Short Communication

Calorie Restriction Increases P-Glycoprotein and Decreases Intestinal Absorption of Digoxin in Mice

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ABSTRACT

There is wide variation in how patients respond to therapeutics. Factors that contribute to pharmacokinetic variations include disease, genetics, drugs, age, and diet. The purpose of this study was to determine the effect of calorie restriction on the expression of Abcb1a in the intestine and whether calorie restriction can alter the absorption of an Abcb1a substrate (i.e., digoxin) in mice. Ten-week-old C57BL/6 mice were given either an ad libitum diet or a 25% calorie-restricted diet for 3 weeks. To determine digoxin absorption, mice were administered [3H]-labeled digoxin by oral gavage. Blood and intestine with contents were collected at 1, 2, 4, and 12 hours after digoxin administration. Concentrations of [3H]-digoxin in plasma and tissues were determined by liquid scintillation. Calorie restriction decreased plasma digoxin concentrations (about 60%) at 1, 2, and 4 hours after administration. Additionally, digoxin concentrations in the small intestine of calorie-restricted mice were elevated at 4 and 12 hours after administration. Furthermore, calorie restriction increased Abcb1a transcripts in the duodenum (4.5-fold) and jejunum (12.5-fold). To confirm a role of Abcb1a in the altered digoxin pharmacokinetics induced by calorie restriction, the experiment was repeated in Abcb1a/b-null mice. Thus, these findings support the hypothesis that calorie restriction increases intestinal Abcb1a expression, leading to decreased absorption of digoxin in mice. Because Abcb1a transports a wide variety of therapeutics, these results may be of important clinical significance.

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ABBREVIATIONS: CYP, cytochrome P450; P-gp, P-glycoprotein; qPCR, quantitative polymerase chain reaction.
Materials and Methods

Chemicals. Digoxin (0.25 mg/ml) was purchased from Westward Pharm Corp (Eaton, NJ). RNA-Be RNA Isolation Reagent was purchased from Tel-Test Inc. (Friendswood, TX), the High Capacity Reverse Transcriptase kit and SYBR Green were purchased from Applied Biosystems (Foster City, CA). [3H]-digoxin and Ultima Gold counting fluid were purchased from PerkinElmer (Shelton, CT), and bovine serum albumin was purchased from Amresco (Solon, OH). Unless otherwise stated, all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Animals. C57BL/6 male mice, eight weeks of age, were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Abcb1a/b-null mice were purchased from Taconic Inc. (Hudson, NY), and were back-crossed into the C57BL/6 background (>99% congenicity). Mice were bred and housed in a light-, temperature-, and humidity-controlled environment in an Association for Assessment and Accreditation of Laboratory Animal Care (Frederick, MD) accredited animal housing facility at the University of Kansas Medical Center. All studies were approved by the University of Kansas Medical Center’s Institutional Animal Care and Use Committee.

Calorie Restriction. After 2-week acclimatization, mice were housed individually and given laboratory Rodent Chow 8604 (Harlan, Madison, WI), either ad libitum or with 75% of the feed consumed by ad libitum feeding (calorie restriction). The average ad libitum daily consumption of feed was 4 g per mouse (determined using the average daily intake from 10 mice). Thus, mice in the calorie restriction group were given approximately 2.7–3.0 g of feed per day. Mice remained on these diets for 3 weeks, after which they were either used for RNA studies or digoxin absorption studies.

RNA Extraction. RNA was isolated from frozen intestine using RNA Isolation Reagent RNA-Be following the manufacturer’s protocol (Tel-Test Inc.). Using a NanoDrop1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), RNA concentrations were quantified at a wavelength of 260 nm.

Messenger RNA Quantification [Reverse Transcription Quantitative Polymerase Chain Reaction (qPCR)]. To perform real-time polymerase chain reaction, we first reverse transcribed RNA to cDNA using an Applied Biosystems High Capacity Reverse Transcriptase kit. In brief, equal volumes of 2X reverse transcriptase, 50 ng/μl RNA, and random primers were mixed and placed in a Mastercycler (Eppendorf, Hauppauge, NY) under the following conditions: 25°C for 10 minutes, 37°C for 120 minutes, and 85°C for 5 minutes. With the resulting cDNA, qPCR was performed as detailed subsequently. The primers for qPCR of Abcb1a (GenBank Accession No. NM_011076) were designed using Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast) (forward, GCCAATCCTGGATACATGGTT; reverse, ACCCTGTAGCCCCTTTCATC) and were synthesized by Integrated DNA Technologies (Coralville, IA). For qPCR, the following were contained per reaction in a 384-well plate (Applied Biosystems): 2.5 μl of 3 μM forward and reverse primer mix, 5 μl of Applied Biosystems SYBR Green PCR master mix, 0.5 μl RNAase-free H2O, and 2 μl of 2 μg/μl cDNA. Fluorescence was quantified with an Applied Biosystems 7300 Real Time PCR System under the following conditions: 50°C for 2 minutes and 95°C for 10 minutes (95°C for 15 seconds, 60°C for 1 minute) × 40 cycles. To ensure primer specificity, melt curves were performed for every reaction. To determine the relative mRNA expression, the comparative ΔΔCt method was applied using 18S as a reference transcript. Values from caloric-restricted mice were normalized to values from mice fed ad libitum.

Digoxin Absorption Experiments. The protocol followed for these experiments was adapted from an earlier study (Mayer et al., 1996). Each mouse was given 0.2 mg/kg digoxin by oral gavage, labeled with [3H]-digoxin (1 μCi/30g b.w.t.). Wild-type mice were euthanized 1, 2, 4, or 12 hours after digoxin administration, and Abcb1a/b-null mice were euthanized 4 hours after digoxin administration. Blood was collected by orbital bleeding into heparinized tubes and centrifuged for 10 minutes at 2000g to isolate plasma. Plasma (100 μl) was transferred to 4 ml Ultima Gold high counting efficiency scintillation cocktail. The entire intestine from stomach to rectum (including fecal matter) was also collected, weighed, and homogenized in 4% (w/v) bovine serum albumin. Thus, all intestine samples included the fecal matter. Henceforth, we will refer to the intestine + contents samples as intestine. Two-hundred μl of intestine homogenate was added to 4 ml Ultima Gold high counting efficiency scintillation cocktail. Radioactivity was quantified by liquid scintillation counting using a Packard Tri-Carb 2100TR Liquid Scintillation Analyzer (Packard Instrument Company, Meriden, CT). Concentrations of digoxin were calculated from a standard curve.

Statistics. Statistical differences between ad libitum and calorie restriction were determined using an unpaired, two-tailed Student’s t test. For the RNA expression data (Fig. 1), the individual values were log transformed to obtain the normal distribution before performing the t test. Comparisons resulting in P < 0.05 were considered statistically significant. All data are presented as the mean ± S.E.M.

Results

Calorie Restriction Decreases Digoxin Absorption in the Small Intestine. The effect of calorie restriction on mRNA expression of Abcb1a in the small intestines of mice was assessed by reverse transcription qPCR (Fig. 1). Calorie restriction significantly (P < 0.05) increased expression of Abcb1a mRNA in the duodenum (4.5-fold) and jejunum (12.5-fold) compared with mice fed the ad libitum diet (control). Expression of Abcb1a was also increased in the ileum; however, it was not statistically different from the controls.

Calorie Restriction Alters Absorption of Digoxin in Mice. To determine whether calorie restriction alters absorption of drugs transported by Abcb1a in the intestine, mice were given [3H]-labeled digoxin and euthanized 1, 2, 4, and 12 hours after administration. The time courses of the intestine-digoxin and plasma-digoxin concentrations are presented Fig. 2. The intestine of calorie-restricted mice contained 1.5-fold more digoxin at 4 and 12 hours than the intestine of ad libitum fed mice. In contrast to the intestine, the plasma concentrations of digoxin were 61%, 69%, and 57% lower in the calorie-restricted mice than in the control mice at 1, 2, and 4 hours, respectively.

Calorie Restriction Does Not Alter Digoxin Absorption in Abcb1a/b-Null Mice. In mice fed ad libitum, compared with wild-type mice, Abcb1a/b-null mice had lower digoxin concentrations in their intestines (974 versus 265 ng digoxin/gram intestine) and higher digoxin concentrations in plasma (0.035 versus 0.100 mean ng digoxin/μl plasma) 4 hours after digoxin administration (Figs. 2 and 3). This result was as predicted because Abcb1a-null mice do not have the capacity to efflux digoxin via P-gp, thus resulting in increased digoxin absorption.

No statistical differences in the concentrations of digoxin in intestine or plasma were observed between ad libitum fed and calorie-restricted Abcb1a/b-null mice (Fig. 3). Thus, these results support the findings that decreased digoxin absorption in calorie-restricted wild-type mice is likely due to increased intestinal P-gp expression.

Discussion

In clinical practice, a challenging problem in drug therapy is individual variations in patient response. Environmental, genetic, and pathophysiological factors affect drug disposition, which is responsible for the variability in clinical response. In this study, we investigated the effects of calorie restriction on drug absorption in mice. Calorie restriction increased the mRNA expression of Abcb1a in the duodenum and jejunum, suggesting increased expression of P-gp, a membrane protein involved in drug efflux. Increased P-gp expression might lead to decreased drug absorption, as observed in the current study.

In conclusion, calorie restriction alters drug absorption by increasing the mRNA expression of Abcb1a, which might be a promising strategy for drug treatment. Further studies are needed to investigate the mechanisms underlying these changes and to validate the findings in clinical settings.
factors are known contributors to variations in drug responses and adverse effects; however, little attention has been given to the impact of food and diet on drug disposition. Thus, the results in this study help further our knowledge of how diet impacts drug absorption. Specifically, the current work revealed that calorie restriction can increase the expression of intestinal Abcb1a and decrease drug absorption. Because Abcb1a transports a wide variety of therapeutics, these results in mice beg the question of whether this phenomenon might also occur in humans.

The gastrointestinal tract functions to digest and absorb nutrients from the diet. However, this organ is also exposed to ingested xenobiotics, and thus also functions as a defense barrier, expressing many metabolic enzymes and efflux transporters. Modulation of efflux transporter expression or function in the intestinal tract can lead to altered systemic and local xenobiotic concentrations (Murakami and Takano, 2008; Huang et al., 2010). The best characterized efflux transporter is P-gp. It is located apically on enterocytes and transports substrates back into the intestinal lumen, resulting in lowered systemic drug concentrations. Increased intestinal P-gp function or expression can markedly affect drug pharmacokinetics, leading to decreased therapeutic effect. In humans, intestinal MDR1 mRNA is inversely correlated with oral tacrolimus concentrations (Masuda et al., 2004), cyclosporine pharmacokinetics (Masuda and Inui, 2006), and talinolol pharmacokinetics (Berndorf et al., 2006). Additionally, xenobiotics that alter the function of P-gp can cause drug-drug interactions; for example, St. John’s Wort induces intestinal P-gp, leading to decreased talinolol area under the curve in human subjects (Schwarz et al., 2007). There are many known therapeutics that inhibit P-gp, leading to drug-drug interactions including cyclosporine A, ketoconazole, quinidine, rifampin, and reserpine, to name a few (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/acm093664.htm). However, little is known about how nutrients in the diet or the amount of calories consumed affect P-gp activity or expression.

Factors in the diet currently suspected to affect P-gp activity include certain flavonoids. In vitro studies have shown that the flavonoids morin, biochanin A, silymarin, and phloretin all have the ability to increase intracellular daunomycin (a P-gp substrate) concentrations in human P-gp positive cells but not in P-gp negative cells. Furthermore, the increase in daunomycin accumulation by these flavonoids is dependent on both flavonoid concentration and P-gp expression, suggesting that these flavonoids inhibit P-gp activity (Zhang and Morris, 2003). Thus, one would anticipate that these flavonoids would increase the absorption of drugs; however, in vivo, biochanin A failed to alter the pharmacokinetics of orally administered P-gp substrates (Zhang et al., 2010). The authors conclude that the disconnect between the in vitro and in vivo data may be due to poor bioavailability and rapid clearance of biochanin A in vivo. It remains to be determined whether flavonoids have the ability to inhibit P-gp in vivo.

Grapefruit juice and orange juice are known to inhibit CYP3A4 and are also suspected to alter P-gp activity (reviewed in Won et al., 2010). Grapefruit juice is well known to increase plasma concentrations of drugs by decreasing CYP3A4, and possibly also by inhibiting P-gp and organic anion-transporting polypeptide activity. Thus, considering that many P-gp substrates are also CYP3A substrates, it is difficult to distinguish the contribution of each factor in grapefruit juice–induced altered drug pharmacokinetics.

The molecular mechanism of how calorie restriction causes an induction of P-gp remains unknown at the present time. In vitro studies using cells derived from intestinal tissues have indicated the pregnane-X receptor as a key player in drug-induced P-gp expression (Maier et al., 2007; Kim et al., 2015). For example, rifampin induces P-gp via casein kinase 2-mediated phosphorylation of heat shock protein 90B, and subsequent stabilization of the pregnane-X receptor (Kim et al., 2015). Thus, increased activation of the pregnane-X receptor might be a good candidate to investigate as a possible mechanism of calorie restriction–induced P-gp expression.

The present study used the P-gp substrate digoxin as an indicator of P-gp activity. This drug is commonly used to evaluate P-gp function in mice because the pharmacokinetic attributes of digoxin are highly P-gp dependent. Additionally, another advantage of using digoxin is that it is not significantly metabolized in mice (Schinkel et al., 1995, 1997; Mayer et al., 1997; Kawahara et al., 1999). Although it is possible that digoxin is transported by other transporters (Taub et al., 2011), our observation was that calorie-restricted Abcb1a/b-null mice did not have
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