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Irinophore C™, a lipid nanoparticulate formulation of irinotecan, improves vascular function, increases the delivery of sequentially administered 5-FU in HT-29 tumors, and controls tumor growth in patient derived xenografts of colon cancer

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ABSTRACT

Purpose: A liposomal formulation of irinotecan, Irinophore C™ (IrC™) is efficacious in a panel of tumor models, normalizes tumor vasculature, and increases the accumulation of a second drug in the same tumor. We now show that Irinophore C™ is also effective against patient derived xenografts (PDX) of colon cancer, and examine the kinetics of vascular normalization in the HT-29 tumor model and assess how these changes might be used with 5-FU sequentially.

Materials and methods: Rag2M mice bearing HT-29 tumors were treated with IrC™ (25 mg/kg; Q7D × 3) for up to three weeks. Groups of tumors were harvested for analysis at 7, 14 and 21 days after the start of treatment. Drug and lipid levels in the tumor were evaluated using HPLC and scintillation counts, respectively. Changes in tumor morphology (H&E), vasculature (CD31), perfusion (Hoechst 33342) and apoptosis (TUNEL) were quantified using microscopy. The accumulation of a second drug ([^{14}C]-5-FU, 40 mg/kg) given 3 h before sacrifice was determined using liquid scintillation. The efficacy of IrC™ (Q7D × 3) followed by 5-FU treatment (Q7D × 3) was assessed in mice bearing established HT-29 tumors. The efficacy of IrC™ was also evaluated in primary human colorectal tumors grown orthotopically in NOD-SCID mice.

Results: Following a single dose of IrC™ the active lactone forms of irinotecan and its metabolite SN-38 were measurable in HT-29 tumors after 7 days. The treatment reduced tumor cell density and increased apoptosis. Hoechst 33342 perfusion and accumulation of [^{14}C]-5-FU in the treated tumors increased significantly on days 7 and 14. This was accompanied by an increase in the number of endothelial cells relative to total nuclei in the tumor sections. Pre-treatment with IrC™ (Q7D × 3) followed by 5-FU (Q7D × 3) delayed the time taken for tumors to reach 1 cm^3 by 9 days ($p < 0.05$). IrC™ was just as effective as free irinotecan when used on patient derived xenografts of colorectal cancer.

Conclusions: Treatment with IrC™ reduces tumor cell viability and appears to normalize the vascular function of the tumor after a single treatment cycle. A concomitant increase in the accumulation of a second drug (5-FU) in the tumor was observed in tumors from IrC™ treated animals and this was correlated with changes in vascular structure consistent with normalization. The treatment effects of sequential 5-FU dosing following IrC™ are additive with no additional toxicity in contrast to previous studies where concurrent 5-FU and IrC™ treatment exacerbated 5-FU toxicity. The studies with PDX tumors also indicate that IrC™ is just as effective as free irinotecan on PDX tumors even though the delivered dose is halved.

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1. Introduction

Treatment regimens for colorectal cancer are based on 5-fluorouracil (5-FU), which together with leucovorin, is used in combination with irinotecan or oxaliplatin [1–3]. These combinations have increased median overall survival to 20–24 months from 6 months with best supportive care. However, the consensus is that a treatment plateau has been reached with the 5-FU, leucovorin, oxaliplatin or irinotecan triad [1,4]. Inherent or acquired drug resistance and adverse drug effects are contributing factors to treatment failure, and adding another cytotoxic agent to this triad will likely exacerbate existing systemic toxicities. The addition of targeted therapeutics, such as agents targeting EGFR to these regimens has therapeutic value [5], but is limited to a subset of patients with KRAS wild-type tumors [6].

In humans, free irinotecan is metabolized by carboxylesterases into SN-38, a drug that is considered more potent than the parent compound [7,8]. Both compounds inhibit topoisomerase I and cause DNA strand breaks during replication which lead to cell death. The lactone forms of irinotecan and SN-38 are therapeutically active. However, under physiological conditions the lactone forms of irinotecan and SN-38 are hydrolyzed by esterases into their biologically inactive carboxylate forms [9,10] thereby reducing their biological availability. High levels of gastrointestinal carboxylesterases in organs such as the gut can result in toxic concentrations of SN-38 that severely damages the intestinal mucosa [11,12]. The resulting diarrhea can be so severe that it is life-threatening or dose-limiting [9,13,14]. The therapeutic efficacy of free irinotecan is thus unfortunately hampered by localized toxicity and limited biological availability.

Our group has shown that encapsulating irinotecan in liposomes with an unbuffered, acidic interior which contains copper (IrC™) helps to maintain the drug in its active lactone form in vivo [15]. This formulation makes more of the active lactone forms of irinotecan and SN-38 available over extended time periods in the blood, and the change in pharmacokinetics is associated with lower irinotecan toxicity and a significant increase in anti-tumor efficacy [16,17]. IrC™ is more efficacious than the free drug in several tumor models including colorectal cancer models [15,18–21]. Irinotecan associated toxicities are markedly reduced in mice; doses equivalent to 350 mg/kg irinotecan in the liposomal form can be administered to immune competent mice without side-effects in contrast to a maximum tolerated dose (MTD) of only 80 mg/kg for free irinotecan [18]. Pre-clinical studies carried out by our group show very specifically in a rat model of late onset diarrhea that IrC™ is less GI toxic then free irinotecan (Waterhouse, private communication). IrC™ therefore has immediate advantages over the free drug since the formulation reduces toxicity in the gut, while prolongation of the active lactone forms in liposomes helps increase exposure of the active drug to the tumor. Further studies in colorectal cancer and glioma tumor models further suggest that tumor perfusion and accumulation of a second drug is improved following treatment with IrC™ treatment [19–21]. Vascular changes in the glioma model also indicate that IrC™ treatment is associated with blood vessel normalization [21] . Vascular normalization has not been observed previously when using irinotecan and this may represent a unique mechanism of action for the IrC™ product.

Recognizing that IrC™, if approved for clinical use, will be used in combination with other drugs, our research team has examined the therapeutic effects when IrC™ and 5-FU are administered concurrently [22]. The data show that any therapeutic potential of the combination is compromised by an unexpected increase in toxicity [22]. The reasons for the enhanced toxicity are not well-understood although it may be due to IrC™ treatment prolonging the circulation lifetime of 5-FU [22]. The observed improvements in tumor perfusion following IrC™ treatment [20,21,23] suggest the possibility that combination treatments with IrC™ and 5-FU might be more efficacious if 5-FU was administered after IrC™ treatment in a sequential, rather than concurrent, manner. We hypothesize that vascular normalization engendered by IrC™ will improve tumor perfusion, increase the accumulation of 5-FU in the tumor and subsequently improve its therapeutic efficacy.

The data presented here show that a single dose of IrC™ is sufficient to improve vascular function in tumors, and that this correlates with enhanced 5-FU accumulation in tumors. Subsequent efficacy studies suggest that sequential administration of 5-FU after IrC™ treatment, in contrast to concurrent administration, is well tolerated, and resulted in at least additive therapeutic effects without additional toxic effects. We also show that doses of IrC™ (equivalent to 25 mg/kg of irinotecan) are as active as free irinotecan (given at 40 mg/kg) against patient derived xenografts (PDX) of colorectal cancer. The data also show that IrC™ is also active against a PDX tumor line that did not respond to free irinotecan.

2. Materials and methods

2.1. Cell culture

All cell lines were tested to verify the absence of mycoplasma before use. The HT-29 human colon adenocarcinoma cell line (ATCC, Manassas, VA, USA) cells were derived from an original stock that was expanded in antibiotic free media, frozen and stored in liquid nitrogen. The cells are maintained in culture for no more than 20 passages and at that time are replaced with frozen stock. The HT-29 cells were cultured in McCoy's 5A with 1% L-glutamine, 1% penicillin/streptomycin and 10% fetal bovine serum (StemCell Technologies; Vancouver, CA). Plated flasks (BD Falcon, BD Sciences, Bedford, MA) were kept in a humidified incubator (5% CO_2, 37 °C) and stocks divided when ~80–90% confluent.

2.2. Irinophore C™

IrC™ was prepared as previously reported [16]. In brief, 1,2-distearoyl-*sn*-glycero-phosphocholine (DSPC; Avanti Polar Lipids, Alabaster, AL) and Cholesterol (Chol) were dissolved in chloroform with DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, Molecular Probes, Oregon). The mixture was spiked with ^{3}H-cholesteryl hexadecyl ether (^{3}H-CHE; Perkin Elmer Life Sciences; Boston, MA). The solvent was removed under vacuum to form a lipid film, which was rehydrated in 300 mM copper sulfate at 70 °C. The resulting multilamellar vesicles were then extruded 10× through polycarbonate membrane filters (100 and 80 nm pore size; Nuclepore, Pleasanton, CA) at 65 °C using a LIPEX™ extruder (Northern Lipids, Vancouver, BC, CA). The liposomes were subsequently incubated with A23187 (0.5 μg/mg lipid) followed by incubation with irinotecan (0.2 mol/mol lipid). Irinotecan concentration was determined by measuring UV–absorbance at 370 nm. The resulting product, IrC™, labeled with DiI and ^{3}H-CHE was used within 3 weeks. IrC™ is stable when maintained at 4 °C for >6 weeks where stability is assessed by no measureable change in product appearance, pH, liposome size, % encapsulated irinotecan, and irinotecan as well as DSPC and Chol content.

2.3. Animals

Studies were approved by the UBC's Institutional Animal Care Committee (IACC) which ensures that work conducted meets the standards defined by the Canadian Council for Animal Care. In the studies, animals were euthanized if tumors ulcerated or reached 1000 mm^3. Mice were also euthanized when their health status was compromised. A standard operating procedure, approved by the IACC, was used to provide comprehensive assessments of body weight, behavioral changes, appearance and level of activity. Mildly or markedly scruffed fur was scored 2 or 5, respectively. A hunched position was given a score of 2 or 5 depending on severity. Signs of lethargy were given a score of 2, 5 or 10 depending on severity. A 6–10% body weight loss (BWL) corresponded to a score of 2, 11–15% BWL corresponded to a score of 5 and 16 to 20%