Irinotecan and radiosensitization in rectal cancer

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Neoadjuvant radiation therapy with concurrent 5-fluorouracil-based chemotherapy is currently considered the standard of care for locally advanced rectal cancer. Pathologically complete response is a desirable outcome and has been associated with increased disease-free survival. There is a need to improve on this approach given that only approximately 10% achieve a pathologically complete response. Irinotecan has an established role in the treatment of metastatic rectal cancer. Both in-vitro and in-vivo data have shown promising radiosensitization properties. This study provides an overview of the published clinical trials evaluating the role of irinotecan as a radiosensitizer in the management of locally advanced rectal cancer. Although early-phase clinical trials initially showed promising results, this did not translate into improved outcome in a larger randomized phase II trial. Increased topoisomerase I expression has recently been identified as a possible predictive marker for improved response to irinotecan-based radiosensitization. This finding could help identify a subset of patients more likely to benefit from the addition of irinotecan in future trials. ***Anti-Cancer Drugs*** **22:324–329 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Current national guidelines for the management of T3–T4 or node-positive, non-metastatic, rectal cancer recommend neoadjuvant radiation therapy with concurrent 5-fluorouracil (5-FU)-based chemotherapy [1]. This recommendation is influenced by the results of a trial from the German Rectal Cancer Study group and the goal with this approach is to down stage the tumor, facilitate the subsequent surgical resection, and to decrease loco-regional recurrence rates [2]. Pathologically complete response (pCR) after completion of neoadjuvant therapy is a desirable outcome in this setting and has been associated with increased disease-free survival [3]. There is, however, a need to improve on this approach given that only 10% of patients achieve a pCR and one-quarter have no response or less than 25% tumor regression after completion of 5-FU-based neoadjuvant chemoradiotherapy [3].

Irinotecan has an established role in the treatment of metastatic colorectal cancer. The purpose of this study is to provide a review of the published literature evaluating the role of irinotecan as a radiosensitizer in the management of locally advanced rectal cancer.

Background and mechanism of activity for irinotecan

Camptothecin is an alkaloid extract from the Chinese tree, *Camptotheca acuminata*. This compound has documented antitumor activity, which is related to the strong inhibition of both DNA and RNA synthesis. Its severe toxicity profile, which included myelosuppression, cystitis, and diarrhea, precluded it from further development [4].

Irinotecan is a semisynthetic analog (7-ethyl-10-piperidino-piperidino-carbonyloxy derivative) of camptothecin, which is more water-soluble than the parent compound. The mechanism of action, metabolism, and toxicity profile have been reviewed extensively elsewhere [4–6] and the following section serves as a short summary of some of the key aspects.

The antitumor mechanism of irinotecan is related to the inhibition of the intranuclear enzyme topoisomerase I (Topo I), which is a 100-kDa protein that relaxes supercoiled DNA. The enzyme produces a single strand break in the DNA, followed by the passing of the intact strand through the break before re-ligation. Irinotecan binds to the Topo I complex and stabilizes it, allowing uncoiling of DNA but preventing re-sealing from taking place. Strand breaks occur mostly at replication forks and the collision between the irinotecan–Topo I complex and the replication fork, results in the formation of double strand breaks, causing G2 phase cell-cycle arrest and cell death [7]. Malignant tissues seem, in general, to have higher levels of Topo I than normal tissues, suggesting that it may be a logical target for anticancer therapy [8].

Irinotecan serves, primarily, as a water-soluble prodrug as it is converted in the liver by carboxylesterase, to the lipophilic and much more active metabolite, SN-38 [4,5,9]. This metabolite is approximately 1000 times more potent than irinotecan in inhibiting Topo I *in vitro*

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[10]. SN-38 seems to be responsible for the vast majority of the in-vivo antitumor activity, even though the parent compound does contribute some smaller amount [6].

The side-effect profile with commonly used doses includes, diarrhea, neutropenia, dehydration, fatigue, nausea, and vomiting. The most common grade 3–4 toxicities are diarrhea and neutropenia, occurring in approximately 20–30% of patients [11].

The precise contribution of SN-38 to the activity and toxicity of irinotecan is currently not known. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. A pH-dependent equilibrium exists between the two forms such that an acid pH promotes the formation of the lactone, whereas a more basic pH favors the hydroxy acid anion form [9]. After intravenous infusion, irinotecan has a mean terminal elimination half-life of approximately 6–12 h and SN-38 has an elimination half-life of approximately 10–20 h. The half-lives of the lactone (active) forms of irinotecan and SN-38 are similar to those of total irinotecan and SN-38, as the lactone and hydroxy acid forms are in equilibrium [5,12]. Irinotecan is widely distributed in body tissues and both irinotecan and SN-38 bind to albumin, irinotecan moderately so (30–68%), and SN-38 highly so, with approximately 95% bound [13,14]. SN-38 is eliminated largely through hepatic glucoronidation by the enzyme UDP–glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite, which is inactive [5,12,13,15].

UGT1A1 is a polymorphic enzyme and approximately 10% of the American population is homozygous for a particular allele, UGT1A1*28. The UGT1A1*28 allele is responsible for Gilbert's syndrome, which clinically is characterized by mild chronic unconjugated hyperbilirubinemia in the absence of liver disease or overt hemolysis [16]. In this condition the hepatic glucoronidating activity is reduced to approximately 30% of normal [17].

There have been several reports indicating that homozygotes for the UGT1A1*28 allele are at increased risk for irinotecan-related hematological and gastrointestinal toxicities [18–21].

Others have, however, cast different opinions with regard to the magnitude and clinical significance of the UGT1A1 polymorphism leading to an increased toxicity in patients receiving irinotecan [22,23]. After the treatment with irinotecan, both diarrhea and neutropenia seem to be dose dependent and not prohibitive with commonly used therapeutic doses [23].

In one study the incidence of grade 3–4 hematological and non-hematological toxicities after the first cycle of irinotecan was 2 and 4%, respectively, for wild-type patients compared with those patients, who are homozygous for the UGT1A1*28 allele, with approximately 14 and 14%, respectively [19]. Increased response rates and a nonsignificant survival advantage was also seen for those homozygous for the UGT1A1*28 allele in the same trial. Routine testing for the UGT1A1*28 allele has not entered common clinical practice, even though a specific assay is commercially available [24]. This is likely because of the relative rarity of homozygosity of the UGT1A1*28 allele and uncertainty about if and how much irinotecan should be dose reduced if found.

Many other factors might contribute to the wide range and sometimes unpredictable nature of irinotecan toxicity is seen in clinical practice. These include other UGTA1A haplotypes and sex, smoking, pretreatment bilirubin levels, and co-medications [25–29].

Irinotecan and radiosensitization

Preclinical data

In-vitro studies found that camptothecin derivatives sensitized log-phased human MCF-7 breast cancer cells to radiation in a schedule-dependent manner. Radiation was used concurrently with, immediately after, or before the treatment with 20(*S*)-10,11-methylenedioxycamptothecin, and resulted in sensitizer enhancement ratios of 1.43, 1.38, and 1.05 [30]. These results suggest that the drug should be given concurrently with or shortly before radiation therapy for any significant benefit of the combination to occur. Lamond *et al.* [31] obtained similar conclusions when treating human melanoma cells (U1-Mel) with 9-aminocamptothecin. The radiation synergy effect was dependent on drug concentration and timing, with enhancement present only when the drug was present at the time of, or shortly after, radiation.

In-vitro studies evaluating radiosensitization of a human lung cancer cell line (H460) and another camptothecin derivative [9-nitro-20(*S*)-camptothecin] found a concentration-dependent dose-enhancement ratio of up to 2.0 [32]. The compound [9-nitro-20(*S*)-camptothecin] also seemed to partially inhibit split-dose recovery in the same cell line suggesting the inhibition of sublethal damage recovery. Further support for the radiosensitizing properties of camptothecins was shown by Omura *et al.* [33] who investigated the effect of SN-38 on spheroids from a human colon cancer cell line (HT-29) exposed to radiation. The results suggested that the mechanism of SN-38 radiosensitization was through the inhibition of potentially lethal damage repair and that the greatest gain of cytotoxicity occurred when SN-38 was given before or just after radiation.

Tamura *et al.* [34] carried out in-vivo studies with human small-cell and mixed small-and-large cell lung carcinoma xenografts exposed to either irinotecan alone, radiation alone, or was given concurrently. Combination therapy resulted in significantly greater tumor regression than either treatment modality on in its own with no excess toxicity observed. Flow cytometric analysis showed that the proportion of cells in the G2/M phase increased 1 h after irinotecan administration, suggesting that some of the radiosensitization properties are cell-cycle mediated.

Clinical data

Neoadjuvant trials

A number of phase I and II trials investigating the addition of irinotecan to neoadjuvant chemoradiation have been reported during the last decade (Table 1). The key findings from these reports are summarized below.

Voelter *et al.* [47] enrolled 28 patients in a phase I dose escalation trial of neoadjuvant irinotecan concurrently given with hyperfractionated–accelerated radiotherapy (41.6, 1.6 Gy twice daily × 13 days). Escalating doses of irinotecan (30–105 mg/m^2) were given on days 1, 8, 15, and radiation therapy was started on day 8 with surgery scheduled to occur within 1 week after the completion of radiation therapy. All except two patients were able to complete the scheduled preoperative therapy and all patients underwent surgery. Dose-limiting toxicity was grade 3 diarrhea occurring at dose level 6 (105 mg/m^2). Postoperative complications occurred in seven patients, with an anastomotic leak rate of 22%. The recommended irinotecan dose for further study was 90 mg/m^2/week, and this was used in a follow-up phase II trial reported by the same group.

In this trial 33 patients with locally advanced rectal cancer (1 cT2, 29 cT3, 3 cT4, and 21 cN +) were enrolled and treated in an otherwise identical manner [35]. Surgery with total mesorectal excision (TME) was performed within 1 week after the completion of radiation therapy. Downstaging of the tumor was seen in 11 patients (35%) despite the short interval between neoadjuvant therapy and surgery. The pCR rate was not reported but the 2-year disease-free survival rate was 66%. The preoperative regimen was well tolerated with grade 3 diarrhea occurring in 24% of patients but severe postoperative complications occurred in 27% of patients, including four cases of pelvic abscess formation and two cases of anastomotic leakage.

Mehta *et al.* [36] enrolled 32 patients in a phase II trial of locally advanced rectal cancer. All patients were staged by endorectal ultrasound (uT3N0 = 19; uT3N1 = 13; uT2N1 = 1). Patients received irinotecan (50 mg/m^2, days 1, 8, 15, and 22) and 5-FU (200 mg/m^2 daily, days 1–33) concurrently with radiotherapy prescribed for draining the lymph nodes (45 Gy in 1.8-Gy daily fractions) and tumor (50.4 Gy in 1.8-Gy daily fractions). Surgical resection was performed 6–10 weeks after the completion of therapy. At surgery, 12 patients (38%) had a pCR and 23 (71%) were down staged. Acute toxicity was frequently observed, and 18 patients (56%) required either a chemotherapy dose reduction or radiation therapy interruption of more than 3 days. The grade 3 toxicities reported were diarrhea (28%), mucositis (21%), rectal sores (21%), and abdominal cramping (9%).

Klautke *et al.* [37] evaluated escalating doses of capecitabine given with irinotecan (40 mg/m^2) during concurrent radiation therapy [1.8 Gy fractions 5 days/week for a total dose of 55.8 (50.4 + 5.4) Gy] in 28 patients with locally advanced rectal cancer. The maximum tolerated dose of capecitabine was 750 mg/m^2 twice daily with dose-limiting toxicity being grade 4 diarrhea and hand–foot syndrome. pCR was seen in four patients (15%); three patients (12%) had only microfocal residual disease and 16 patients (62%) had a partial response on resection.

Another phase I trial from Germany evaluated two different dose levels of capecitabine given in addition to weekly irinotecan (50 mg/m^2) and pelvic radiotherapy to 50.4 Gy (including a 5.4 Gy boost) [38]. Dose-limiting diarrhea was seen with capecitabine (625 mg/m^2, twice daily) and (500 mg/m^2) was therefore recommended for future trials. A total of 19 patients were enrolled with pCR observed in four patients (21%) and another five patients (26%) had only microfoci of residual tumor.

A larger Spanish phase II study of neoadjuvant chemoradiotherapy in patients with resectable T3–T4 rectal cancer was reported by Navarro *et al.* [39]. Weekly irinotecan (50 mg/m^2) and infusional 5-FU (225 mg/m^2/day; 5 days/week) were concurrently administered with radiation therapy (45, 1.8 Gy/day, 5 days/week) for 5 weeks. Seventy-four patients were enrolled and 73 underwent operation with a pCR rate of 14% and down staging occurring in 49%. In addition, 67% of patients with nodal involvement per staging endorectal ultrasound were found to have no pathological involvement after the completion of therapy. The treatment was relatively well

Table 1 Summary of clinical trials evaluating irinotecan-based neoadjuvant chemoradiation for locally advanced rectal cancer

References	Chemo	Phase	Patients (*n*)	pCR	Down staging
[35]	Irinotecan	II	33	NR	35%
[36]	Irinotecan/5-FU	II	32	38%	71%
[37]	Irinotecan/capecitabine	I/II	28	15%	NR
[38]	Irinotecan/capecitabine	I	19	21%	68%
[39]	Irinotecan/5-FU	II	74	14%	49%
[40]	Irinotecan/5-FU	I/II	57	21%	41%
[41]	Irinotecan/capecitabine	II	36	15%	NR
[42]	Irinotecan/capecitabine	I	46	24%	NR
[43]	Irinotecan/capecitabine	II	48	25%	NR
[44]	Irinotecan/5-FU	II	106	26%	78%
[45]	Irinotecan/S-1	II	43	21%	50%
[46]	Irinotecan/capecitabine/cetuximab	II	50	8%	NR

5-FU, 5-fluorouracil; NR, not reported; pCR, pathologically complete response rate.

tolerated with grade 3–4 toxicity mainly consisting of diarrhea (14%), asthenia (9%), rectal mucositis (8%), abdominal pain (8%), and neutropenia (7%).

Glynne-Jones *et al.* [40] carried out a phase I/II trial in patients with borderline/unresectable locally advanced rectal cancer. Escalating doses of irinotecan were given together with bolus 5-FU (350 mg/m^2) and leucovorin (20 mg/m^2) D1–5 and D29–33 with concurrent preoperative pelvic radiation (45 Gy in 25 fractions of 1.8 Gy). TME was planned 6–10 weeks later. The maximum tolerated dose of irinotecan in this trial was 20 mg/m^2 with grade 3–4 diarrhea being the dose-limiting toxicity. Eight of the 57 enrolled patients did not proceed with the planned surgery. The reasons were development of metastatic disease or surgical unresectability in six patients, one patient was deemed medically inoperable, and one died because of complications from radiation. Of the 57 patients undergoing neoadjuvant therapy, down staging was observed in 41% and the pCR rate was 21%.

A German phase II trial reported by Willeke *et al.* [41] evaluated weekly irinotecan (50 mg/m^2) and capecitabine (500 mg/m^2, twice daily) D1-38 given concurrently with pelvic radiation therapy up to 50.4 Gy. A total of 36 patients with T3–4, Nx, or N + rectal carcinoma were enrolled. This included three patients with locally recurrent disease. Two patients did not proceed with surgery, one declined the intervention and one developed metastatic disease. pCR was seen in five patients (15%) and nine patients (26%) had only microfoci of residual tumor present in the resected specimen. The chemoradiation regimen was relatively well tolerated with grade 3–4 toxicity mainly consisting of leucopenia (25%) and diarrhea (11%).

Gollins *et al.* [42] used MRI scanning to identify high-risk rectal cancer patients, defined as involvement or threatening involvement (1 mm or less) of the mesorectal fascia. Neoadjuvant radiation therapy up to 45 Gy with concurrent escalating doses of both daily capecitabine and weekly inrinotecan was given from day 1–35. The maximum tolerated dose was 650 mg/m^2, twice daily for capecitabine and 60 mg/m^2 for irinotecan with grade 3 diarrhea occurring in 21% of patients at this dose level. Forty-one of the 46 enrolled patients underwent resection with a pCR rate of 24%.

A phase II trial reported from South Korea by Hong *et al.* [43] evaluated preoperative radiotherapy (45 Gy in 25 fractions followed by a 5.4 Gy cone-down boost) with concurrent weekly irinotecan (40 mg/m^2) and capecitabine (825 mg/m^2 twice daily, weekdays only). TME was planned 4–8 weeks after the conclusion of radiotherapy. Forty-four of the 48 enrolled patients underwent TME with a reported pCR rate of 25% and near total tumor regression in an additional 18%. The treatment was overall well tolerated with grade 3 toxicity mainly consisting of neutropenia, diarrhea, infection, and alanine transferase elevation in one patient each. No grade 4 toxicity or treatment-related death was reported.

The largest study investigating incorporation of irinotecan in the neoadjuvant setting with concurrent 5-FU-based chemoradiation was reported by Mohiuddin *et al.* [44]. One hundred and six patients with T3–4 distal rectal cancers were randomly assigned to receive continuous venous infusion of 5-FU (225 mg/m^2/days/7 days per week) and pelvic hyperfractionated radiation of 45.6 at 1.2 Gy twice daily with a 9.6 Gy boost for T3 and 14.4 Gy boost for T4 tumors, respectively (arm1), or continuous venous infusion of 5-FU at the same dose but given only 5 days per week along with 4-week doses of irinotecan (50 mg/m^2) plus pelvic radiation therapy of 45 at 1.8 Gy/days with a boost of 5.4 Gy for T3 and 9 Gy to T4 tumors (arm2). TME was recommended 4–10 weeks after the completion of therapy and the primary endpoint of the study was the pCR rate. The resectability rate was 93% with 103 patients being assessable for response. The results were essentially identical in the two treatment arms with a pCR rate of 26% and tumor down staging occurring in 78% in both groups. Grade 3 and 4 acute hematologic and nonhematologic toxicities occurred in 13 and 38% in arms 1 and 12 and 45% in arm 2, and the rate of late toxicity was 6% in both arms.

The oral fluoropyrimidine S-1 was used together with weekly irinotecan for neoadjuvant concurrent radiotherapy in two patients with locally advanced rectal cancer [48]. Both patients showed a subsequent pCR on resection. A subsequent phase II trial evaluated the combination of weekly irinotecan (40 mg/m^2), oral S-1 (70 mg/m^2/days/weekdays only), and three-dimensional conformal radiotherapy (50.4 in 1.8 Gy fractions) [45]. Forty-two of the 43 enrolled patients underwent resection with down staging reported in 50% and pCR seen in nine patients (21%). The side-effect profile was similar to that observed with capecitabine and irinotecan combinations.

Combination with biologics and tissue markers

In the phase II, MARGIT trial, the addition of cetuximab to neoadjuvant chemoradiation therapy was evaluated in 50 patients with locally advanced rectal cancer (T3–4 or N +) [46]. Pelvic radiotherapy was given up to a dose of 50.4 Gy (45 + 5.4 Gy) with concurrent cetuximab (400 mg/m^2/day 1, 250 mg/m^2/days 8, 15, 22, 29) in combination with weekly irinotecan (40 mg/m^2) and capecitabine (500 mg/m^2 twice daily, days 1–38). All patients underwent surgery and only four patients (8%) had a pCR. The treatment was relatively well tolerated with grade 3 nonhematologic toxicity dominated by diarrhea (30%), transaminase elevations (10%), and acne-like skin rash (6%).

The same group reported a correlative tissue study in patients with locally advanced rectal cancer undergoing neoadjuvant chemoradiotherapy with capecitabine and irinotecan [49]. Normal tissue and tumor tissue samples

were collected before the start of the treatment and during surgical resection in 38 patients. Topo I and thymidylate synthase expressions were measured using real-time PCR. The results of gene expression levels were compared between responders ($n = 18$) and nonresponders ($n = 20$). The biopsies of the untreated tumor tissue of responding patients showed a significant higher expression of Topo I compared with nonresponding patients ($P = 0.015$). No such correlation was seen for thymidylate synthase expression. This suggests that Topo I expression could be considered for further study as a possible predictor of response to irinotecan-based neoadjuvant chemoradiotherapy.

Conclusion

Irinotecan has shown activity as a radiosensitizer in preclinical models and early-phase clinical trials of neoadjuvant chemoradiation showing promising results initially for the treatment of locally advanced rectal cancer. However, this did not translate into any additive or synergistic clinical effect compared with 5-FU-based chemoradiotherapy in a larger randomized phase II trial [44].

The addition of cetuximab to chemoradiotherapy with capecitabine and irinotecan also failed to show any substantial clinical benefit [46]. The limited data regarding irinotecan-based chemoradiotherapy in the adjuvant and locally recurrent setting has also been disappointing [50,51]. Future trials should attempt to define the subset of patients that are most likely to benefit from chemoradiation with irinotecan. The finding of increased Topo I expression being a possible predictive marker for increased response to irinotecan-based chemoradiotherapy is intriguing and deserves further study [49].

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