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Impact of diet on irinotecan toxicity in mice

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ABSTRACT

Irinotecan is highly effective in the treatment of metastatic colorectal cancer as well as many other cancers. However, irinotecan is known to cause severe diarrhea, which pose significant problems in patients undergoing irinotecan based chemotherapy. Dietary and herbal components have shown promise in improving gastrointestinal health. Therefore, we compared the effect of grain-based chow diet containing phytoestrogens and corn/alfalfa as fat source to purified diets containing either animal-derived fat source (lard) or plant-derived fat source (soybean oil) on irinotecan-induced toxicities in mice. The concentration of the toxic metabolite, SN-38, was measured in the serum, and the activity of main enzyme, carboxylesterase (CEs) involved in biotransformation of irinotecan to SN-38 formation was measured in the liver. We found that the grain-based diet was protective against irinotecan-induced diarrhea. Interestingly, purified diet containing lard caused fatty liver in mice, while grain-based chow diet containing corn/alfa-alfa or purified diet with soybean oil did not cause fat deposition in the liver. Serum SN-38 concentration was significantly higher in the mice fed with purified diets compared to the chow-fed mice. Hepatic CEs activity was induced in the presence of irinotecan in mice on purified diets, but not chow diet. These results indicate that components of grain-based natural diet (presumably phytoestrogens and/or the macronutrients balance) compared to purified diets may have a beneficial effect by controlling the adverse effects of irinotecan in cancer patients.

1. Introduction

The topoisomerase inhibitor, irinotecan (CPT-11, Camptosar®), is approved by the FDA to treat metastatic colorectal cancer and is also used in many other cancers. Although irinotecan is highly effective, its use is severely limited due to its adverse effects [1]. Irinotecan causes diarrhea in ~88% patients, and up to 40% patients experience severe late onset (grade 3–4) diarrhea which can be life threatening [2–6]. Irinotecan-based chemotherapy also causes non-alcoholic fatty liver disease (NAFLD), that worsens postoperative outcome like morbidity and mortality in colorectal liver metastases (CLM) patients [7,8].

Understanding of irinotecan's complex pharmacology and metabolism has provided clues to the etiology of irinotecan-induced diarrhea. Irinotecan is a prodrug that is converted to the active form, 7-ethyl-10-hydroxycamptothecin (SN-38), by carboxylesterases (CEs). SN-38 is further metabolized by glucuronosyltransferases (UGTs) to inactive SN-38 glucuronide (SN-38G) [9], which can be deconjugated back to SN-38 by bacterial β-glucuronidases in the gut. Both the parent compound and metabolites undergo enterohepatic circulation and accumulation of toxic SN-38 in blood and intestine due to this process has been correlated with diarrhea in humans [3,10,11].

Significant effort has been made to reduce severity of diarrhea with irinotecan. Different strategies have been tested in preclinical models and clinical studies including dose modification, intestinal alkalization, structural modification, pharmacological therapies, drug metabolizing enzymes and transporter inhibitors, probiotics, antibiotics, and other miscellaneous agents [12–18]. Currently, loperamide, an anti-diarrheal drug, is being used to control diarrhea associated with irinotecan [19,20]. Although, high dose of loperamide is moderately effective in uncomplicated diarrhea, its utility as monotherapy for severe diarrhea is limited [21]. Therefore, new approaches to prevent intestinal toxicity of irinotecan-based regimens remains an on-going effort. At present,

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Table 1
Major components in the diets used in the experiment [40,44–46].

Compositions		Chow	PD-1	PD-2
Type		Grain/cereal based diet	Purified diets	
Protein	Kcal (%)	20	20.	20
	Source	Multiple[a]	Casein, Lactic, 30 Mesh	
Carbohydrate	Kcal (%)	64	64	70
	Source	Soya bean, plant	Corn starch, sucrose	
	Sucrose (%)	0	8	35
	Fiber	High (4X) fiber content: cellulose, dextrins, inulin, lignin, waxes, chitins, pectins, beta-glucans and oligosaccharides	Low fiber content 5% cellulose	
Fat	KCal %	13	16	10
	Source	Ground wheat/corn/oats/alfalfa (Veg)	Soya bean oil (Veg)	Lard (Animal)
	Saturated (g/100 g)	15	16	39
	Monosaturated (g/100 g)	28	23	45
	Polyunsaturated (g/100 g)	55–68	58	11
phytoestrogens	–	[b]Isoflavones (398 Aglycone Units, AU)	Not detected	

[a] Complete ingredient contents that are source of protein and others can be found in the diet specific sheets.
[b] Data on phytoestrogen content was provided by Lab diet (personal communication).

there are no interventions to counteract the NAFLD caused by irinotecan, and preoperative assessment for significant liver pathology with the currently available tools is a challenging clinical problem. Therefore, strategies need to be developed to reduce/prevent irinotecan gut and liver toxicity to improve therapeutic outcome in patients.

Specific dietary elements have attracted attention towards improving the therapeutic outcomes of chemotherapeutic agents by reducing toxicities. In this context, n-3 polyunsaturated fatty acids (PUFA) eicosapentaneoic acid, and prebiotic oligosaccharides have been proposed to be potential modulators of gut injury related to cancer chemotherapy [22–24]. Significant interest has been given towards prebiotics for managing diarrheas involving disruption of intestinal flora balance. Also, dietary monounsaturated fatty acids (MUFAs) when replaced with dietary carbohydrate and saturated fat, have shown to prevent the development of NAFLD [25]. Several studies also support the protective effects of n-3 polyunsaturated fatty acids (PUFAs) in NAFLD [26,27]. Natural phenols, including resveratrol and anthocyanins have shown positive effects on animal NAFLD models [28], however their benefits in patients with NAFLD either have failed to show any significantly improvements [29] or has not yet been well elucidated [30].

Dietary compounds improve the efficiency of cytotoxic agents [31] and alleviate adverse effects [31–33]. Therefore, nutritional approach to reduce adverse side effects and improve efficacy of irinotecan by reducing its dose-limiting intestinal toxicity has been of prime interest recently. Dietary supplementation with the non-essential amino acids, glutamine showed improvement in irinotecan-induced grade 4 diarrhea in small study series with five patients [34]. In rats, glutamine treatment reduced the incidence of severe diarrhea induced by irinotecan [35]. Likewise, reduced apoptosis in the crypts of the small intestine with irinotecan was observed in mice fed with 3% AAFA, a omega-3 polyunsaturated fatty acid product containing a high concentration of long chain fatty acids [36,37]. In addition, these mice were also protected against irinotecan-induced liver hypertrophy (enlargement of the liver) [36]. However, optimal strategies to translate these findings into clinical care remain to be elucidated.

In our current study, we tested the influence of grain-based and purified diets on irinotecan associated toxicities. Our study revealed a diet-dependent toxic effect of irinotecan, as the grain based chow-fed mice were protected from irinotecan-induced toxicities, whereas mice on purified diets that are non-grain based developed toxicities with irinotecan.

2. Materials & methods

2.1. Chemicals

Camptothecin (CPT; internal standard (I.S)), 7-ethyl-10-hydroxycamptothecin (SN-38), p-nitrophenyl-β-D-glucuronide (Cat # N1627), and 4-Nitrophenyl acetate (PNPA; Cat # N8130) were purchased from Sigma-Aldrich (St. Louis, MO). Irinotecan hydrochloride (NDC-0703-4437-11) for injection was procured from Martin surgical (Houston, TX). SN-38G was prepared in our lab using cell lines we previously reported [38]. Acetonitrile and waters (LC-MS grade) were purchased for VWR international, LLC (Suwanee, GA, USA). Unless specified, all other materials were purchased from Sigma-Aldrich (St Louis, MO, USA.).

2.2. Animals and diets

The C57BL/6 J mice (male, 12-weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME). Three different diets were used in the current study, primarily categorized as grain/cereal based and purified ingredients based; (a) Chow diet (#0007688); (b) American Institute of Nutrition modified (AIN) 93G (# D10012G); and (c) Low fat diet (# D12450B). Chow diet was purchased from Pico Lab and purified diet from Research Diets, Inc. For understanding the basic differences among the diets used, only the major components of these diets are listed in Table 1 but the breakdown of ingredients for each of the diet can be found in product specific sheets. Chow diets are grain or cereal based and typically contain ingredients such as ground corn, ground oats, alfalfa meal, soybean meal and ground wheat. Chow diets are 'closed' formulas, thus exact amount of each ingredient is mostly unknown [35,39]. In comparison to chow diet, the purified diets are considered open formulas and have well-characterized definition of the lipid, protein, and carbohydrate constituents. American Institute of Nutrition (AIN) formulation, AIN-76 A is commercial source for semi-purified diets and AIN-93G used in our study is redefined AIN-76 A formulation [40] and is referred throughout the manuscript as purified diet 1 (PD-1). Low-fat diet is control diet used as matched controls for purified high-fat diet [41] and has defined, and flexible formulation similar to AIN-93G. However, this low-fat purified diet predominantly differs from AIN-93G in lard being the source of fat instead of soybean oil (Table 1). Throughout the manuscript, this low-fat diet is referred as purified diet 2 (PD-2). A major difference between grain-based chow diet and purified diets is that, they differ greatly in their phytoestrogen content. Though the exact amount of phytoestrogen is unknown for chow diet, this grain-based diet is primarily made with ground plants