 1994). The TLC data are consistent with our finding of increased expression of GIT cytochrome P450 metabolic enzymes.

Critique 2: Sucralose does not induce cytochrome P450 family of enzymes

Our response: Unpublished non-peer reviewed laboratory data on hepatic CYP (Hawkins et al., 1987) were cited to support the lack of cytochrome P450 induction. However, these data lacked methodological descriptions and corroborative data in the open scientific literature. Furthermore, hepatic CYP-mediated metabolism does not predict intestinal CYP-mediated metabolism because catalytic activities in the liver and GIT are independently regulated (Lown et al., 1994). Our study demonstrated dose-related increases in intestinal CYP3A4 and CYP2D1 protein expression subsequent to Splenda. (CYP2D1 is the rat analog of human CYP2D6 (Laurenzana et al., 1995)). The increased CYP3A4 and CYP2D1 expression likely results from "autoinduction" by which sucralose enhances it own metabolism. Xenobiotics often induce proteins involved in their own detoxification (Schuetz et al., 1996). Our results are significant because: (a) most ingested sucralose reportedly remains in the gut (US FDA, 1998), and (b) increased expression of intestinal CYP by sucralose can significantly alter the bioavailability of concomitantly administered drugs.

Critique 3: Increases in Cytochrome P450 levels were only normal biological variations

Our response: We observed linear and dose-dependent increases in intestinal CYP3A4 and CYP2D1 expression that were statistically significant, indicating that increased protein levels were not random. CYP3A4 is co-localized with P-gp in enterocytes, and there is extensive overlap in substrate specificities and co-inducibility of CYP3A4 and P-gp (Benet and Cummins, 2001). Thus

0273-2300/$ - see front matter
http://dx.doi.org/10.1016/j.yrtph.2012.05.002

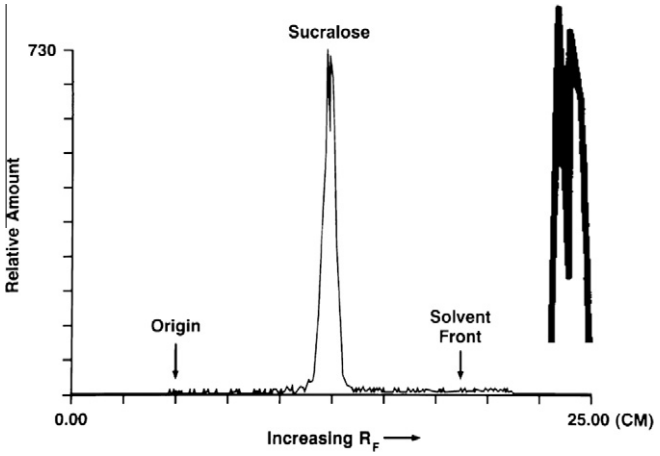

Fig. 1. Thin-layer radiochromatographic profile of a methanolic fecal extract from a male rat that received an oral dose of ^{14}C-sucralose (100 mg/kg) (from Fig. 2b, Sims et al., 2000 with permission). An enlargement of the multiple peaks in the profile is given to the right.

CYP3A4 and P-gp act together as a concerted barrier to absorption of xenobiotics (e.g. sucralose, drugs, pesticides) in the small intestine. In the case of sucralose, we found that Splenda containing sucralose at doses of 3.3 and 5.5 mg/kg/day significantly increased the expression of both CYP3A4 and P-gp. The combined effect of CYP3A4 and P-gp induction greatly increases the probability of first pass metabolism of sucralose by CYP3A4 in the gut because it enhances the pattern of repeated cycling into and out of the gut enterocytes via passive diffusion and active efflux by P-gp (Benet and Cummins, 2001; Benet, 2009). The concerted induction of CYP3A4 and P-gp by sucralose also has the potential to limit the intestinal absorption of drugs that are substrates of CYP3A4 which constitute ~50% of clinically used therapeutics (Gonzalez and Tukey, 2006).

Critique 4: CYP3A4 and CYP2D1 protein expression was measured in the colon

Our response: This critique is incorrect. As stated in our methodology, measurements were made in the distal regions of the small intestine (i.e. the jejunum and ileum), not the colon.

Critique 5: P-gp is not involved in the disposition of sucralose because the response was not dose-dependent

Our response: This critique has no scientific merit. As the dosage of sucralose increased from 3.3 to 11 mg/kg/day, there was a monotonic decrease in the mean values for P-gp expression. Conversely, as the dosage of sucralose increased from 3.3 to 11 mg/kg/day, there was a monotonic increase in the mean values for CYP3A4 and CYP2D1 expression. See Critique 3 for a discussion of the interplay between the expression of P-gp and CYP. One possible explanation for the significantly reduced expression of P-gp at the 11 mg/kg/day dosage is that sucralose is predominantly metabolized at elevated dosages due to induction of intestinal CYP; that is, sucralose no longer exists as an intact molecule at a concentration sufficient to increase P-gp expression.

Critique 6: P-gp expression has no effect on nutrients

Our response: This critique is incorrect. Published data indicate that P-gp interacts with lipids (Eckford and Sharom, 2005), cholesterol (Eckford and Sharom, 2008), phytonutrients and other food nutrients (Deferme and Augustijns, 2003).

Critique 7: Sucralose is a small molecule with low potential for chemical reactivity

Our response: Small molecules can be reactive and are not immune from metabolism. The TLC radiochromatograms of Sims et al. (2000) and Roberts et al. (2000) indicate that sucralose is metabolized. See our response to Critique 1.

Critique 8: Sucralose is stable at elevated temperatures

Our response: Barndt and Jackson (1990) performed a radiolabeled baking study and claimed that sucralose is a heat stable compound that minimally degrades in baked goods (e.g. cakes, cookies and graham crackers). However, the data reported in this McNeil publication do not support a "heat-stable" conclusion because the TLC profiles of the extracts (e.g. from cookies) in the Barndt and Jackson paper reveal multiple closely eluting peaks. Rahn and Yaylayan (2010) noted that the findings of Barndt and Jackson "lack credibility due to inefficient separation technique used in analysing the degradation products." In contrast to the conclusions of Barndt and Jackson, three independent laboratories in the United States (Hutchinson, 1996; Hutchinson et al., 1999), Canada (Yaylayan, 2009; Rahn and Yaylayan, 2010), and Brazil (Bannach et al., 2009) have concluded that sucralose is not heat stable but rather undergoes thermal decomposition. Furthermore, Rahn and Yaylayan (2010) urged caution regarding use of sucralose in baked products containing glycerol or lipids due to the production of potentially toxic chloropropanols.

Critique 9: Sucralose is highly water-soluble and not expected to be bioaccumulative

Our response: Sucralose is an amphiphilic molecule as reported by the discoverers of sucralose (Hough and Khan, 1978); therefore it is soluble in water, lipids, and non-polar solvents. The fact that sucralose has lipophilic as well as hydrophilic properties is illustrated by the following. First, the sucralose molecule is comprised of hydrophobic domains (–C–CH_2Cl) as well as hydrophilic domains (hydroxyl groups). Second, sucralose is readily soluble in methanol and ethanol (Li et al., 2010). Third, the extraction of sucralose from excreta required laboratory solvents commonly utilized to extract lipid components from mixtures such as methanol, butanol, diethylether, ethyl acetate, and ethylmethylketone (see Sims et al., 2000; Roberts et al., 2000 for description of sucralose extraction procedures). Fourth, sucralose must diffuse through the phospholipid membrane bilayer of enterocytes to interact with P-gp and CYP isozymes. This explains why compounds that interact with both P-gp and CYP are either amphiphilic or lipophilic (Wang et al., 2002). Fifth, the lipophilic as well as hydrophilic properties of sucralose were acknowledged by the US FDA (1998) and the EU (2004) since these regulatory bodies gave their approval for the use of sucralose in both fat- and water-based products.

Critique 10: Decreases in bacterial counts were normal variation

Our response: The decrements in bacterial counts were not due to random variation but rather showed a methodical pattern of continued reduction over the 12-week period of daily sucralose dosing. The decreases at Week 12 were highly statistically significant, e.g. for *Bacteroides*, the decreases were statistically significant at $p < 0.00001$ for all four sucralose doses. Furthermore, the burgeoning scientific literature on probiotics suggests that the magnitude of the change in bacterial counts from sucralose delivered in Splenda is biologically significant, i.e. the magnitude of the