

Selective Modulation of the Therapeutic Efficacy of Anticancer Drugs by Selenium Containing Compounds against Human Tumor Xenografts

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ABSTRACT

Purpose: Studies were carried out in athymic nude mice bearing human squamous cell carcinoma of the head and neck (FaDu and A253) and colon carcinoma (HCT-8 and HT-29) xenografts to evaluate the potential role of selenium-containing compounds as selective modulators of the toxicity and antitumor activity of selected anticancer drugs with particular emphasis on irinotecan, a topoisomerase I poison.

Experimental Design: Antitumor activity and toxicity were evaluated using nontoxic doses (0.2 mg/mouse/day) and schedule (14–28 days) of the selenium-containing compounds, 5-methylselenocysteine and seleno-L-methionine, administered orally to nude mice daily for 7 days before i.v. administration of anticancer drugs, with continued selenium treatment for 7–21 days, depending on anticancer drugs under evaluation. Several doses of anticancer drugs were used, including the maximum tolerated dose (MTD) and toxic doses. Although many chemotherapeutic agents were evaluated for toxicity protection by selenium, data on antitumor activity were primarily obtained using the MTD, 2 × MTD, and 3 × MTD of weekly ×4 schedule of irinotecan.

Results: Selenium was highly protective against toxicity induced by a variety of chemotherapeutic agents. Furthermore, selenium increased significantly the cure rate of xenografts bearing human tumors that are sensitive (HCT-8 and FaDu) and resistant (HT-29 and A253) to irinotecan. The high cure rate (100%) was achieved in nude mice bearing HCT-8 and FaDu xenografts treated with the MTD of irinotecan (100 mg/kg/week × 4) when combined with selenium. Administration of higher doses of irinotecan (200

and 300 mg/kg/week × 4) was required to achieve high cure rate for HT-29 and A253 xenografts. Administration of these higher doses was possible due to selective protection of normal tissues by selenium. Thus, the use of selenium as selective modulator of the therapeutic efficacy of anticancer drugs is new and novel.

Conclusions: We demonstrated that selenium is a highly effective modulator of the therapeutic efficacy and selectivity of anticancer drugs in nude mice bearing human tumor xenografts of colon carcinoma and squamous cell carcinoma of the head and neck. The observed *in vivo* synergic interaction is highly dependent on the schedule of selenium.

INTRODUCTION

The ultimate goal of chemotherapy cure is rarely achieved in patients with advanced epithelial malignancies. Resistance and the lack of therapeutic selectivity to conventional drugs used alone and in combination remain major obstacles to curative therapy. For example, irinotecan/5-fluorouracil (FU) combination therapy has an overall response rate of ~50% in patients with advanced colorectal cancer, with a significant impact on overall survival (1, 2). However, this combination is associated with significant dose-limiting toxicities (1, 2). The clinical challenge faced today is to develop new drugs and treatment modalities that will impact cure rates significantly by reversing drug resistance, with minimal toxicity.

During the last several years, this laboratory has been developing new treatment modalities based on selective modulation of the therapeutic efficacy of clinically active drugs, irinotecan and FU, alone and in combination. Results generated in nude mice bearing human tumor xenografts indicate that irinotecan, but not FU, induces molecular changes associated with apoptosis and cell cycle perturbation in a dose- and time-dependent manner (3, 4). Administration of a DNA synthesis inhibitor (*e.g.*, FU) after optimal molecular alterations are induced by irinotecan significantly increases cure rates (from 20% to 100%) of human HCT-8 (colon) and FaDu (human squamous cell carcinoma of the head and neck; HSCCHN) tumor xenografts. The high cure rates achieved with sequential weekly administration of irinotecan, followed 24 h later by FU could not be achieved when FU was administered before or concurrently with irinotecan (the most common clinical practice). We have demonstrated that the high cure observed irinotecan followed by FU is associated with poly(ADP-ribose) polymerase (PARP) cleavage, Bax activation, induction of apoptosis, and recruitment of cells into S phase. These molecular changes induced by irinotecan

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were optimal at 24 h before FU therapy was initiated. Lack of alteration of these specific markers by irinotecan was associated with resistance to the irinotecan/FU combination.¹

Although the sequential combination of irinotecan, followed 24 h later by FU, yielded high cure rates in HCT-8 (colon) and FaDu (HSCCHN) human tumor xenografts, two human tumor xenografts, A253 (HSCCHN) and HT-29 (colon carcinoma), are relatively resistant to this combination. Thus, there is a critical need to develop new approaches that reverse drug resistance, while enhancing therapeutic selectivity and cure rates.

Selenium is at various stages of clinical development as a chemopreventive agent based on published *in vitro* data demonstrating its ability to induce specific molecular perturbation associated with apoptosis and angiogenesis (5–11). 5-Methylselenocysteine (MSC) and seleno-L-methionine (SLM) are stable, water-soluble compounds, which are quantitatively absorbed orally (12), and hydrolyzed by β -lyase to methylselenol, the presumed selenium active metabolite responsible for the activity of selenium (6, 12–14). Mammals readily metabolize MSC to methylselenol in a stoichiometric manner as soon as it enters into cells. Studies by Wang *et al.* (6, 8) demonstrate the antimutagenic and proapoptotic activities of methylselenic acid in vascular endothelial cells. These researchers found that the specific molecular alteration and cell cycle perturbation induced by selenium are functions of selenium dose and schedule. High concentrations of selenium induce high levels of apoptosis and DNA fragmentation. Selenium-induced apoptosis was associated with increased phosphorylation of p53 mitogen-activated protein kinase, dephosphorylation of Akt and extracellular signal-regulated kinase 1/2, and PARP cleavage (5, 15–17).

PARP is a nuclear protein activated by single- and double-strand DNA damage (18). Induced cleavage of PARP by Topoisomerase (Topo) I poisons, such as irinotecan, has been demonstrated *in vitro* and is associated with programmed cell death (19, 20). Internucleosomal DNA fragmentation, caspase-mediated cleavage of PARP, and degradation of key cytoskeletal proteins principally underlie these cellular and nuclear changes (21, 22). Cleavage of PARP inactivates the enzyme and its ability to respond to DNA strand breaks, and directs the cell toward an apoptotic death (23). PARP cleavage has been recognized as a sensitive marker of caspase-mediated apoptosis. Caspase-3 has a much higher specific activity for PARP cleavage than other caspases (24, 25).

Selenium is in clinical trial for prevention in cancers of the skin, lymphoma, thyroid, head and neck, prostate, pancreatic, lung, and colon, and had a significant reduction in total cancer incidence and mortality rates (14, 26–35). Selenium (sodium selenite) can reduce cisplatin-induced toxicity without reducing the antitumor activity in mice bearing yolk sac and Prima breast tumors (36, 37). Data from our laboratory demonstrate that MSC potentiate cell growth inhibition induced by SN-38, and the potentiation is associated with activation of GADD153,

dephosphorylation of Akt, PARP cleavage, and induction of apoptosis.¹

On the basis of these published reports and preliminary data generated in our laboratory, studies were initiated in nude mice bearing irinotecan-sensitive and -resistant [no cures by the maximum tolerated dose (MTD) of irinotecan] tumor xenografts to evaluate the potential role of selenium-containing compounds as selective modulators of the therapeutic selectivity and efficacy of irinotecan.

Data presented here indicate that nontoxic doses and schedules of selenium-containing compounds, in combination with irinotecan, achieved higher cure rates, with reduced host toxicity in nude mice bearing human tumor xenografts resistant to the combination of irinotecan/FU combination.

MATERIALS AND METHODS

Animals. Female athymic nude mice (*nu/nu*, body weight, 20–25 g), 8–12 weeks of age, were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and kept 5 mice/cage with water and food *ad libitum* according to Institutional Animal Care and Use Committee approval.

Tumors. Head and neck squamous cell carcinoma xenografts A253 (well differentiated) and FaDu (poorly differentiated), and colorectal xenografts HCT-8 (poorly differentiated) and HT-29 (moderately differentiated) were purchased from American Type Culture Collection (Manassas, VA). The cell lines were maintained as a monolayer in RPMI 1640 supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA). Xenografts were initially established by implanting s.c. 10^6 cultured individual cell lines and passed several generations by transplanting ~50 mg non-necrotic tumor tissues before treatment. Treatments in the treated mice were started when tumors reached ~200 mg.

Tumor Measurement. Two axes of the tumor (L, longest axis; W, shortest axis) were measured with a vernier caliper. Tumor weight (mg) was calculated as: $\frac{1}{2}(L \times W^2)$ (mm). Relative tumor volume (percentage) was calculated by actual tumor weight (ATW) over initial tumor weight (ITW, day 0) as follows: $ATW \div ITW \times 100\%$. Measurements were taken once a day during the first 10 days and two to three times a week thereafter.

Drugs. MSC and SLM were purchased from Sigma (St. Louis, MO) and dissolved in sterile saline at a concentration of 1 mg/ml. Irinotecan (PNU-101440E) was supplied by Pharmacia Corp. (Kalamazoo, MI), and the injectable formulation of irinotecan was obtained by dissolved irinotecan (20 mg/ml), D-sorbitol (45 mg/ml; Sigma), and D-lactic acid (0.9 mg/ml; Sigma) in double-distilled water heated 80–90°C for 10 min. The pH was adjusted to 3.5. The solution was sterile-filtered and stored protected from light until administration. FU was purchased from Hoffmann-La Roche Inc. (Nutley, NJ) as a solution of 50 mg/ml in 10-ml vials. Taxol and *cis*-diamminedichloroplatinum(II) were purchased from Bristol-Myers Squibb Co. (Princeton, NJ) as a solution of 6 mg/ml in 5-ml and 1 mg/ml in 50-ml vials, respectively. Oxaliplatin was purchased from Sanofi-Synthelabo (New York, NY) as a solution of 5 mg/ml in 10-ml vials. Doxorubicin was purchased from Novaplus (Bed-

¹ Y. M. Rustum, S. Cao, F. A. Darrani, M. B. Yin, R. Azrak, and K. Toth, unpublished observations.

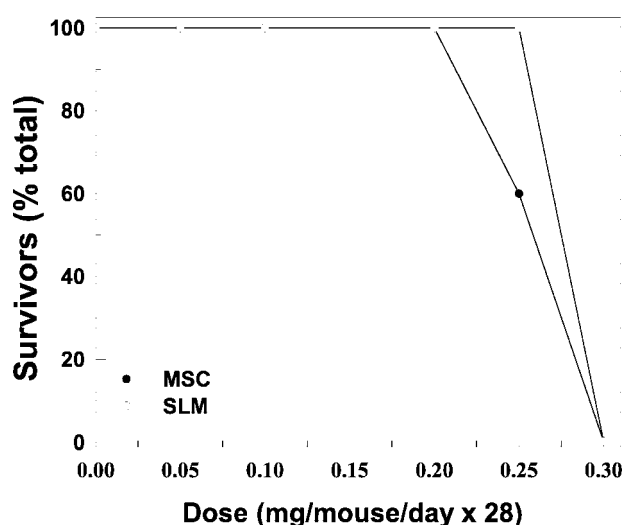


Fig. 1 Dose response of 5-methylselenocysteine (MSC) and seleno-L-methionine (SLM) in nude mice, identification of the maximum tolerated dose of MSC and SLM in nude mice. MSC and SLM were administered p.o. daily for 28 days. MSC 0.25 mg death from day 7 to day 10. MSC 0.3 mg death from day 5 to day 6. SLM 0.3 mg death from day 7 to day 13.

ford, OH) as a solution of 2 mg/ml in 25-ml vials. All of the drugs were diluted in sterile saline.

Drug Doses and Schedules. MSC and SLM were administered by daily oral route at various doses (from 0.01 to 0.6 mg/mouse/day) from 14 to 42 days. All of the chemotherapeutic agents were administered by i.v. push. Two treatment schedules were used: (a) i.v. push once a week for 4 weeks (weekly \times 4) with irinotecan, FU, and oxaliplatin at the MTD or above; (b) a single i.v. injection (i.v. \times 1) with cisplatin, Taxol, and doxorubicin at the MTD or above. For the combination of MSC or SLM with chemotherapeutic agents, MSC or SLM were given 7 days before therapy for a total of 28 days with weekly \times 4 schedule and 14 days with i.v. \times 1. For irinotecan combination, various schedules of MSC were used simultaneously for (same day) 1, 3, 7, and 21 days before irinotecan in a total of 21–42 days. Each experiment was repeated at least twice.

MTD and Toxicity Evaluation. The MTD was defined as the maximum dose that caused no drug-related lethality and which produced animal body weight loss of $<20\%$ of original weight. The kinetics of drug-induced toxicities (body weight loss, diarrhea, and lethality) were determined daily for a minimum of 4 weeks and observed at least twice a week thereafter.

Antitumor Activity. When tumors reached approximately 200–250 mg (7–8 days after tumor transplantation), the mice were separated into different treatment groups of 5 mice each. Antitumor activity was assessed by MTGI, which is mean tumor weight (MTW) of the treated group (TG) compared with the untreated control group (CG) at same time (most time is day 10–12 when control group was sacrificed because the large tumor was >2000 mg), which is calculated as $MTGI = (MTWTG - MTWCG) \div MTWCG \times 100\%$. The tumor doubling time was defined as the mean time for the tumor to reach twice its initial weight (at treatment beginning, day 0). Tumor

response was expressed as partial response when tumor weight was temporarily reduced by at least 50% compared with initial tumor size and as complete response when tumor was undetectable by palpation at site of transplant. Cure was defined as animal surviving with no tumor for at least 3 months after treatment, at which time the animals were sacrificed. Under these conditions, all of the tumor cells were killed; otherwise, even if one single tumor cell survived treatment, it would have developed into a large tumor within 2–3 weeks after termination of therapy. The response rate was expressed as the percentage of animals in the group. All of the studies were performed in accordance with Institutional Animal Care and Use Committee and under an approved Institute protocol.

RESULTS

Identification of MTD of Selenium Containing Compound in Nude Mice. The MTDs of MSC and SLM orally administered daily for 28 days to mice were determined. The results are shown in Fig. 1. The data indicate that the MTDs for MSC and SLM are 0.2 and 0.25 mg/mouse/day \times 28, respectively. The data also indicate a relatively steep dose response, with 100% lethality achieved with 0.3 mg/kg/day \times 28 of either agent. Thus, for evaluation of therapeutic selectivity of chemotherapeutic agents in combination with MSC or SLM, 0.2 mg/mouse/day \times 28 was determined to be a safe and nontoxic dose and schedule.

Identification of the MTD of Chemotherapeutic Agents with and without SLM or MSC. Using clinical relevant schedules for irinotecan, oxaliplatin, and FU (weekly \times 4), and cisplatin, Taxol, and doxorubicin (single i.v. injection), the MTD of these agents in combination with MSC was determined in nude mice. The results are shown in Table 1. The data indicate that the MTD for each agent with MSC is higher, suggesting that MSC is offering significant protection against toxicity induced by these agents, as well as the potential for increased selectivity and efficacy of irinotecan and FU.

Optimization of Selenium Schedule. Because we determined that 0.2 mg/mouse/day \times 28 is a nontoxic dose and schedule as shown in Fig. 1, and because the duration of treatment of selenium may vary dependent on the schedule of the anticancer drug under evaluation, the optimal duration of

Table 1 MTD^a of chemotherapeutic agents alone and in combination with MSC (0.2 mg/mouse/day)

Drug	Schedule	MTD (mg/kg)	
		– MSC	+ MSC ^b
Irinotecan	i.v. weekly \times 4	100	200
FU	i.v. weekly \times 4	100	125
Irinotecan + FU	i.v. weekly \times 4	50 (each)	ND
Oxaliplatin	i.v. weekly \times 4	7.5	12.5
Cisplatin	i.v. \times 1	8	15
Taxol	i.v. \times 1	35	75
Doxorubicin	i.v. \times 1	10	12.5

^a MTD, maximum tolerated dose; MSC, methylselenocysteine; FU, 5-fluorouracil; ND, not determined.

^b For the combination, MSC was administered p.o. 7 days before therapy in total 28 days for the weekly \times 4 schedule and 14 days for the i.v. \times 1 schedule.

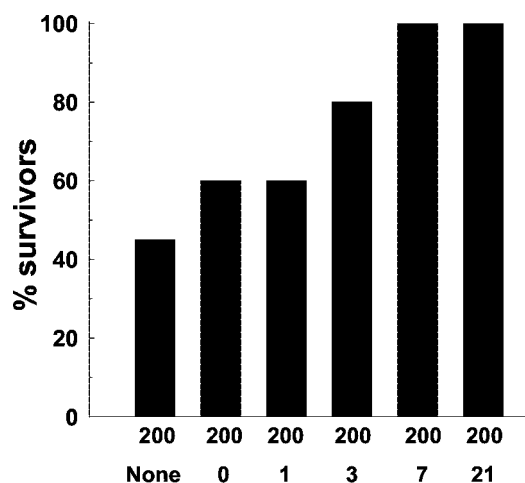


Fig. 2 Schedule-dependency of 5-methylselenocysteine (MSC) against irinotecan induced toxicity, defining duration of selenium treatment for optimal protection from irinotecan-induced toxicity in normal nude mice. Selenium (0.2 mg/mouse/day) was administered p.o. either with no prior selenium treatment (administered together with selenium on the same day), 1, 3, 7, or 21 days before irinotecan treatment. Selenium treatment was continued for 21 days during irinotecan treatment. Minimum of 5 animals/group with experiment repeated at least twice.

pretreatment with selenium before drug administration was determined (Fig. 2). The data in Fig. 2 were generated using toxic doses of irinotecan (200 mg/kg/week \times 4) alone and with selenium orally administered simultaneously with irinotecan, 1, 3, 7, and 21 days before the administration of 200 mg/kg/week \times 4 of irinotecan. With all of the treatment modalities, selenium treatment continued for 21 days during the treatment. The data clearly demonstrated that the optimal protection against irinotecan induced toxicity, 7 days selenium pretreatment before irinotecan administration were required, the protective effect was comparable to 21 days selenium pretreatment (Fig. 2). Thus, for all of the subsequent experiments, the 28 days of daily oral administration of 0.2 mg/mouse/day with the first dose were administered 7 days before drug treatment was used.

Comparative Evaluation of MSC and SLM as Modulators of Toxicity Induced by Irinotecan in Nude Mice. To determine whether MSC protection of irinotecan-induced toxicity is specific for MSC, a comparative study of MSC and SLM was carried out. Irinotecan was administered by i.v. push once a week for 4 weeks. MSC and SLM (0.2 mg/mouse/day) were given p.o. daily for 28 days, with the first dose administered 7 days before the weekly \times 4 treatment with irinotecan. The results are shown in Fig. 3. With irinotecan alone at doses of 200 and 300 mg/kg/week \times 4, only 45% and 0% of the animals survived, respectively. In contrast, 100% and 80% of animals survived treatment with irinotecan at doses of 200 and 300 mg/kg/week \times 4, in combination with either MSC or SLM. These data demonstrate that with the dose and schedule used, both MSC and SLM were equally effective in protection from toxicity induced by high doses of irinotecan equivalent to two and three times the MTD. In combination with MSC or SLM, the MTD of irinotecan is 200 mg/kg/week \times 4, in contrast to 100 mg/kg/week \times 4 for irinotecan when administered alone.

Role of Selenium as Modulator of the Toxicity of Chemotherapeutic Agents. The effects of MSC on toxicity induced by chemotherapeutic agents with different structures and mechanisms of action (e.g., irinotecan, FU, cisplatin, oxaliplatin, and Taxol) were evaluated in nude mice. Irinotecan, FU, and oxaliplatin were administered by i.v. push weekly \times 4 schedule, whereas Taxol, *cis*-diamminedichloroplatinum(II), and doxorubicin were administered by a single i.v. injection. In all of the cases, drug doses used for evaluation in combination with MSC were toxic and above the MTD. The results shown in Fig. 4 indicate that MSC offered significant protection against toxicity induced by each chemotherapeutic agent. Thus, these data support the potential general applicability of MSC as a modulator of host toxicity induced by various classes of chemotherapeutic agents with different mechanisms of action.

Modulation of the Antitumor Activity of the MTD of Irinotecan by MSC. The effects of MSC on the antitumor activity of irinotecan at its MTD (100 mg/kg/week \times 4) in nude mice bearing human tumor xenografts were evaluated. The data summarized in Fig. 5 indicate that the cure rates increased from 20% to 100% in HCT-8 tumors, from 0% to 20% in HT-29, from 30% to 100% in FaDu, and from 10% to 60% in A253. This significant increase in antitumor activity of irinotecan was observed with decreased toxicity (less body weight loss).

Modulation of the Antitumor Activity in Tumor Xenografts Resistant to Irinotecan. Because the MTD of irinotecan (100 mg/kg) against HT-29 and A253 was less active (0–10% cures), the effects of higher doses of irinotecan with or without MSC were evaluated in nude mice bearing both HT-29 and A253 tumor xenografts. Using the MTD of irinotecan in combination with MSC (200 mg/kg), cure rates increased from 0% to 40% in HT-29 and from 20% to 80% in A253, without increased toxicity (Fig. 6). Furthermore, using 300 mg/kg irinotecan (three times the MTD), the overall cure rate of surviving

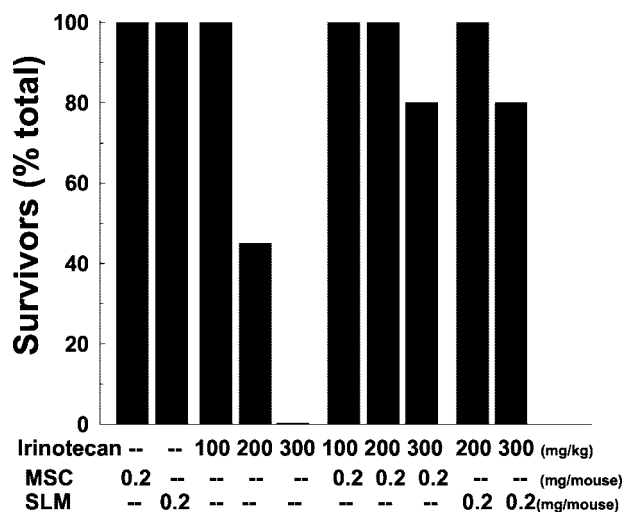


Fig. 3 5-Methylselenocysteine (MSC) and seleno-L-methionine (SLM) are equally effective ameliorators of irinotecan induced toxicity in nude mice, modulation of irinotecan toxicity by MSC and SLM. Irinotecan was administered by i.v. push once a week for 4 weeks, MSC and SLM were given p.o. daily for 28 days, and the first dose started 7 days before irinotecan treatment.

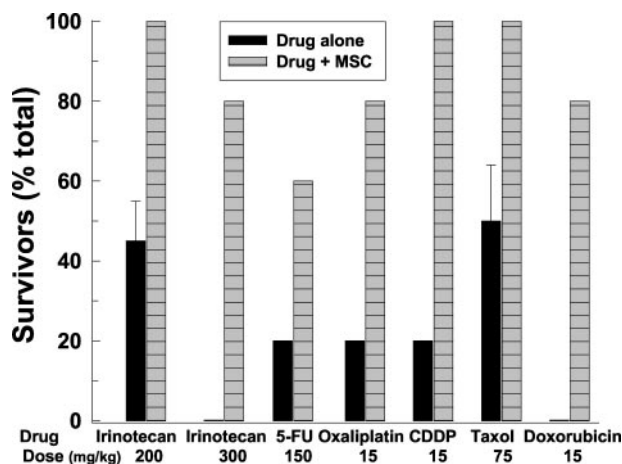


Fig. 4 Toxicity of chemotherapeutic agents \pm 5-methylselenocysteine (MSC) in nude mice; irinotecan, 5-fluorouracil (FU), and oxaliplatin were administered by i.v. push weekly \times 4 and MSC (0.2 mg/mouse/day) was given p.o. daily for 28 days; the first dose started 7 days before drug treatment. Taxol, cisplatin (CDDP), and doxorubicin were administered by a single i.v. injection and MSC by p.o. daily for 14 days, and the first dose started 7 days before drug treatment; bars, \pm SD.

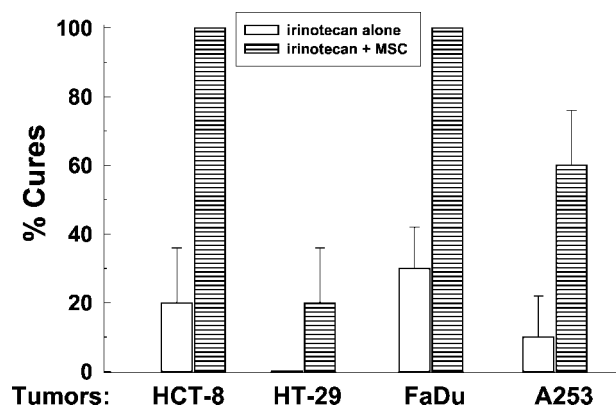


Fig. 5 5-Methylselenocysteine (MSC) potentiates the cures of human tumor xenografts treated with the maximum tolerated doses of irinotecan. Antitumor activity of the maximum tolerated dose of irinotecan (100 mg/kg/week \times 4) alone and in combination with MSC (0.2 mg/kg/day \times 28); bars, \pm SD.

animals bearing HT-29 increased to 60% and to 100% in animals bearing A253.

Kinetics of Response to Irinotecan with and without MSC in Nude Mice Bearing Human Tumor Xenografts.

The data in Fig. 7 demonstrate the heterogeneity in the kinetics of antitumor activity of irinotecan modulated by nontoxic dose and schedule of MSC in human A253, FaDu (HSCCHN), HT-29, and HCT-8 (colon) tumor xenografts. The data indicate that complete tumor response in HCT-8 and FaDu xenografts is achieved within 1–2 weeks after termination of the 4 weeks of irinotecan/MSC therapy. The data also indicate that heterogeneity in response to the MTD of irinotecan was completely eliminated when combined with MSC in HCT-8 and FaDu tumor xenografts. For xenografts with less-sensitive A253 and

HT-29 tumors, whereas antitumor activity of irinotecan at its MTD (100 mg/kg/week \times 4) was enhanced by MSC, optimal response (cures) without increasing toxicity was achieved at higher doses of irinotecan (200 mg/kg/week \times 4). Whereas 300 mg/kg/week \times 4 irinotecan was toxic with 100% lethality in treated animals, in combination with MSC, lethality was observed in only 20% of treated animals. The data in Fig. 7 indicate that maximum cure rates in HT-29 and A253 xenografts were achieved only when higher doses of irinotecan were administered in combination with MSC; 80% of the animals survived 300 mg/kg irinotecan in combination with MSC, and 80% and 100% of these HT-29 and A253 xenografts were cured of this tumor, respectively.

Comparative Antitumor Activity of Irinotecan \pm MSC with Standard Chemotherapy of Irinotecan/FU Combination.

We have demonstrated previously that irinotecan, followed 24 h later by FU, is superior to FU being administered 24 h before or concurrently with irinotecan. The data in Fig. 8 compare the cure rates achieved with irinotecan or FU alone, in combination with MSC or FU, administered at their respective MTDs. All of the therapy was carried out against four advanced transplantable tumors, two human colorectal carcinomas [poorly differentiated (HCT-8) and well-differentiated (HT-29) tumors] and two human squamous cell carcinomas of the head and neck [poorly differentiated (FaDu) and well-differentiated (A253) tumors] transplanted into nude mice (xenografts).

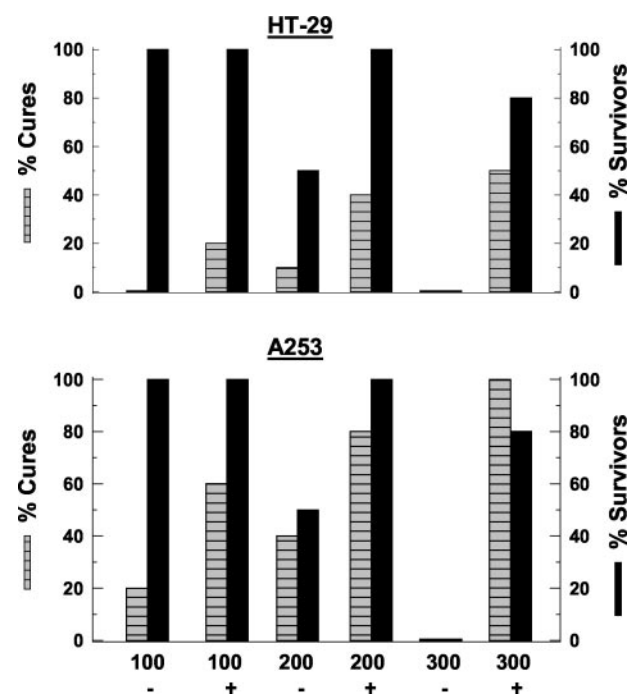


Fig. 6 5-Methylselenocysteine (MSC) protection of normal tissues is essential for maximizing cures of human tumor xenografts resistant to the maximum tolerated dose of irinotecan alone, antitumor activity of irinotecan alone and in combination with MSC in nude mice bearing human A253 and HT-29 tumor xenografts. The maximum tolerated dose of irinotecan alone is 100 mg/kg/week \times 4 and in combination with MSC is 200 mg/kg/week \times 4. Whereas 300 mg/kg/week \times 4 of irinotecan alone is toxic in 100% lethality in treated animals, in combination with MSC lethality was reduced to 20% of treated animals.

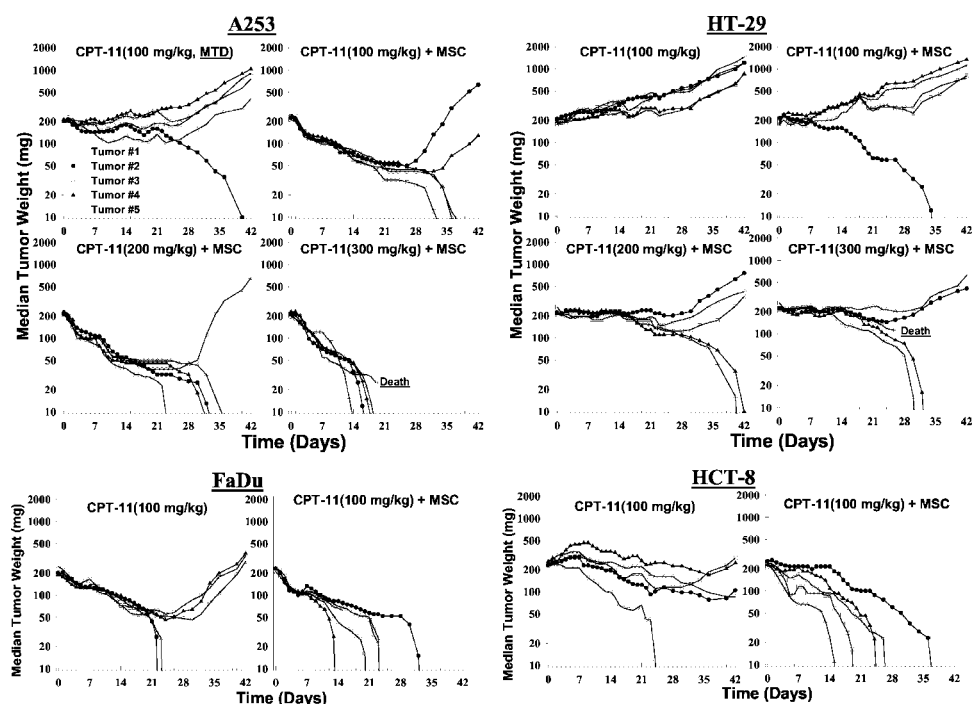


Fig. 7 Individual tumor xenografts response to irinotecan with and without 5-methylselenocysteine (MSC), kinetics of xenografts treated with irinotecan alone and in combination with MSC. The maximum tolerated dose (MTD) of irinotecan alone is 100 mg/kg/week \times 4 and in combination with MSC is 200 mg/kg/week \times 4.

The data indicate that treatment of HCT-8 and FaDu xenografts with the MTD of irinotecan (100 mg/kg/week \times 4) produced 20% and 30% cure rates, respectively. In contrast, administration of the MTD of irinotecan in combination with MSC yielded 100% cure rates in both tumors with no toxicity. The data in Fig. 8 also show that whereas HT-29 and A253 were resistant to the MTD of irinotecan (0–10% cures), the combination with MSC produced 20% and 60% cure rates, respectively. The data also indicate that all four of the tumors are resistant to the MTD of FU (100 mg/kg/week \times 4). Furthermore, the combinations of irinotecan/MSC was more effective than irinotecan/FU in all four tested tumor xenografts. The data clearly demonstrate that higher cure rates can be achieved, without toxicity, with irinotecan/MSC compared with irinotecan/FU in HT-29 and A253 xenografts resistant to irinotecan, FU, and the combination of the two drugs. These impressive therapeutic results in resistant xenografts were obtained with the administration of the MTD of irinotecan (100 mg/kg) in combination with nontoxic doses and schedules of MSC. In fact, similarly high cure rates can be achieved with 50% of the MTD of irinotecan in combination with MSC (data not shown). Whereas the MTD of the combination of irinotecan and FU is 50% of the MTD of each drug alone, the MTD of the combination of irinotecan/MSC is twice the MTD of irinotecan administered alone.

MSC Potentiates the Cure Rates of Irinotecan of Xenografts Bearing Drug Sensitive and *de Novo* Resistant Tumors: Overall Summary of the Results Obtained. The MTDs of irinotecan alone and in combination with MSC (0.2 mg/mouse/day \times 28) are 100 and 200 mg/kg/week \times 4, respectively (Table 1). In xenografts bearing animals treated with the MTD of irinotecan, the addition of MSC increased the cure rates

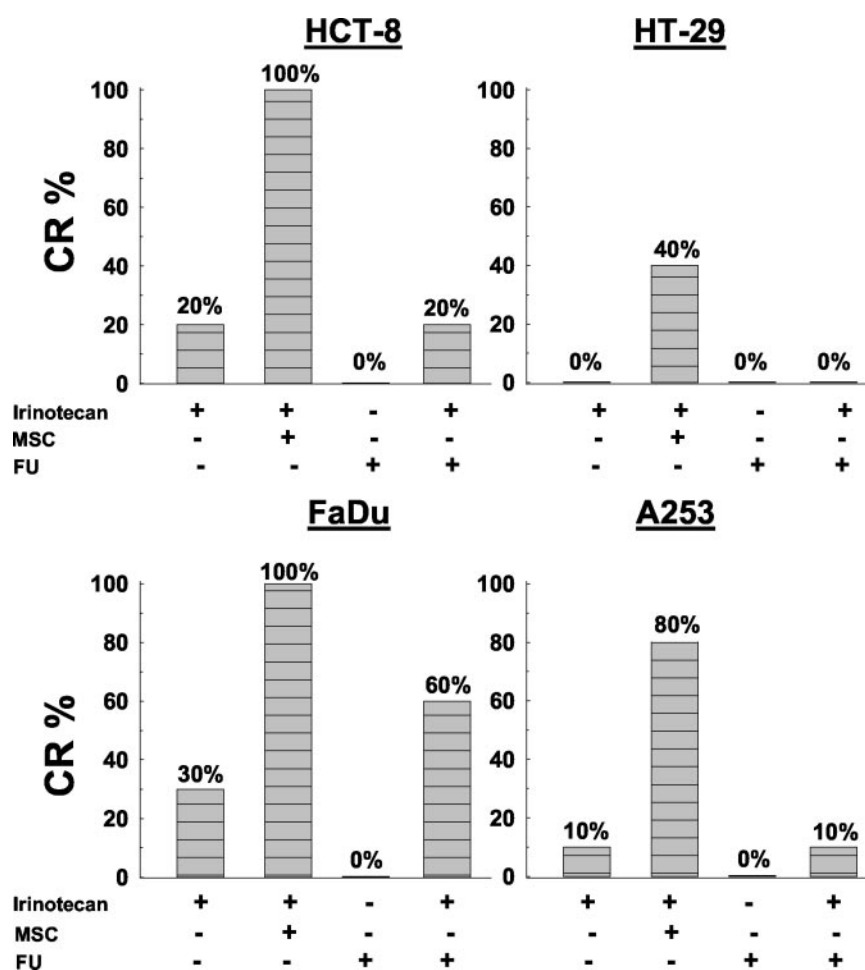
from 20% to 100% in HCT-8 and from 30% to 100% in FaDu. In contrast, cure rates of *de novo*-resistant xenografts (0% cures) were increased by the addition of MSC from 0% to 40% in HT-29 and from 10% to 80% in A253 tumors (Fig. 8).

The therapeutic results generated to date clearly demonstrate that modulation of the cure rates in irinotecan-sensitive and *de novo*-resistant tumor xenografts by MSC is highly selective. The data also indicate that optimal therapeutic cure in the less irinotecan-sensitive tumor xenografts (A253 and HT-29) was only achieved when higher doses of irinotecan protected by MSC were administered. These data suggest that in *de novo*-sensitive tumors such as HCT-8 and FaDu, maximum cure rates can be achieved with the MTD of irinotecan (100 mg/kg/week \times 4) when combined with MSC. In contrast, in the less-sensitive xenografts (A253 and HT-29), maximum cure rates can only be achieved with higher doses of irinotecan combined with MSC that can be administered without increasing toxicity.

DISCUSSION

Chemotherapy with anticancer drugs used alone and in combination is limited by resistance, lack of significant therapeutic selectivity, heterogeneity of tumor tissues, and drug delivery. Studies were carried out in our laboratory to identify and develop new approaches aimed at overcoming these therapeutic obstacles to curative therapy. Studies were carried out in well-characterized human tumor xenografts with respect to toxicity profile and antitumor response to anticancer drugs representing Topo I poison (irinotecan), antimetabolite (FU), DNA intercalator and Topo II inhibitor (Doxorubicin), microtubulin inhibitor (Taxol), and DNA intercalator (cisplatin and oxaliplatin). The

Fig. 8 5-Methylselenocysteine (MSC) in combination with irinotecan yields higher cures than irinotecan/5-fluorouracil (FU) combination against human tumor xenografts [maximum tolerated doses (MTD) comparison], comparative antitumor activity of the MTD of irinotecan/FU (50 mg/kg/week \times 4 of each) with the MTD of irinotecan/MSC (200 mg/kg/week \times 4) in nude mice bearing human HCT-8/HT-29 (colon), and FaDu/A253 (human squamous cell carcinoma of the head and neck) tumor xenografts.



potential modulators of drug response selected in this study were selenium-containing compounds, namely, MSC and SLM. Results reported herein demonstrate that selenium: (a) protects against organ-specific anticancer drug induced *in vivo* toxicity; and (b) simultaneously augments cure rates in drug-sensitive and more importantly in drug-resistant tumors. Thus, the data presented offer a new and novel approach developed preclinically and under evaluation of validation clinically at Roswell Park Cancer Institute.

Although the data reported herein evaluated the role of selenium as a selective modulator of *in vivo* toxicity induced by multiple chemotherapeutic agents, proof-of-principle that selenium augments the antitumor activity of anticancer drugs and protects against drug-induced toxicity was confirmed using irinotecan, a Topo I poison.

Irinotecan is a prodrug activated by a carboxylesterase enzyme to the active metabolite SN-38 that is \sim 100 times more potent than the parent compound. Irinotecan and its active compound SN-38 interact with the enzyme Topo I that relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and SN-38 bind the Topo I-DNA complex and prevent religation of these single-strand breaks. Cytotoxicity of irinotecan and SN-38 is due in part to the double-strand

DNA damage produced during DNA synthesis when replication enzymes interact with the ternary complex formed by Topo I, DNA, and SN-38. The cytotoxic effect of irinotecan and its metabolite SN-38 is specific to the S phase of the cell cycle. However, the presence of high quantities of Topo I in dividing and resting cells suggests that the mechanism of action is partially independent of the percentage of dividing cells, and that consequently irinotecan could be active on tumors with rapid and slow cell proliferation.

Irinotecan is clinically approved for the treatment of metastatic colorectal cancer patients used alone or in combination with other chemotherapeutic agents, FU, capecitabine, oxaliplatin, and Iressa. The 20% overall response rate with no significant impact on overall survival with irinotecan in colorectal cancer also is associated with grade 3 diarrhea, neutropenia, and mucositis in 20–30% of patients. Thus, there is a critical need to develop new approaches for therapy of colorectal cancer and other solid tumor malignancies.

Selenium is an essential trace element with an average nutritional intake of 50–350 μ g/day. Dietary selenium is predominantly in the form of organic compounds, primarily selenomethionine and selenocysteine, ingested in grains, meat, yeast, and vegetables. Selenium deficiency has been implicated

in an increased risk of carcinogenesis. Studies on selenium have focused recently on its chemopreventive activity (38, 39). Selenium plays an important role in a number of biological functions, and it affected multiple markers, including p53, the transcriptional factor, activator protein P, nuclear factor κ B, cyclooxygenase 2, protein kinases C and A, c-Jun NH₂-terminal kinase, and others (13, 40–57).

Although selenium compounds have been extensively evaluated as chemopreventive agents, little has been published regarding the potential of these agents in modifying toxicity and therapeutic efficacy of anticancer drugs against established tumor xenografts.

To demonstrate the effect of MSC on the antitumor activity of chemotherapy, nude mice were transplanted s.c. with tumor fragments of HSCCHN (A253 and FaDu) and colon carcinoma (HCT-8 and HT-29). Drug treatments were then initiated when tumor sizes approached about 200–250 mg. Irinotecan was administered at the MTDs of 100 mg/kg, and 200 or 300 mg/kg/week \times 4 in the presence or absence of MSC or SLM (0.2 mg/mouse/day).

The preclinical data presented here demonstrate that selenium-containing compounds, MSC and SLM, offered selective protection against toxicity induced by chemotherapeutic agents with different structure(s) and mechanism(s) of action. The results also demonstrate that the protective effects extend to other chemotherapeutic agents (paclitaxel, FU, *cis*-diamminedichloroplatinum(II), oxaliplatin, doxorubicin, and irinotecan). Despite the protective effects of selenium compounds on normal tissues during treatment with irinotecan, no evidence of antagonistic effects on antitumor activity was noted. On the contrary, the coadministration of MSC was associated with an improved antitumor activity resulting in high cure rates in all of the tumor xenografts with irinotecan-sensitive (HCT-8 and FaDu) and -resistant tumors (A253 and HT-29).

The results reported herein are novel, because it is the first demonstration that selenium-containing compounds are selective modulators of the therapeutic efficacy of anticancer drugs, representing several classes of chemotherapeutic agents, in human tumor xenografts. Selenium is the first modulator reported to date that results in significant improvement of the therapeutic index of anticancer drugs. This improvement is achieved through protection of normal tissues against toxicity induced by anticancer drugs, and simultaneously augments the antitumor activity and high cure rate. Of significant interest, the combination of MSC and irinotecan increased cure rates in xenografts for the relatively irinotecan-resistant tumors (A253 and HT-29) from 0–10% to 40–80%.

On the basis of the therapeutic data generated to date, a Phase I clinical trial is now in progress at Roswell Park Cancer Institute to validate the clinical usefulness of this approach and to identify associated mechanism(s).

Although the mechanism of action of selenium has been reported to be multifactorial, mechanisms associated with observed therapeutic selectivity of selenium in combination with anticancer drugs needs to be delineated. Preliminary results from our laboratory indicate that increased phosphorylation of the DNA damage regulating kinase chk2, and down-regulation of cdc6 expression resulting in increased level of preapoptotic DNA fragmentation are relevant parameters. Furthermore,

increased poly(ADP-ribose) polymerase and activation of caspase-3 are additional markers altered by selenium in combination with irinotecan. Our preliminary results also indicate that in tumor tissues (FaDu) with high vascular endothelial growth factor expression, selenium down-regulates the expression of this factor in a time- and dose-dependent manner. Studies are under way to confirm the initial findings and to identify critical mechanism(s) associated with the observed increased therapeutic efficacy of anticancer drugs by selenium.

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