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Pre-clinical Evaluation of the Activity of Irinotecan as a Basis for Regional Chemotherapy

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Abstract. *Background: Irinotecan alone and in combination with 5-fluorouracil (5-FU) displays potent activity in advanced colorectal cancer. The aim of this study was to estimate the potential efficacy of irinotecan for hepatic arterial infusion (HAI) chemotherapy. Materials and Methods: We investigated the anti-proliferative effects of irinotecan alone and in combination with 5-FU in HT29 and NMG64/84 colon and COLO-357, MIA PaCa-2 and PANC-1 pancreatic cancer cell lines and in fresh tumors from patients with primary colon cancer (n=2) and colorectal liver metastases (n=11) in vitro, using the MTT growth assay and the human tumor colony-forming assay (HTCA), mimicking conditions which are achievable during HAI. Results: Irinotecan displayed concentration- and time-dependent cytotoxic effects in all tested cell lines. Treatment of cell lines with irinotecan followed by 5-FU did not result in synergistic anti-proliferative effects. In the HTCA, the sensitivity of each cell line varied depending on the incubation times (30, 90, 180 and 1440 min). Independent of the individual sensitivity, the* IC_{50} *concentration and time products were lowest when incubating with irinotecan for 30 min for all cell lines. The* IC_{50} *of irinotecan in HT29, NMG64/84, COLO-357, MIA PaCa-2 and PANC-1 cells at 30 min were 200, 160, 100, 400 and 150 µg/ml, respectively, in the HTCA. All isolated tumor samples displayed concentration-dependent inhibition of colony formation after exposure to irinotecan for 30 min. The* IC_{50} *of irinotecan of 5 of the 11 liver metastases was <100 µg/ml. Conclusion: Irinotecan seems to be suitable for HAI therapy phase II studies. Due to the observation that several liver metastases had* IC_{50} *values that may be clinically achievable by HAI, patients with such tumors may benefit in the future from HAI using irinotecan.*

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Camptothecin is a natural plant alkaloid that was isolated from the Chinese bush *Camptotheca acuminata* in the early 1960s (1). Because of severe and unpredictable side-effects including hemorrhagic cystitis, clinical development did not progress until the discovery of its cellular target topoisomerase I (2). Camptothecin inhibits topoisomerase I, a nuclear enzyme that relaxes super coiled DNA and is therefore required for DNA synthesis in S-phase (3). The emerging interest in topoisomerase I inhibitors resulted in the subsequent synthesis of various camptothecin derivates including irinotecan (3).

Irinotecan (CPT-11) is chemically unique due to the presence of a side chain on the core structure resulting in reduced, more predictable and manageable clinical toxicity in comparison to camptothecin, but keeping a broad spectrum of anti-tumor activity *in vitro* and *in vivo* (4). Its action is dependent on a host of enzymes involved in metabolic transformation and active transport (3). One mechanism to obtain pharmacological activity is the cleavage of the side chain by carboylesterase resulting in SN-38 (3). Although SN-38 is thought to be the main active metabolite and has a 1000-fold higher ability than irinotecan to inhibit topoisomerase I (5), a recent comparison of the anti-tumor activity revealed that irinotecan is the more potent agent in solid tumors including colorectal cancer (6).

In accordance with the pre-clinical results, irinotecan was found to display response rates of 10 – 35% in phase II monotherapy trials in patients with previously treated and untreated metastatic colorectal cancer (2). In addition to its effectiveness as a single agent, recent studies have also shown that irinotecan may share additive or synergistic cytotoxic properties with several well established chemotherapeutic compounds including 5-fluorouracil (5-FU) (2). Two large phase III trials in

advanced metastasized colorectal cancer reported objective response rates of 39% and 49% when combining irinotecan with 5-FU and folinic acid (7, 8).

Despite these promising response rates, long-term survival of patients with metastatic colorectal cancer can only be achieved in those who undergo surgical resection of metastases. Patients with isolated colorectal liver metastases (CRLM) display 5-year survival rates of 40% after liver resection and 25% are totally cured (9). In contrast, the 5-year survival rate is only 9% for all patients with advanced colorectal cancer (10). Unfortunately, primary surgical resection can only be offered to approximately 10% of these patients at the time of diagnosis (9). However, patients with colorectal cancer whose liver metastases can be surgically removed after tumor shrinkage by neoadjuvant chemotherapy also benefit from the secondary resection (9). The best response rates of CRLM, using fluoropyrimidine-based regimens without irinotecan or oxaliplatin, are presently achieved with regional hepatic arterial infusion (HAI) chemotherapy (11). Based on intensive *in vitro* concentration response studies and *in vitro* phase II tests with various agents using the human tumor colony-forming assay (HTCA), a protocol was developed for regional treatment of isolated CRLM using mitoxantrone, 5-FU/folinic acid and mitomycin C, resulting in a response rate of 66%, a median survival of 27.4 months and a secondary achievement of complete resectability in 12% of the patients (12, 13). Since irinotecan could improve the activity of 5-FU-based regimens in the systemic treatment of metastatic colorectal cancer (7, 8), the addition of irinotecan to HAI protocols may also improve this treatment option for isolated colorectal liver metastases.

The aim of this study was to estimate the potential efficacy of irinotecan for HAI chemotherapy for future phase II trials. On the basis of the available pre-clinical and clinical data, we investigated the anti-tumor effects of irinotecan in established colon and pancreatic cancer cell lines and in fresh tumor cell suspensions from patients with colorectal liver metastases and primary tumors *in vitro* in the HTCA, using infusion times and drug concentrations that mimic clinical HAI conditions. We now report that irinotecan displayed dose- and time-dependent cytotoxicity in all the tested cell lines and an inhibition of colony formation in mono-therapy at clinically relevant concentrations in 5 out of the 11 tested liver metastases.

Materials and Methods

Materials. HT29 human colon carcinoma cells and MIA PaCa-2 and PANC-1 human pancreatic cancer cells were purchased from the American Type Culture Collection (Rockville, MD, USA); irinotecan from Aventis GmbH (Bad Soden, Germany); 5-FU from Medac GmbH (Hamburg, Germany); HAM F12, DME, penicillin/streptomycin solution and fetal bovine serum from Invitrogen GmbH (Karlsruhe, Germany); Cycle Test Plus DNA Reagent Kit for FACS analysis was purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA, USA); 3-(4,5-methylthiazol-2-yl)-2,5-diaphenyltetrazolium-bromide (MTT) and all other reagents were from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany). NMG 64/84 human colon cancer cells were established in our laboratory from a primary adenocarinoma of the colon. COLO-357 human pancreatic cancer cells were a generous gift from Prof. M. Korc of Dartmouth Medical School (Lebanon, New Hampshire, USA).

Cell lines and cell culture. HT29 and NMG64/84 cells were cultured in HAM F12 medium and COLO-357, MIA PaCa-2 and PANC-1 cells in DME medium and maintained at 37˚C in 5% CO_2. The media were supplemented with 7.5% fetal bovine serum and penicillin/streptomycin and termed complete medium.

Patients. Patients with liver metastases receiving hepatic resection or primary tumor resection were entered into this study. All patients had given written consent for tumor resection and the additional *in vitro* drug testing. The study was approved by the Ethics Committee of the University of Ulm, Germany (174/2001).

Tumor tissue. Tumor tissue was obtained at laparotomy without expanding the scope of surgery. Immediately after the removal of the tumor tissue, one part was processed to obtain a routine histology and the other part was immediately processed for the *in vitro* chemosensitivity assay. For this purpose, a single cell tumor suspension was prepared by digestion of macroscopically viable tumor tissue overnight, as described (14).

Human tumor colony-forming assay (HTCA). Individual drug sensitivity testing was performed using a soft-agar double layer system, as described (14). In the case of the cultured cell lines, 1.0 x 10^5 cells/test point were incubated for the indicated times (30 min, 90 min, 180 min, 1440 min) with the indicated concentrations (0.1 to 1000 μg/ml) of irinotecan. In the case of primary tumor cell suspensions, trypan blue staining was carried out and viable tumor cells were adjusted to a density of 3 x 10^6/ml. Cells (1.5 x 10^6/test point) were then incubated for 30 min with the indicated concentrations (0.1 to 1000 μg/ml) of irinotecan and seeded into dishes (5 x 10^5 cells/dish) using the double-layer technique. Depending on the cell line and tumor, colony formation was evaluated between 2 to 4 weeks after drug exposure. All test points were performed in triplicate and the results are shown as mean colony formation in relation to the mean of the untreated control. The assay was regarded as evaluable when on average 30 colonies consisting of at least 30 cells had formed in the untreated control dishes (12). *In vitro* sensitivity was defined as an inhibition of colony formation of at least 50% (12).

MTT growth assay. To determine the effects of irinotecan in combination with 5-FU, the MTT (3-(4,5-methylthiazol-2-yl)-2,5-diaphenyltetrazolium-bromide) assay was used, as described (15). Depending on the cell lines, 10,000 to 20,000 cells per well were seeded in 96-well plates and incubated for 24 h in complete

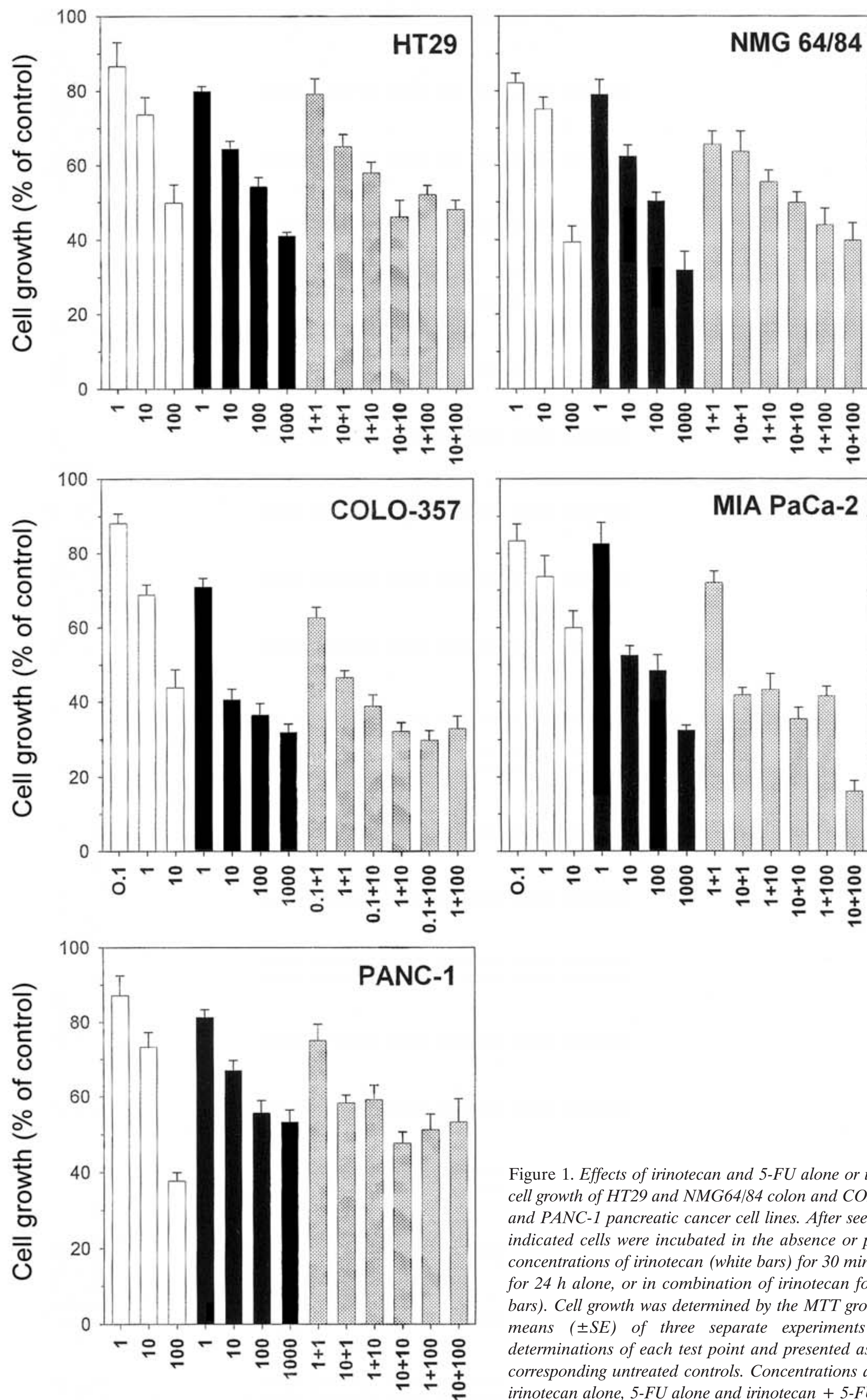

Figure 1. *Effects of irinotecan and 5-FU alone or in combination on the cell growth of HT29 and NMG64/84 colon and COLO-357, MIA PaCa-2 and PANC-1 pancreatic cancer cell lines. After seeding in 96-well plates, indicated cells were incubated in the absence or presence of increasing concentrations of irinotecan (white bars) for 30 min or 5-FU (black bars) for 24 h alone, or in combination of irinotecan followed by 5-FU (gray bars). Cell growth was determined by the MTT growth assay. Results are means (±SE) of three separate experiments with quadruplicate determinations of each test point and presented as growth in percent of corresponding untreated controls. Concentrations are shown as μg/ml of irinotecan alone, 5-FU alone and irinotecan + 5-FU.*

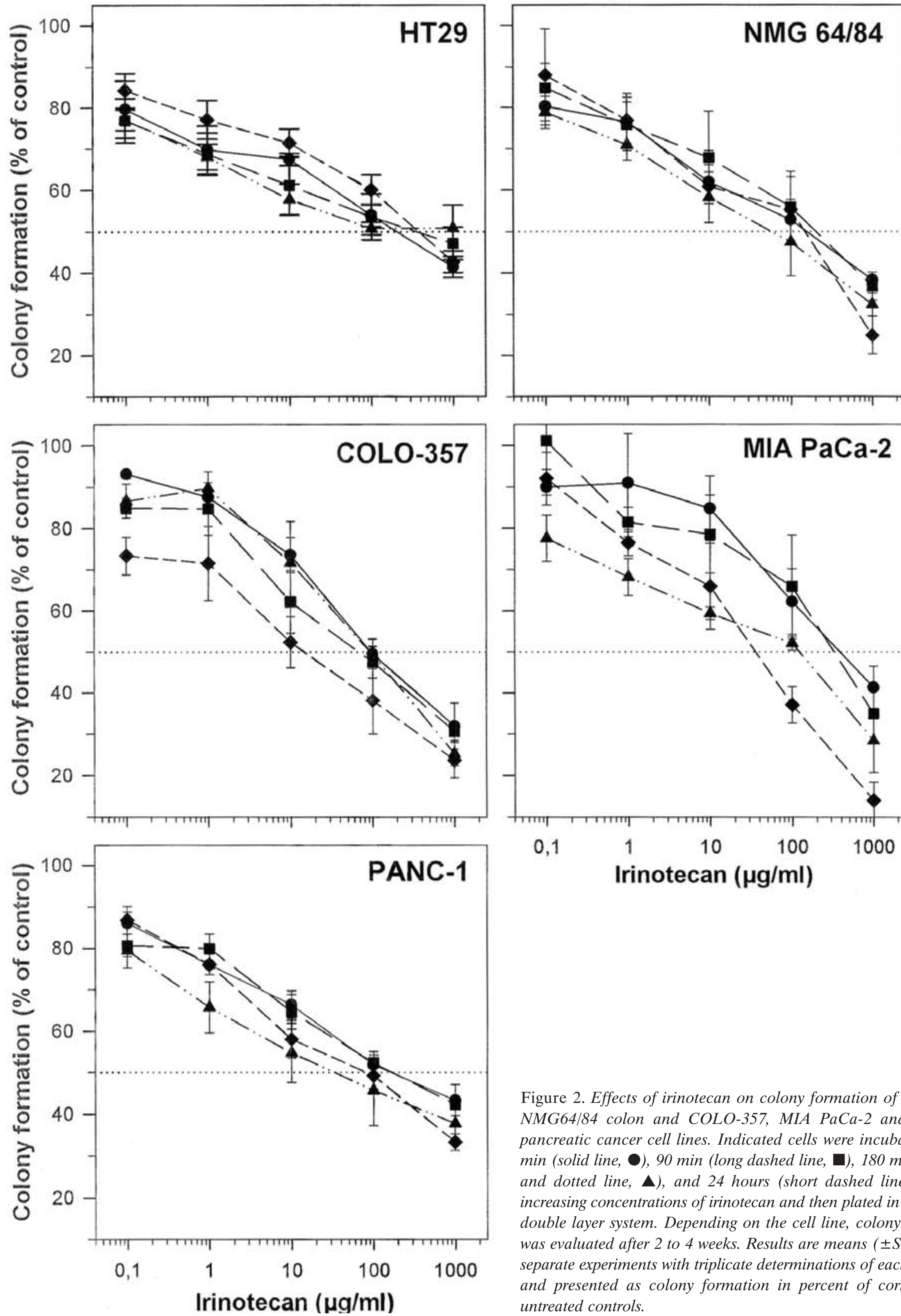

Figure 2. *Effects of irinotecan on colony formation of HT29 and NMG64/84 colon and COLO-357, MIA PaCa-2 and PANC-1 pancreatic cancer cell lines. Indicated cells were incubated for 30 min (solid line, ●), 90 min (long dashed line, ■), 180 min (dashed and dotted line, ▲), and 24 hours (short dashed line, ◆) with increasing concentrations of irinotecan and then plated in a soft-agar double layer system. Depending on the cell line, colony formation was evaluated after 2 to 4 weeks. Results are means (±SE) of three separate experiments with triplicate determinations of each test point and presented as colony formation in percent of corresponding untreated controls.*

Table I. *IC_{50} and IC_{50} x time products of irinotecan in cultured human colon and pancreatic cancer cell lines.*

	IC_{50} (μg/ml) – IC_{50} x time products (μg • ml^{-1} • min)			
Cell lines	30-min incubation	90-min incubation	180-min incubation	24-h incubation
HT29	200 - 6000	400 – 36000	>100 - >18000	400 - 576000
NMG64/84	160 - 4800	200 - 18000	60 - 10800	150 - 216000
COLO-357	100 - 3000	100 - 9000	70 - 12600	13 -18720
MIA PaCa-2	400 - 12000	330 - 29700	120 - 21600	35 - 50400
PANC-1	150 - 4500	150 - 13500	35 - 6300	80 - 115200

medium. On day 2, cells were incubated in the absence or presence of irinotecan for 30 min followed by 5-FU for 24 h. After another 24 h in complete medium without any additives, MTT reagent was added on day 4 to initiate the assay and the cells were incubated for an additional 4 h at 37˚C. After removal of the medium and dissolving the crystals with acidified isopropanol, the samples were analyzed using an ELISA plate reader at 570 nm. The value at 650 nm was subtracted as background.

Cell cycle analysis. To determine the effect of irinotecan on cell the cycle, cells were seeded in 6-well plates and incubated for 24 h in complete medium to 70% confluency, followed by serum-free medium for another 24 h. Then, indicated concentrations of irinotecan were added for 30 min. After the removal of irinotecan and washing twice with serum-free medium, the cells were incubated for another 24 h in serum-free medium. FACS analysis was then performed using a CycleTest Plus kit, according to the instructions of the manufacturer, and FACScan (Becton Dickinson) analysis system equipped with a FACStation, MAC PowerPC computer and CellQuest acquisition software, as previously described (16).

Statistics. The results are shown as median and range, absolute and relative frequencies, and means (± SD or ± SE) in comparison to untreated controls. Statistical analysis was performed with SigmaStat 2.0 software.

Results

Effect of irinotecan and 5-FU on cell growth of colon and pancreatic cancer cell lines. Several clinical studies have suggested a synergistic effect of irinotecan and 5-FU (7, 8). In order to characterize the anti-proliferative effects of irinotecan in combination with 5-FU, the MTT test was used, adapting the incubation times of irinotecan (90 min) and 5-FU (24 h) from the clinical settings (7, 8). Increasing concentrations of irinotecan inhibited the growth of all cell lines in a dose-dependent manner (Figure 1). COLO-357 cells were most sensitive and HT29 most resistant to irinotecan in the MTT assay on incubation for 90 min. The IC_{50} concentrations were 100, 50, 5.4, 23 and 46 μg/ml irinotecan for HT29, NMG 64/84, COLO-357, MIA PaCa-2 and PANC-1, respectively. 5-FU alone for 24 h also inhibited the growth of all cell lines in a dose-dependent manner (Figure 1). The subsequent treatment of the cells with irinotecan (90 min) followed by 5-FU (24 h) did not result in synergistic effects (Figure 1). Additive effects were observed in HT29, NMG 64/84, MIA PaCa-2 and PANC-1 cells, especially at 10 μg/ml of irinotecan followed by low or medium concentrations of 5-FU. In the most sensitive COLO-357 cells, additive effects were only observed for 1 μg/ml of irinotecan followed by 1 μg/ml of 5-FU (Figure 1).

Effect of irinotecan on colony formation of colon and pancreatic cancer cell lines. In order to determine irinotecan concentrations and incubation times resulting in efficient inhibition of colony formation, HT29 and NMG64/84 human colon cancer cell lines and COLO-357, MIA PaCa-2 and PANC-1 human pancreatic cancer cell lines were exposed to increasing concentrations of irinotecan (0.1 to 1000 μg/ml) for 30 min, 90 min, 180 min and 24h. Irinotecan inhibited the colony formation in all the cell lines in a dose-dependent manner (Figure 2). Prolongation of the exposure time resulted in an enhancement of the inhibitory effects in pancreatic cancer cell lines, decreasing the concentration necessary to achieve an inhibition of colony-formation of 50% (IC_{50} values) at an exposure time of 24 h by 1.9- to 11-fold compared to the 30-min exposure time (Table I). In contrast, in colon cancer cell lines the IC_{50} values slightly increased for HT29 cells and remained stable in NMG 64/84 cells, comparing the 30-min with the 24-h incubation time (Table I). The IC_{50} concentrations of irinotecan and the irinotecan IC_{50} concentration and time products (c x t) are summarized in Table I. Comparable to the MTT, COLO-357 cells were most sensitive and HT29 most resistant to irinotecan in the HTCA on incubation for 90 min. However, the sensitivity of each cell line varied depending on the incubation time. Nevertheless, independent of the individual sensitivity, the IC_{50} concentration and time products were lowest for all cell lines when incubating with irinotecan for 30 min (Table I), suggesting that this may be the most effective incubation time.

Effect of irinotecan on the cell cycle of colon and pancreatic cancer cell lines. Irinotecan inhibits DNA synthesis in S-phase by inhibition of topoisomerase I (3). Thus, the effects of irinotecan on the cell cycle were next characterized using 100 and 1000 μg/ml for 30 min. In colon cancer cell lines, irinotecan decreased the cell number in G0/G1- and increased the cell number in

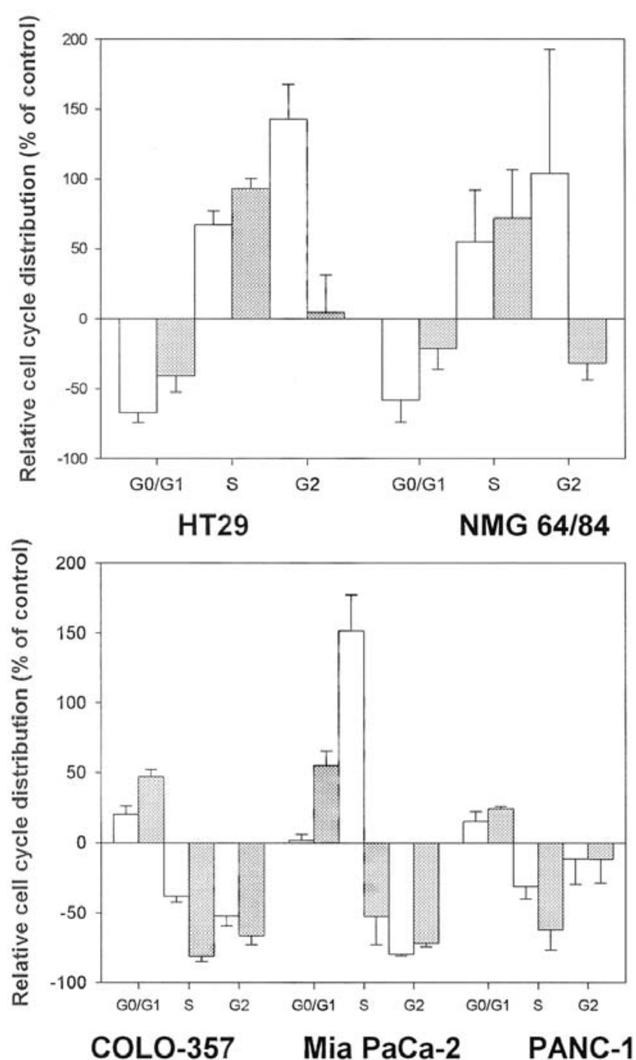

Figure 3. *Effects of irinotecan on cell cycle of HT29 and NMG64/84 colon and COLO-357, MIA PaCa-2 and PANC-1 pancreatic cancer cell lines. Indicated cells were incubated in the absence or presence of 100 μg/ml (white bars) and 1000 μg/ml (gray bars) irinotecan for 30 min. After removal of irinotecan and an incubation of the cells for an additional 24 h in complete medium, cell cycle analysis (FACS) was performed. Results are means (±SD) of three experiments with duplicate determinations of each test point and presented as percent difference in relative cell cycle distribution compared to corresponding untreated controls.*

S-phase. The cell number in G2 was increased at the low concentration of irinotecan, while it was unchanged or decreased at the high concentration of irinotecan in HT29 and NMG 64/84, respectively (Figure 3A). In contrast, in all three pancreatic cancer cell lines irinotecan increased cell number in G_0/G_1- and decreased cell number in S- and G_2-phase (Figure 3B).

Table II. *Characteristics of patients with colorectal liver metastases and primary tumors receiving tumor resection and their in vitro response to irinotecan.*

ID	Primary	Liver metastases	Age/Sex	Prior treatment	IC_{50} of irinotecan (μg/ml)
01/02	Colon	Synchronous	70/f	-	1000
06/02	Rectum	-	69/m	-	400
07/02	Rectum	-	69/m	-	150
08/02	Rectum	Metachronous	62/m	Adjuvant 5-FU *i.v.*	200
10/02	Colon	Synchronous	60/f	5-FU + oxaliplatin *i.v.*	35
11/02	Colon	Metachronous	69/m	Adjuvant 5-FU *i.v.*	300
12/02	Rectum	Metachronous	67/f	Adjuvant 5-FU *i.v.*	43
03/03	Colon	Synchronous	61/m	-	180
04/03	Colon	Metachronous	42/m	HAI*	23
05/03	Colon	Metachronous	53/m	Adjuvant 5-FU *i.v.*	74
06/03	Rectum	Metachronous	61/m	Adjuvant 5-FU *i.v.*	200
07/03	Colon	Metachronous	61/m	-	105
09/03	Colon	Metachronous	58/f	Adjuvant 5-FU *i.v.*	30

*Hepatic arterial infusion (HAI) chemotherapy consisted of mitoxantrone, 5-FU and folinic acid, and mitomycin C and was administered for 13 cycles (13).

HTCA of freshly isolated tumor specimens. After characterization of the anti-proliferative effects of irinotecan in cultured human colon and pancreatic cancer cell lines, freshly isolated tumor specimens were exposed to irinotecan. A total of 18 colorectal liver metastases and 3 primary colorectal cancers were subjected to the HTCA. Six samples (5 metastases, 1 primary tumor) did not form colonies and 2 samples were contaminated. Overall, 11 out of the 18 metastases (61%) and 2 out of the 3 primary tumors (66%) were evaluable. The characteristics of the 13 evaluable patients, including their HTCA results, are summarized in Table II. Eleven patients had synchronous or metachronous isolated colorectal liver metastases and 2 patients primary rectal cancer. Seven patients received adjuvant 5-FU-based chemotherapy and 1 patient regional hepatic arterial infusion (HAI) chemotherapy prior to liver resection (Table II).

Effect of irinotecan on colony formation of freshly isolated colorectal cancer cells. Based on pre-clinical and clinical data (17) and on our results of the cell lines, we chose to expose the freshly explanted human tumor specimens to 1, 10, 100

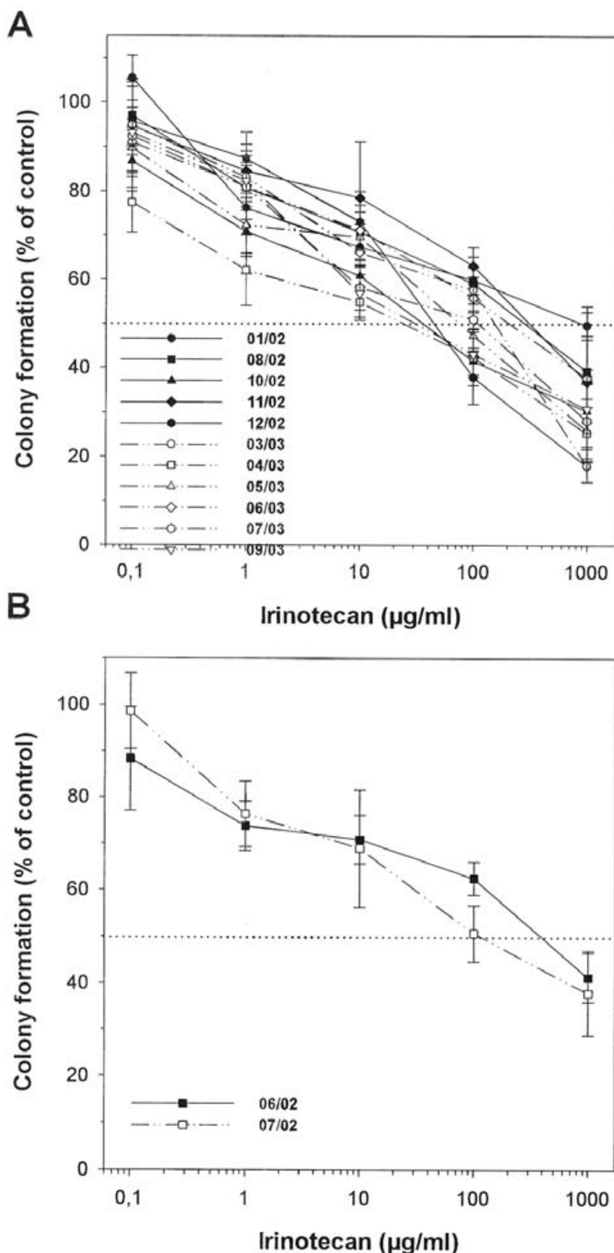

Figure 4. *Effects of irinotecan on colony formation of freshly isolated tumor cells from colorectal liver metastases (A, n=11) and primary colorectal cancers (B, n=2). Indicated specimens were incubated for 30 min with increasing concentrations of irinotecan and then plated in a soft-agar double layer system. Colony formation was evaluated after 2 to 4 weeks. Results are means (±SD) of triplicate determinations of each test point and presented as colony formation in percent of corresponding untreated controls.*

and 1000 µg/ml of irinotecan for 30 min. Similar to the observations in the cultured cell lines at the 30- min exposure time, all tumor specimens displayed an obvious concentration response effect (Figure 4). The median IC_{50} concentration of irinotecan was 105 µg/ml (range: 23 to 1000 µg/ml) for the liver metastases and the IC_{50} concentrations were 150 and 400 µg/ml for the two primary tumor samples (Table II). Five of the 11 metastatic specimens had an IC_{50} $<$89 µg/ml, a concentration clinically achievable during HAI treatment (Table II).

Discussion

The liver is the major target site for metastases in colorectal cancer and almost 50% of the patients with colorectal cancer will eventually develop liver metastases (18). Unfortunately, at this tumor stage cure is generally only possible after liver resection, which, however, can be only performed in about 10% of the patients at the time of diagnosis (9). Interestingly, it has been demonstrated that patients may profit to a similar extent from a secondary liver resection after neo-adjuvant chemotherapy (19). The goal of neo-adjuvant treatment should, therefore, be the reduction of the number and size of liver metastases to enable a secondary resection. Hepatic arterial infusion chemotherapy can achieve higher response rates than systemic chemotherapy (11). In order to further increase the efficacy of HAI treatment with subsequent down-sizing of the liver lesions to enable a secondary resection, drugs that have been successfully introduced into systemic treatment of colorectal cancer, like oxaliplatin and irinotecan, should also be evaluated for HAI treatment.

Based on *in vitro* chemosensitivity testing using the human tumor colony-forming assay and mimicking clinical conditions, we could demonstrate that patients who received *in vitro* sensitive tested drugs achieved significantly higher response rates compared to patients only receiving *in vitro* resistant drug combinations (14). Based on the HTCA, an effective protocol was designed for HAI, chemotherapy using mitoxantrone, 5-FU/folinic acid and mitomycin C, which achieved high survival and secondary resection rates (13). More recently, based on *in vitro* HTCA results, oxaliplatin was introduced to HAI, substituting for mitoxantrone in the HAI protocol (20). Preliminary phase II results of this protocol demonstrated a response rate of 80% with tolerable toxicity (21). Comparable to oxaliplatin, irinotecan could also significantly improve the activity of 5-FU-based regimens in the systemic treatment of metastatic colorectal cancer (7, 8) and, therefore, may also improve the efficacy of HAI chemotherapy protocols of isolated colorectal liver metastases.

Despite its well-known effectiveness as a single agent and in combination with 5-FU for systemic treatment of metastasized colorectal cancer, not many studies have been published about the possible role of irinotecan for

HAI treatment. Therefore, the aim of this study was to characterize the potential efficacy of irinotecan alone and in combination with 5-FU for HAI chemotherapy for future phase II trials. To date, three studies have been published combining systemic irinotecan with HAI treatment using 5-fluoro-deoxyuridine (FUdR) (11) and one study combining systemic irinotecan with HAI using 5-FU and folinic acid (22). The results about direct intra-arterial infusion of irinotecan are currently rare, comprising case reports (23), two phase I and pharmacokinetic studies (24, 25) and one small phase II study (26).

Our initial experiments revealed that no synergistic effects were detectable using irinotecan in combination with 5-FU, though under certain conditions weak additive actions were present. The MTT assay (27) was used in this setting because its combines easy and rapid handling and evaluation, even when using drug combinations. Due to the fact that no synergistic effects were seen, we decided to focus on the effect of iriotecan alone at the onset of the study. Using our well-established HTCA system, we could demonstrate that irinotecan exerts potent dose-dependent *in vitro* cytotoxic activity against cultured colon and pancreatic cancer cell lines and against colorectal liver metastases and primary tumors. Our results demonstrated that a broad range of sensitivity toward irinotecan exists in the cell lines and, especially, in the fresh tumor specimens analyzed. This is in concordance with the data obtained by Guichard and colleagues, demonstrating variable effects of irinotecan on carboxylesterase and topoisomerase I activities in primary colorectal cancers and colorectal liver metastases (28).

A pharmacokinetic study of continuous HAI treatment using irinotecan has demonstrated no difference in the systemic SN-38 levels and toxicities compared to systemic application (25). Nevertheless, the dose-dependent anti-tumor effects of irintecan favor HAI application, allowing high local drug concentrations in the liver (11). The infusion times in the trials of systemic treatment for irinotecan were 30 – 90 min at a dose of 80 -100 mg/m^2 body surface area weekly (17). However, there are also studies using 350 mg/ m^2 body surface area every 21 days (17). Based on the recommended time for clinical use and on theoretical considerations regarding regional chemotherapy (12), we decided to expose the cells to irinotecan at concentrations ranging from 0.1 to 1000 μg/ml. In order to determine the influence of the duration of exposure to irinotecan, we also tested these concentrations for 30, 90 and 180 min and 24 h in the cell lines. The increase in exposure times resulted in the enhancement of cytotoxicity. However, the concentration and time products to achieve a 50% inhibition of colony formation were lowest in all cell lines on incubation with irinotecan for 30 min, suggesting that irinotecan is most effective during a short incubation. Currently, two HAI regimens for irinotecan have been published, one using continuous irinotecan infusion (15-25 mg/m^2/d for 5 days) (25) and one using 200 mg/m^2 for 30 min every 3 weeks (26). Our present results favor the latter regimen. However, more patients need to be evaluated to determine which regimen might be superior.

Similar to regional chemotherapy of colorectal liver metastases, patients with locally advanced pancreatic cancer may receive regional chemotherapy using mitoxantrone, 5-FU/folinic acid and cisplatin *via* the celiac artery (29). This regimen has been shown to reduce the occurrence of liver metastases in patients with pancreatic cancer, but is very laborious and costly. Our results in the cell lines have demonstrated that pancreatic cancer cell lines are also growth inhibited using irinotecan with sensitivities in the range of colon cancer cell lines. Therefore, it is possible that irinotecan may also play a role in regional chemotherapy of pancreatic cancer in the future.

It is well known that patients receiving regional chemotherapy with agents that had been tested *in vitro* sensitive by the HTCA display a significantly better response rate than patients only treated with *in vitro* resistant HTCA agents (14, 30). This demonstrates that *in vitro* response, using our established HTCA conditions, correlates well with clinical response to HAI chemotherapy, mimicking clinical conditions *in vitro*. Therefore, considering the observations in cultured cell lines and the clinically recommended infusion times, we decided to expose the tumor specimens to irinotecan for 30 min. Under these conditions, we could also show a dose-dependent inhibition of colony formation in all specimens. Assuming a hepatic arterial blood flow of 250 ml/min (12), a theoretical concentration of 91 μg/ml for 30 min could be achieved by HAI therapy of irinotecan administering 350 mg/m^2 (17) to a patient with 1.95 m^2 body surface area as a 30-min infusion. Under the present conditions, the IC_{50} values of 5 out of the 11 of the metastatic specimens were below this theoretically achievable concentration, underscoring the potential efficacy of irinotecan for regional chemotherapy. Only one sample displayed a relatively high IC_{50} concentration of 1000 μg/ml, which, interestingly, did not receive any prior chemotherapy.

Although the pharmacokinetic and phase I study using irinotecan for HAI could not demonstrate lower systemic SN-38 levels or side-effects compared to systemic administration, HAI resulted in a higher metabolic ratio of irinotecan in the liver (25). In view of these pharmacokinetic and phase I data and our present results, we conclude that irinotecan seems to be suitable for phase

II studies of HAI therapy and that selected patients with colorectal liver metastases may benefit in the future from HAI protocols including irinotecan.

Acknowledgements

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