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SEQUENCE-DEPENDENT ACTIVITY OF THE IRINOTECAN-5FU COMBINATION IN HUMAN COLON-CANCER MODEL HT-29 *IN VITRO* AND *IN VIVO*

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Irinotecan, a DNA-topoisomerase-I inhibitor, is active against metastatic colon carcinoma. We investigated the effects of irinotecan and 5FU combinations in human colon-carcinoma cell line HT-29, both *in vitro* and *in vivo.* Cytotoxicity of 24-hr exposure was evaluated by SRB technique and the nature of interactions were determined by median-effect analysis. Strong synergism between irinotecan and 5FU was observed after sequential exposure, and only additivity after simultaneous exposure. At 50% level of kill, the mean sums of fractional effects were 0.13 ± 0.05 and 0.18 ± 0.02 respectively for the 2 sequential schedules, indicating that the combined amount of the 2 drugs necessary to kill 50% cells was only 0.18 and 0.13 times respectively, as much as would be required if they demonstrated purely additive behavior. In nude-mice xenografts, schedule-dependent toxicity was observed: the schedule in which irinotecan was administered i.v. 6 hours before 5FU was the most toxic. Higher anti-tumoral activity was noted when 20 mg/kg/day of each drug was administered sequentially (a delay of 6 hr between the 2 drugs) to mice over 5 days, in comparison with simultaneous administration. *In vivo* data confirmed those obtained *in vitro* in the same human colon-cancer model. These results suggest that irinotecan and 5FU combinations are of clinical interest and that the schedule of administration is a critical parameter for chemotherapeutic efficacy. *Int. J. Cancer* 73: 729–734, 1997.
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An established principle of chemotherapy for neoplasia is that multidrug treatments are frequently superior to single agents. This concept is based on the view that resistance to any single agent could be overcome by using multiple agents with distinct mechanisms of action. An alternative rationale for using combinations of anti-neoplastic agents is the potential additive or synergistic cytotoxicity they could engender. Irinotecan, a semi-synthetic water-soluble derivative of camptothecin, has demonstrated *in vitro* cytotoxicity against various types of human cell lines and anti-tumoral efficacy against experimental tumor models, against P-glycoprotein expressing multidrug-resistant cell lines, and against various xenograft models. This drug inhibits, via its first metabolite SN-38, the nuclear enzyme topoisomerase I. The DNA topoisomerases are enzymes which modify DNA topology by transiently breaking strands of the DNA, passing a single- or double-stranded DNA through the break and finally resealing either one or two breaks. Topoisomerase I is of particular importance in replication and transcription. Camptothecin derivatives inhibit topoisomerase-I activity through reversible binding to co-valent DNA-topoI intermediate called cleavable complex, which inhibits DNA religation (Rivory and Robert, 1995).

Irinotecan has recently emerged as a promising active agent for the treatment of metastatic colorectal cancer (Rougier *et al.,* 1997). Until now, 5FU, an analog of uracil basis, was considered as the most active single agent. Its anti-tumor activity is the result of inhibition of thymidylate-synthase activity and incorporation into both DNA and RNA (Lonn and Lonn, 1984). The difference in mechanisms of action and resistance of irinotecan and 5FU gives a rationale for testing their combination. Moreover, the sequence of administration of drugs with different mechanisms of action might be critical in terms of cytotoxicity, of anti-tumoral activity and/or of toxicity.

Synergistic interactions between irinotecan and other drugs have been described *in vitro* (Kano *et al.,* 1992), but few studies to date described interactions between irinotecan and FU *in vitro* (Aschele *et al.,* 1996; Erlichman *et al.,* 1996; Mans *et al.,* 1996) or *in vivo* (Houghton *et al.,* 1996).

Here we describe the synergistic cytotoxicity and the enhancement of the anti-tumor activity between irinotecan and 5FU in the human HT-29 colon-carcinoma cell line and in xenografts when cells or tumors are sequentially exposed to both drugs in whatever order.

MATERIAL AND METHODS

Drugs

Irinotecan was kindly provided by Rhône Poulenc Rorer (Neuilly sur Seine, France). 5FU was purchased from Roche (Neuilly sur Seine, France). For animal studies, the drugs were dissolved in 0.9% sodium-chloride solution immediately before injection on each day of treatment. Drugs were administered as about 0.2 ml volume of the appropriate solution per mouse according to the body weight. RPMI 1640 and FCS were purchased from Seromed (Strasbourg, France).

Cell cultures

The HT-29 colon-cancer cell line was obtained from the ATCC (Rockville, MD). Cells were grown as monolayers in RPMI-1640 medium supplemented with 5% FCS at 37°C in a humidified atmosphere containing 5% CO_2. Cells were trypsinized once a week with trypsin/EDTA (0.25%/0.02%) and medium was changed once a week. Doubling time of the cell line was 22 ± 5 hr.

Cytotoxicity assays

Determination of the IC_{50} was performed using the sulforhodamine-B technique (Skehan *et al.,* 1990). On day 1, 2500 cells/well in a volume of 150 µl were plated in 96-well plates. In each plate, one column contained cells not exposed to drugs, and 9 columns contained cells exposed to increasing concentrations of drugs. For each drug or drug combination, 6 wells were used.

On days 2 and 3, 5FU or irinotecan were added in a volume of 50 µl, resulting in a series of final concentrations ranging from 0.4 µM to 200 µM for 5FU and from 0.8 µM to 400 µM for irinotecan. The concentrations in the combinations were reduced 10-fold with a 5FU:irinotecan ratio of 1:2 (molar ratio of individual IC_{50}). After drug exposure, the medium in the control and drug-containing wells was replaced by 200 µl of fresh drug-free medium and the cells were cultured for 72 hr after the end of drug exposure. At the end of the culture, the cells were precipitated with 50 µl ice-cold 50% TCA and fixed for 60 min at 4°C, rinsed 6 times with water, and air-dried. Fixed cells were colored with 50 µl of solution (0.4% sulforhodamine B/0.1% acetic acid), rinsed with 0.1% acetic-acid solution, and air-dried. Sulforhodamine was re-dissolved in 150

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μl/well of 10 mM Tris buffer, pH 10, and 540 nm OD was measured in a Multiscan Multisoft apparatus (Labsystems, Les Ulis, France).

Growth-inhibition curves were plotted relative to control cells (First column) and IC50 was determined by interpolated graph. The cells were exposed to the 2-drug combination using 3 different modalities (Fig. 1): sequence 1, 5FU for 24 hr, then irinotecan for 24 hr; sequence 2, irinotecan for 24 hr, then 5FU for 24 hr; sequence 3, 5FU and irinotecan simultaneously for 24 hr. The cytotoxicity of combinations was compared with the cytotoxicity of each drug alone in every experiment, and each experiment was performed 3 times.

Median-effect analysis

Median-effect analysis was used to determine the interactions between irinotecan and 5FU. Dose-response interactions (antagonism, additivity, synergism) were expressed as a non-exclusive case combination index (CI) for every fraction affected (FA), using the method of Chou and Talalay (1984), processed by a computer program developed by Chou and Chou (Biosoft). CI <1 indicates synergism, >1 indicates antagonism, and a CI value of 1 indicates additivity of the drugs.

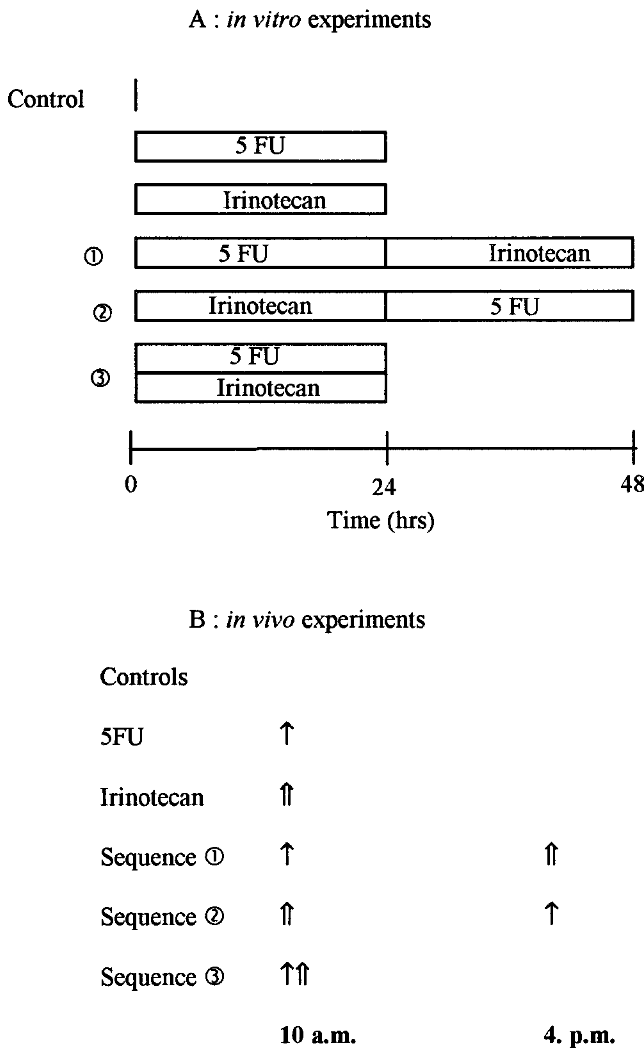

FIGURE 1 – Schematic representation of drug-exposure schedules. Sequence 1, 24 hr of 5FU exposure, then 24 hr of irinotecan exposure for *in vitro* study; for in vivo study, 6-hr delay between i.v. administration of 5FU and of irinotecan. Sequence 2, irinotecan exposure for 24 hr, then 5FU exposure for 24 h; for *in vivo* study, 6-hr delay between i.v. administration of irinotecan and of 5FU. Sequence 3, simultaneous exposure of irinotecan and 5FU; for *in vivo* study, simultaneous i.v. administration of the 2 drugs.

Animals

Female Swiss athymic mice, 4 to 5 weeks old, purchased from Iffa Credo (Saint Germain sur l'Arbresle, France), were housed in filter-capped cages kept in sterile facility and maintained in accordance with the usual standards. After a 2-week quarantine, they were used for chemotherapy testing.

Tumor model

A xenograft in nude mice was obtained after s.c. implantation of 8×10^6 cells of the HT-29 tumor cell line maintained *in vitro* in our laboratory.

In vivo *experimental design*

Drug activity was evaluated only against advanced-stage tumors. For each experiment, 8×10^6 cells were xenotransplanted s.c. in mice aged 6 to 7 weeks. Seven days after cell inoculation (day 0 of the treatment), mice bearing 100- and 250-mm^3 s.c. tumors were pooled and randomly assigned to different groups of 6 to 9 mice. Two perpendicular diameters of the tumors were measured 3 times weekly with a calliper square by the same investigator. Each tumor volume was calculated according to the following equation: $V(mm) = d^2\ (mm^2) \times D\ (mm)/2$, where d and D are the smallest and the largest perpendicular tumor diameters respectively. Animal body weights were recorded 3 times a week, and mortality was checked daily. The experiments lasted until tumor volumes reached 5 times the initial volume.

Treatment

In a first experiment, irinotecan was administered i.v. via a caudal vein to mice at 5 dose levels, namely 20, 30, 40, 50 and 60 mg/kg in 0.2 ml, given 5 times per week. 5FU was administered in the same conditions at 3 dose levels, namely 20, 30 and 40 mg/kg. This experiment was performed to determine the maximum tolerated dose of each drug.

In a second experiment, different combinations of irinotecan and 5FU following the above schedules and routes were tested. Simultaneous injections or sequential administrations of 5FU and irinotecan (Fig. 1), with a 6-hr delay between administration of the 2 drugs were performed. The drugs were also given alone. Each treated group and the control group comprised 9 mice. Different dose levels of each drug were also tested in combination treatment: 40 mg/kg irinotecan with 30 mg/kg 5FU, 30 mg/kg irinotecan with 20 mg/kg 5FU, and 20 mg/kg irinotecan with 20 mg/kg 5FU. Control groups were treated with saline.

Evaluation of toxicity

The toxicity of each drug or each combination of drugs was evaluated according to 2 criteria: (i) number of dead mice; (ii) maximum body-weight loss (MBWL).

Evaluation of anti-tumor activity

The activity of each drug or each combination of drugs was evaluated by the mean time for a tumor to reach a volume 5 times greater than the initial volume (MTR).

Statistical analysis

Comparison of the different administration schedules was performed by Kaplan-Meier estimates and by the log-rank test. Comparison of mean body-weight loss and time to reach 5 times the initial tumor volume among the different schedules of association was performed by variance analysis and multiple median comparison, after verification of variance homogeneity. For all analyses, differences of $p < 0.05$ were considered as significant.

RESULTS

Analysis of cytotoxic effects of 2-drug combinations

The IC_{50} values were 8.3 ± 4.8 μM and 13.8 ± 10 μM for 5FU and irinotecan respectively. Figure 2 shows the median-effect analysis of the 3 combinations, and Table I summarizes the CI values.

When cells were simultaneously exposed to irinotecan and 5FU (sequence 3), the CI was near 1, indicating additivity. By contrast, when cells were sequentially exposed to irinotecan and 5FU, strong and significant synergism was observed.

For sequences 1 and 2, CI_{50} values were 0.18 ± 0.02 and 0.13 ± 0.05 respectively, indicating that the combined amount of the 2 drugs necessary to kill 50% cells was only 0.18 and 0.13 times as much as would be required if they demonstrated purely additive behavior.

Determination of maximum tolerated doses of 5FU and irinotecan in nude mice

Acute toxicity-related deaths were observed at the 60 mg/kg/day × 5 irinotecan dose level, with 3 out of 10 mice dying on days 7 to 10. This dose level was considered as toxic. The maximum tolerated dose was 40 mg/kg/day × 5 (total dose 200 mg/kg), with one toxicity-related death out of 15 treated mice and a maximum body-weight loss ranging from 4 to 28% (mean = 15.8%).

With 5FU, toxicity-related deaths were observed at the 40 mg/kg/day × 5 dose levels, with 5 out of 6 mice dying on days 7 to 10. This dose level was considered as toxic. The maximum tolerated dose was 30 mg/kg/day × 5 (total dose 150 mg/kg), with no toxicity-related death and a maximum body-weight loss ranging from 0 to 4.3% (mean = 1.5%).

Toxicity of combined irinotecan/5FU chemotherapy

The optimal doses of irinotecan (40 mg/kg/day) and 5FU (30 mg/kg/day) were combined according to the 3 administration schedules. Out of 8 mice, all died on days 7 to 13.

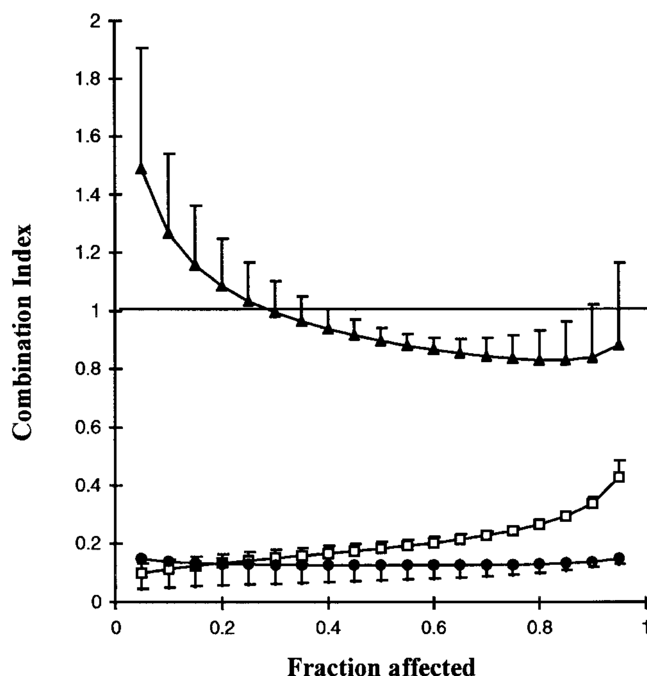

FIGURE 2 – Median-effect analysis of interaction between irinotecan and 5FU in HT-29 cell line. Sequence 1, □; sequence 2, ●; sequence 3, ▲. CI values are: CI > 1, antagonism; CI = 1, additivity; and CI < 1, synergism. Values are mean ± SD of 3 independent experiments.

TABLE I – VALUES OF COMBINATION INDEX (CI) FOR THE INTERACTION BETWEEN IRINOTECAN AND 5FU AGAINST HT-29 CELL LINE *IN VITRO*

Schedule	CI 20%	CI 50%	CI 80%
Sequence 1	0.13 ± 0.03	0.18 ± 0.02	0.26 ± 0.06
Sequence 2	0.13 ± 0.03	0.13 ± 0.05	0.13 ± 0.03
Sequence 3	1.08 ± 0.16	0.89 ± 0.04	0.85 ± 0.10

CI 20%, CI 50%, CI 80% = combination index indicating the combined amount of the 2 drugs necessary to kill 20, 50 and 80% of the cells respectively. Sequence 1: 24 hr of 5FU exposure, then 24 hr of irinotecan exposure; Sequence 2: irinotecan exposure for 24 hr, then 5FU exposure for 24 hr; Sequence 3: simultaneous exposure of irinotecan and 5FU. Values represent the average of 3 independent determinations using triplicate cultures for each point data. Means ± SD are given. CI <1 indicates synergy.

The dose levels of irinotecan and 5FU were decreased to 30 mg/kg for irinotecan and to 20 mg/kg for 5FU. Figure 3 illustrates the life span of the different groups of mice: in sequence 2, the toxicity was significantly higher, with 5 out of 9 mice dying. In sequences 1 and 3, 0 death and 1 toxicity-related death, respectively, occurred.

Finally, when 20 mg/kg/day × 5 day irinotecan was combined with the same dose of 5FU, no toxicity-related death was observed whatever the schedule. However, the maximum body-weight loss was significantly higher in sequence 2 (15.8 ± 7.3%) than in sequence 1 (8.4 ± 7%) (p = 0.028) or sequence 3 (4.9 ± 5.3%) (p = 0.0001) (Fig. 4).

Anti-tumoral activity of irinotecan/5FU chemotherapy

Table II and Figure 5 summarize the anti-tumoral activity obtained when combining irinotecan and 5FU. When 30 mg/kg/day irinotecan and 20 mg/kg/day 5FU were administered simultaneously, the mean time to reach 5 times the initial volume was 35.7 ± 3.7 days. When 5FU was administered 6 hr before irinotecan, MTR was 36.7 ± 5.2 days. In the other schedule (irinotecan before 5FU), no conclusion could be drawn in terms of anti-tumoral activity, due to toxicity (5 out of 9 mice died). When 20 mg/kg/day irinotecan was simultaneously combined with 20 mg/kg/day 5FU, MTR was 28.3 ± 4.3 days. It reached 32.5 ± 3.7 and 34.9 ± 3.8 days for sequences 1 and 2 respectively. These data significantly differed from simultaneous administration (p = 0.014 and 0.0029 respectively).

DISCUSSION

Inhibitors of DNA topoisomerase I represent a new class of anti-cancer agents currently being used in early clinical trials. Preliminary studies suggest that irinotecan will be useful in metastatic colorectal carcinoma (Rougier *et al.,* 1997), raising the question of how to rationally combine topoisomerase-I inhibitors with 5FU, the cornerstone anti-cancer drug in metastatic colorectal cancer.

While irinotecan is thought to exert its anti-tumoral activity following transformation to a highly potent metabolite, SN-38, via a carboxylesterase, it appears to have its own inhibitory effects on DNA and RNA synthesis at high concentrations (about 10 μM), corresponding to the concentrations we used (Kawato *et al.,* 1991). Moreover, the use of irinotecan might be important, since 5FU pre-treatment might modify the cellular pharmacology of irinotecan and consequently its transformation into SN-38. We therefore used this drug for our *in vitro* experiments, which clearly demonstrate that the cytotoxic interaction between irinotecan and 5FU is schedule-dependent. Simultaneous exposure of these 2 agents for 24 hr was only additive. Sequential exposure to 5FU for 24 hr followed by irinotecan for 24 hr, or vice versa, had dramatic synergistic effects. Until now, *in vitro* studies on interactions between irinotecan and 5FU have not been conclusive: Kano *et al.* (1992) reported an additive effect between the 2 drugs when human T-cell-leukemia cell line MOLT-3 was exposed simultaneously for

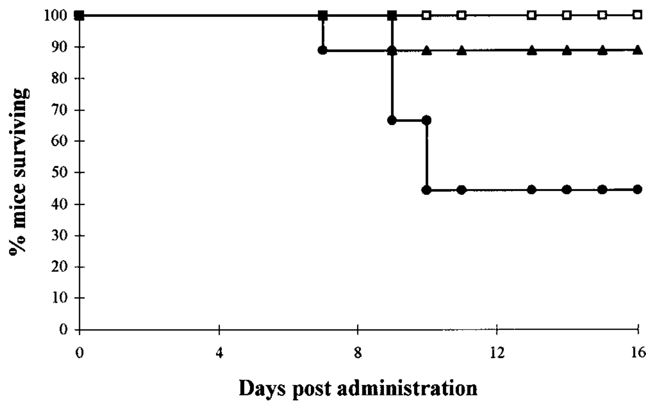

FIGURE 3 – Acute toxicity of the different schedules of association between 5FU (20 mg/kg/day × 5 days) and irinotecan (30 mg/kg/day × 5 days) □, sequence 1: 5FU administered 6 hr before irinotecan; ●, sequence 2: irinotecan administered 6 hr before 5FU; ▲, sequence 3: irinotecan and 5FU administered simultaneously.

TABLE II – ANTI-TUMORAL ACTIVITY AND TOXICITY OF THE DIFFERENT SCHEDULES OF ASSOCIATION BETWEEN 5FU AND IRINOTECAN

Schedule	MTR (days)[1]	Number of toxic deaths/Number of treated mice
Controls	14.9 ± 3.7	0/50
Irinotecan 20 mg/kg/day	25.5 ± 3.9	0/21
Irinotecan 30 mg/kg/day	32.1 ± 5.5	1/15
5FU 20 mg/kg/day	17.1 ± 4.7	0/28
Irinotecan 30 mg/kg + 5FU 20 mg/kg		
Sequence 1	36.7 ± 5.2	0/9
Sequence 2	N.E.	5/9
Sequence 3	35.7 ± 3.7	1/17
Irinotecan 20 mg/kg + 5FU 20 mg/kg		
Sequence 1	32.5 ± 3.7	0/9
Sequence 2	34.9 ± 3.8	0/9
Sequence 3	28.3 ± 4.3	0/17

[1]MTR, mean time (days) to reach 5 times the initial tumor volume, ± SD. Each group comprised at least 9 mice. In sequence 1, 5FU was administered i.v. 6 hr before irinotecan; in sequence 2, irinotecan was administered i.v. 6 hr before 5FU; in sequence 3, 5FU and irinotecan were administered simultaneously.

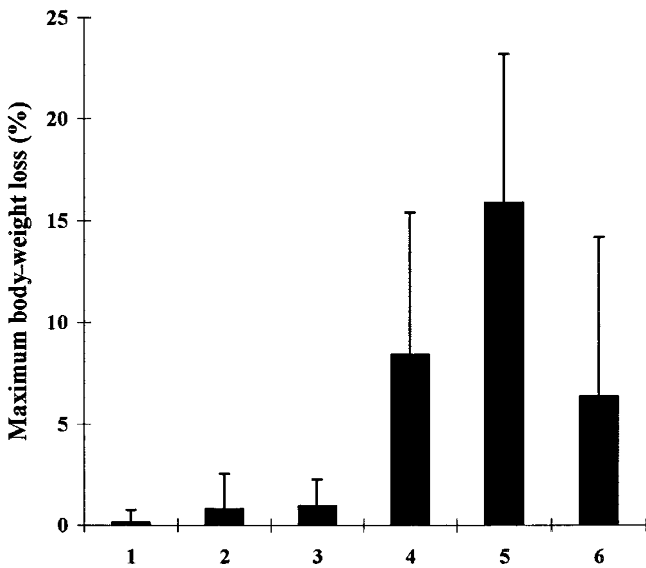

FIGURE 4 – Acute toxicity of the different schedules of association between 5FU (20 mg/kg/day × 5 days) and irinotecan (20 mg/kg/day × 5 days). Toxicity was determined by mean maximum body-weight loss occurring during 14 days following drug administration. 1, controls; 2, 5FU alone; 3, irinotecan alone; 4, sequence 1: 5FU, then irinotecan; 5, sequence 2: irinotecan, then 5FU; 6, sequence 3: simultaneous administration.

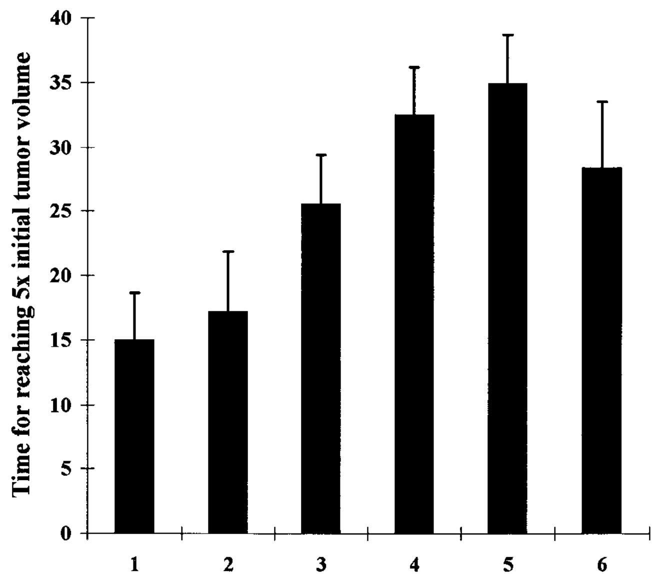

FIGURE 5 – Anti-tumoral activity of the different schedules of association between 5FU (20 mg/kg/day × 5 days) and irinotecan (20 mg/kg/day × 5 days). MTR represents the number of days necessary to reach 5 times the initial tumoral volume, expressed as the mean ± SD. Each group comprised at least 9 mice. 1, controls; 2, 5FU alone; 3, irinotecan alone; 4, sequence 1: 5FU, then irinotecan; 5, sequence 2: irinotecan, then 5FU; 6, sequence 3: simultaneous administration.

3 days to 5FU and either irinotecan or SN-38. Mans *et al.* (1996) showed in a panel of 4 human colon-carcinoma cell lines, that 5FU cytotoxicity was potentiated following 48 hr pre-treatment with irinotecan. In HCT-8 cell lines, a sequential interaction was noted between 5FU plus leucovorin (LV) and SN-38: median-effect analysis revealed antagonism in simultaneous 24-hr exposure, also in sequential 24-hr exposure to 5FU/LV and SN-38 (Erlichman *et al.,* 1996). However, the sequence SN-38 followed by 5FU/LV showed marked synergy. In the same model, Aschele *et al.* (1996) showed synergy between Raltitrexed, a specific inhibitor of thymidylate synthase, and SN-38, especially when cells were exposed sequentially to both drugs. In contrast, no positive interaction between topotecan, another topoisomerase-I inhibitor, and 5FU occurred in the same cellular model with simultaneous exposure (Kaufmann *et al.,* 1996).

In general, synergism was always observed when cells were first exposed to topoisomerase-I inhibitors irinotecan or SN-38. Inconsistent results were obtained with the other sequential-exposure schedule. These apparent discrepancies would be cell-line-dependent, even if the sensitivity of different cell lines to irinotecan was not related to the cellular carboxylesterase activity (Jansen *et al.,* 1997).

Since inhibitors of topoisomerase I seem to be highly S-phase-specific, their cytotoxicity is likely a function of exposure time to a drug above some critical level (Rivory and Robert, 1995). Consequently, we used repeated schedules of drug for *in vivo* studies, as suggested by Houghton *et al.* (1995). Moreover, administration of high dosage by i.v. bolus was not possible in a mouse model: when a single i.v. dose of irinotecan exceeded 60 mg/kg, immediate life-threatening toxicity occurred, due to a cholinergic syndrome as

reported by Vassal *et al.* (1996). Because irinotecan at 20 to 40 mg/kg/day given on the 5-day i.v. schedule results in acceptable toxicity in mice, we used this administration schedule as a platform for combining this agent with 5FU. We also administered 5FU daily doses, the recommended dose being 30 mg/kg/day *i.e.,* 150 mg/kg total dose, in agreement with data of Naguib *et al.* (1994).

As experiments had shown, *in vitro,* the sequence and time interval appear to be important factors for the anti-tumor activity and toxicity of the irinotecan + 5FU combination. In combination, irinotecan and 5FU could be administered at only 33% and 50% respectively of MTDs as a single agent (toxicity index ranging from 0.83 to 1). The combination was highly toxic, but the toxicity was schedule-dependent. When irinotecan (30 mg/kg/day × 5 days) was administered before 5FU (20 mg/kg/day × 5 days), there were toxicity-related deaths (5 out of 9 mice). By contrast, with the other sequences (simultaneous or 5FU before irinotecan), there were no toxic deaths. This sequence-related toxicity was confirmed when the dose level of irinotecan was decreased: the maximum body-weight loss was significantly higher when irinotecan was administered before 5FU. These results are in marked contrast to the data obtained when combining irinotecan and etoposide or 5FU. (Houghton *et al.,* 1996). However, the administration schedule was not the same: 5FU (56 mg/kg/day) was administered on days 1, 7 and 14 in combination with irinotecan given over 2 × 5 days in 2 consecutive weeks (days 1–5 and 8–12). The observed toxicity was less than additive. This discrepancy can be explained by the fact that the drugs were not administered daily and sequentially. Our data strongly suggest that toxicity is schedule-dependent, being highest when irinotecan was given before 5FU.

Simple anti-tumoral additivity was observed against the HT-29 tumor model, when the 2 drugs were administered simultaneously. These results confirm the data obtained by Houghton *et al.* (1996) on 4 different colon xenografts and showing that irinotecan and 5FU, whether combined or not with leucovorin, were not superior to irinotecan alone. Interestingly, our data demonstrate that higher anti-tumoral activity was obtained when drugs were administered sequentially to mice with a delay of 6 hr, thus confirming the results of our *in vitro* study. Hence, in trying to put this all together, one might conclude that sequential administration of irinotecan and 5FU, whatever the order, produced much more anti-tumoral activity than simultaneous administration, for equal or only slightly higher toxicity.

The underlying basis for the sequence-dependent activity reported here is not totally known, but different hypotheses are possible. There are at least 2 possible explanations for the effect of irinotecan in sequential treatment. One is that irinotecan treatment induced an increase in S-phase cells and a decrease in G_1 phase (Rivory and Robert, 1995), and this induced synchronization could be the basis of the increase in 5FU cytotoxicity. Another is that tissue-specific factors, including growth factors and oncogenes induced by irinotecan-stabilized cleavable DNA/topo-I complexes may modulate the cell-cycle phase, as discussed by Baserga (1990). It might also be postulated that 5FU causes single-strand DNA (Lonn and Lonn, 1984), which may involve direct incorporation into DNA or may occur as a consequence of accumulation and incorporation of dUTP with subsequent excision repair (Schuetz *et al.,* 1984). Topoisomerase I has an active role in repair, and the basis of interaction between 5FU and irinotecan would be the same as for topoisomerase-I inhibitors and ionizing radiations (Boothman *et al.,* 1994) or cisplatin (Goldwasser *et al.,* 1996).

At the clinical level, different phase-I clinical trials have been performed in Japan, Europe and the United States (Rougier and Bugat, 1996). Depending on the administration schedule of irinotecan (once weekly or every 3 weeks), of 5FU (bolus or continuous infusion), and the association or not with leucovorin, concurrent administration of substantial doses is feasible in terms of toxicity, and clinically-relevant dose intensity is achievable for each compound. Moreover, no pharmacokinetic interaction appeared between the different compounds, and encouraging preliminary response data were achieved (Saltz *et al.,* 1996). The results of the present study, suggesting that irinotecan and 5FU should be administered sequentially for obtaining optimal anti-tumoral effect, are of interest for further clinical development of this type of regimen in patients with metastatic colorectal cancer.

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