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ORIGINAL ARTICLE

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Antitumor activity of cryptophycins: effect of infusion time and combination studies

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Abstract *Introduction/Purpose*: Cryptophycins are a family of antitubulin antitumor agents. A synthetic cryptophycin derivative (LY355703, CRYPTO 52) is in early clinical evaluation. The effect of infusion time on the antitumor activity of four cryptophycins was assessed in rats bearing the 13762 mammary carcinoma and combination treatment regimens were assessed in nude mice bearing human tumor xenografts. *Methods*: The cryptophycins were prepared in 2% PEG300/8% cremophor/90% normal saline and delivered by jugular vein catheter on days 7, 9 and 11 post tumor implant to 13762 tumor-bearing rats. The cryptophycins prepared in the same formulation were administered by intravenous bolus injection on an alternate day schedule for five doses to human tumor xenograft bearing nude mice. *Results*: An infusion time of 2 h in the rats increased the tumor growth delay produced by CRYPTO 52 and CRYPTO 55, while increasing the infusion time to 6 h continued to increase the tumor growth delay for CRYPTO 292 and CRYPTO 296. Administering CRYPTO 292 at a higher dose two times was more effective than administering it at a lower dose three times. The tumor growth delays produced by the cryptophycins in the rat 13762 mammary carcinoma were greater than those with cisplatin, doxorubicin, 5-fluorouracil and 5×3 Gray and comparable with cyclophosphamide and gemcitabine. Combination studies were carried out in human tumor xenografts including the MX-1 breast carcinoma, the Calu-6 non-small cell lung carcinoma, the H82 small cell lung carcinoma and the SW-2 small cell lung carcinoma. CRYPTO 52 and CRYPTO 55 combined with doxorubicin, paclitaxel and 5-fluorouracil to form highly effective regimens against the human MX-1 breast carcinoma. CRYPTO 52 and CRYPTO 55 were also highly effective against the three lung carcinoma xenografts when combined with the antitumor platinum complexes, cisplatin, carboplatin or oxaliplatin. *Conclusions*: Cryptophycins represent a promising new class of antitumor agents that may be optimally administered by intravenous infusion and in combination with doxorubicin, paclitaxel and 5-fluorouracil.

Key words Cryptophycins · Cryptophycin 52 · Microtubulin inhibitors

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Introduction

Cryptophycin-1, a desipeptide isolated from the cyanobacterium *Nostoc* sp., was initially described as an antifungal agent [21] and later was shown to have antimitotic activity, cytotoxicity toward tumor cells in culture and anticancer activity against murine solid tumor models and human tumor xenografts [2, 17, 18, 34]. Subsequently many cryptophycins have been isolated and prepared by chemical synthesis [29]. The mechanism of anticancer action of the cryptophycins has been associated with their action on intracellular microtubules [9, 22, 23, 32, 33]. In cell culture, the cryptophycins maintained activity against ovarian and breast carcinoma cells that overexpress the multidrug resistance efflux pump P-glycoprotein (MDR-1) as well as the MRP multi-drug resistance protein and in in vivo tumor models, the cryptophycins lacked cross-resistance with paclitaxel and doxorubicin [2, 22, 23, 29].

Microtubules are dynamic assemblies involved in the maintenance of cell structure, regulation of membrane transport processes, cell motility and proliferation. Agents such as paclitaxel promote tubulin assembly and hyperstabilize microtubules, while agents such as colchicine and *Vinca* alkaloids inhibit tubulin polymerization and destabilize microtubules [7]. The action of the cryptophycins resemble those of the *Vinca* alkaloids so that it is likely that the cryptophycins interact with the

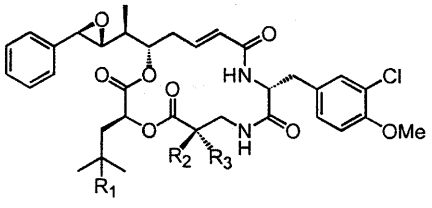
Vinca alkaloid binding domain or at a site that overlaps with the *Vinca* alkaloid binding domain of tubulin [1, 14, 15, 16, 22, 23].

Cryptophycin 52 (LY355703), a synthetic cryptophycin, is currently undergoing Phase I clinical trial [24]. CRYPTO 52 has demonstrated very potent activity against human tumor cell lines in culture [20, 30, 31] and a broad range of antitumor activity against both murine and human tumors. CRYPTO 52 demonstrated marked antitumor activity against both early and well-established tumors [2, 18, 34]. The current studies were undertaken in order to begin to explore the use of CRYPTO 52 in combination chemotherapy treatment regimens in human tumor xenograft models of small cell and non-small cell lung cancer and breast cancer, and to explore the effect of rate of administration of CRYPTO 52 in rat 13762 mammary carcinoma. In these studies, two pairs of cryptophycins (CRYPTO 52/55 and CRYPTO 292/296) were evaluated. CRYPTO 296 and CRYPTO 55 are the corresponding chlorohydrin derivatives of the β-epoxides, CRYTPO 292 and CRYPTO 52 (Fig. 1).

Materials and Methods

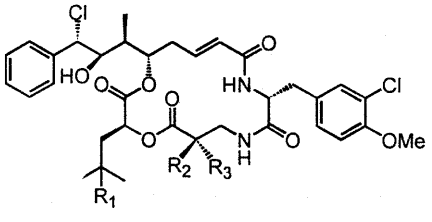
Drugs

Cisplatin, carboplatin, doxorubicin, paclitaxel, 5-fluorouracil and cyclophosphamide were purchased from Sigma Chemicals (St. Louis, Mo., USA). Vinorelbine (Navelbine) was purchased from the Indiana University Medical Center pharmacy. Oxaliplatin was purchased from Nescott Fine Chemicals (Santiago, Chile). Cryptophycin 52 (LY355703), cryptophycin 55, cryptophycin 292 and cryptophycin 296 were produced by total chemical synthesis at Lilly Research Laboratories (Indianapolis, Ind., USA; Fig. 1). Each of the cryptophycins, paclitaxel, vinorelbine and doxorubicin was formulated in 2% PEG 300/8% cremophor/90% normal saline for intravenous administration to the animals and prepared at concentrations so that the volume of injection was 0.1 ml per 10 g body weight of the animals. The other agents were formulated in normal saline and administered by intraperitoneal injection.



Cryptophycin 52 (LY355703) (R_1 = H, R_2 = R_3 = CH_3)

Cryptophycin 292 (R_1 = R_2 = CH_3, R_3 = H)



Cryptophycin 55 (R_1 = H, R_2 = R_3 = CH_3)

Cryptophycin 296 (R_1 = R_2 = CH_3, R_3 = H)

Fig. 1 Chemical structures of the cryptophycins

Infusion rate studies

The rat 13762 mammary adenocarcinoma is a carcinogen-induced (DMBA) tumor of the female Fischer 344 rat. Female Fischer 344 rats (120–140 g; Harlan-Sprague-Dawley, Indianapolis, Ind.) were housed with food and water ad libitum. The tumor can metastasize to the lungs and abdominal organs. The tumor is composed of epithelial tissue folds and acini. The tumor grows to 100 mm^3 in about 14 days when implanted subcutaneously in the hind legs of female rats [27, 28].

Rat 13762 mammary carcinoma cells (5×10^6 cells) prepared from a brei of donor tumors were implanted subcutaneously into a hind leg of Fischer 344 female rats on day 0. The surgery for jugular vein cannula implantation was performed under aseptic conditions on day 5 or 6. The cannulas were permanently implanted and remained in place through the duration of the study. Control groups included both surgical and non-surgical controls. Tumors grew faster in the surgical controls than in the non-surgical controls for about 1 week post surgery, likely due to angiogenesis associated with wound healing. Controls were vehicle treated. The cryptophycin compounds were administered by intravenous infusion over 2 or 6 h through the jugular vein cannula (infusion volumes were such that 0.15 ml compound preparation was administered per 10 g body weight) or by intravenous injection (injection volumes were such that 0.1 ml compound preparation was administered per 10 g body weight) into a tail vein on days 7, 9 and 11 post tumor cell implantation. The treatments were: cryptophycin 52 (0.3 mg/kg) ×3; cryptophycin 55 (2 mg/kg) ×3; cryptophycin 292 (0.2 mg/kg) ×3 or (0.3 mg/kg) ×2 and cryptophycin 296 (0.55 mg/kg) ×3.

Separate groups of four rats bearing the 13672 mammary carcinoma implanted subcutaneously in a hind leg beginning on day 7 post tumor cell implantation were treated with each of the following therapies: cisplatin (8 mg/kg) i.p. on day 7; doxorubicin (1.5 mg/kg) i.p. on days 7 through 11; cyclophosphamide (100 mg/kg) i.p. on days 7, 9 and 11; 5-fluorouracil (25 mg/kg) i.p. on days 7 through 11; gemcitabine (60 mg/kg) i.p. on days 7, 10 and 13; fractionated radiation (3 Gray) locally to the tumor-bearing limb on days 7 through 11 (GammaCell 40, Nordion, Ottawa, Ont., Canada).

The progress of each tumor was measured three times per week until it reached a volume of 2000 mm^3. The tumor growth delay was calculated as the number of days for each individual tumor to reach 1000 mm^3 compared with the appropriate control (surgical or non-surgical). Each treatment group had four animals and the experiment was repeated twice. Days of tumor growth delay are the mean ± SEM for the treatment group compared with the control [25, 27, 28].

Human tumor lines

The Calu-6 anaplastic lung carcinoma originated from a 61-year-old female and was established in 1976 [3]. The NCI-H82 small cell lung carcinoma originated from the pleural fluid of a heavily pretreated 40-year-old male and was established in 1978 [6]. The SW2 small cell lung carcinoma originated from the pleural fluid of a male patient and was established in 1980 [4, 5]. The MX-1 breast carcinoma originated as a poorly differentiated mammary carcinoma in a 29-year-old female. Calu-6 and H82 cells were purchased from ATCC (Rockville, Md., USA). The MX-1 breast carcinoma was obtained from the National Cancer Institute-Frederick Cancer

Research Facility, DCT Tumor Repository. Each of these lines is tumorigenic in nude mice.

Nude mice, male and female, were purchased from Charles River Laboratories (Wilmington, Mass., USA) at 5 to 6 weeks of age. When the animals were 7 to 8 weeks of age they were exposed to 4.5 Gray of total body radiation delivered using a GammaCell 40 irradiator (Nordion). Twenty-four hours later, MX-1, Calu-6, SW2 or H82 tumor cells (5×10^6) prepared from a brei of several donor tumors were implanted subcutaneously in a 1: 1 mixture of RPMI tissue culture media and Matrigel (Collaborative Biomedical Products, Bedford, Mass.) in a hind leg of the animals. MX-1 tumors grow to 500 mm^3 in 34.7 ± 2.9, Calu-6 tumors grow to 500 mm^3 in 19.0 ± 3.4, SW2 tumors grow to 500 mm^3 in 24.0 ± 2.5 days and H82 tumors grow to 500 mm^3 in 14.0 ± 0.8 days.

Xenograft tumor growth delay experiments: lung carcinomas

Treatments were initiated on day 7 post tumor cell implantation when the Calu-6, SW2 [4, 5] and H82 tumors were 100–200 mm^3 in volume. Cryptophycin 52 (5 mg/kg) and cryptophycin 55 (15 mg/kg) were administered by intravenous injection on days 7, 9, 11, 14, 16 and 18 post tumor cell implantation. Cisplatin (10 mg/kg) and carboplatin (50 mg/kg) were administered by intraperitoneal injection on day 7; and oxaliplatin (12.5 mg/kg) was administered by intraperitoneal injection on days 7 and 14 post tumor cell implantation.

Breast carcinoma

Treatments were initiated on day 7 when the MX-1 tumors were 50–100 mm^3 in volume. Cryptophycin 52 (5 mg/kg) and cryptophycin 55 (15 mg/kg) were administered by intravenous injection on days 7, 9, 11, 13 and 15. Similarly, doxorubicin (1.75 mg/kg) was administered on days 7 through 11, paclitaxel (24 mg/kg) was administered on days 7, 9, 11 and 13 and vinorelbine (7.5 mg/kg) was administered on days 7, 10 and 13 by intravenous injection. Gemcitabine (60 mg/kg) i.p. was administered on days 7, 10, 13 and 16 and 5-fluorouracil (30 mg/kg) i.p. on days 7 through or on days 12 through 16.

The progress of each tumor was measured three times per week until it reached a volume of 2000 mm^3. Tumor growth delay (TGD) was calculated as the time taken by each individual tumor to reach 500 mm^3 compared with the time in the untreated controls. Each treatment group included five animals, and each experiment was repeated two times. TGD times (days) are the means ± SEM for the treatment group compared with those for the control group [25, 26].

Assurances

All studies involving animals were carried out in accordance with NIH Guidelines and with institutional IACUC approved protocols. The animal facilities are ALAAC accredited and pathogen-free. A sentinel animal program is in place and all tumors are MAP tested.

Results

To compare the antitumor activity of several cryptophycin analogs (Fig. 1) administered by intravenous infusion or intravenous injection female Fischer 344 rats were implanted with jugular vein cannulas. The cryptophycins were administered at maximally tolerated doses on alternate days three times or two times. Tumor cells were implanted on day 0 and allowed to grow for 7 days prior to cryptophycin administration. The surgery for jugular vein cannula implantation was performed on day 5 or 6. Control groups included both surgical and non-surgical controls receiving infusions or injections of vehicle.

All of the cryptophycins tested were active antitumor agents against the rat 13762 mammary carcinoma. Although there was no significant difference in the antitumor activity of the cryptophycins administered by intravenous injection, differences in activity became evident as the administration time was extended to 2 h and then to 6 h (Fig. 2). When administered as a 6-h infusion, the chlorohydrin, cryptophycin 55, was a significantly more active antitumor agent than the corresponding epoxide, cryptophycin 52, when both agents were administered on days 7, 9 and 11. This trend did not pertain to the mono-methyl series, however; the epoxide, cryptophycin 292, had greater activity than the chlorohydrin, cryptophycin 296, when administered on the three-dose regimen.

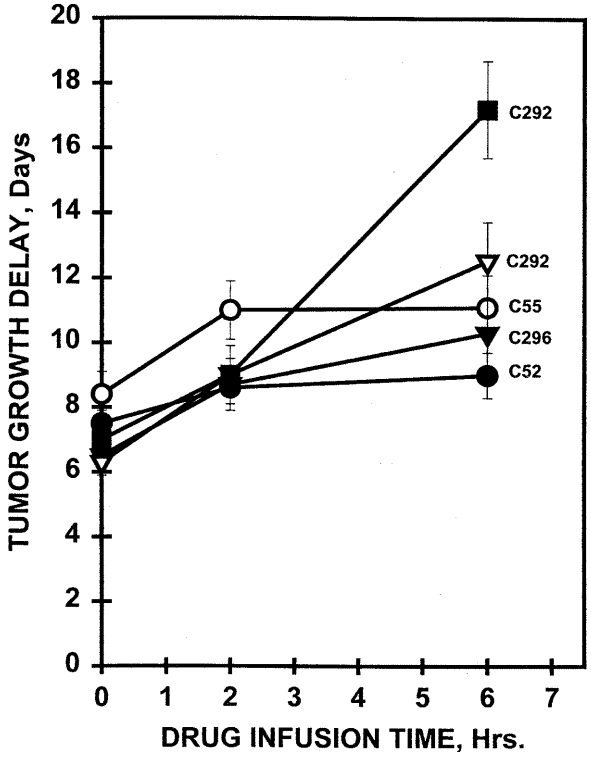


Fig. 2 Growth delay of the rat 13762 mammary carcinoma grown as a subcutaneous tumor in a hind leg of female Fischer 344 rats after treatment with: CRYPTO 52 (0.3 mg/kg) i.v., days 7, 9 and 11 (●); CRYPTO 55 (2 mg/kg) i.v., days 7, 9 and 11 (○); CRYPTO 292 (0.2 mg/kg) i.v., days 7, 9 and 11 (▼); CRYPTO 296 (0.55 mg/kg) i.v., days 7, 9 and 11 (▽) or CRYPTO 292 (0.3 mg/kg) i.v., days 7 and 9 (■). The compounds were administered by intravenous injection into the tail vein or by intravenous infusion through a jugular vein cannula surgically implanted on day 5 or 6. Intravenous infusion times were 2 or 6 h. Tumor growth delay was determined when the tumors reached a volume of 1000 mm^3. Points are the means of at least two independent experiments; bars are SEM

To determine whether the efficacy of the cryptophycins could be increased by administering a higher dose of the compound in a shorter treatment regimen, the antitumor activity of a two dose regimen of cryptophycin 292 was compared with a three-dose regimen of the same compound (Fig. 2). The antitumor activity of the compound was significantly increased when on the higher dose: shorter duration regimen. The tumor growth delay for cryptophycin 292 (0.2 mg/kg ×3, 6 h) was 12.5 days, while the tumor growth delay for cryptophycin 292 (0.3 mg/kg ×2, 6 h.) was 17.2 days.

The antitumor activity of six currently used anticancer treatments was compared with the antitumor activity of the cryptophycins in the rat 13762 mammary carcinoma (Fig. 3). In Fig. 3, the activity for the cryptophycins is from the 3-day regimen using the 6-h infusion schedule. Each of the anticancer treatments was administered on a standard maximally tolerated regimen for that agent except for radiation therapy, which was delivered locally to the tumor-bearing limb, thus modeling 1 week of clinical radiation therapy (5 × 3 Gray). The most effective of the standard treatment agents were cyclophosphamide, which produced a tumor growth delay of 9.3 days and gemcitabine, which produced a tumor growth delay of 8.6 days. These compare well with the tumor growth delay for the epoxides, cryptophycin 52 of 9.0 days and cryptophycin 292 of 12.5 days, administered on the three-dose schedule. The most effective agent overall, however, was cryptophycin 292, which produced a tumor growth delay of 17.2 days using the dose-intensive two-treatment regimen.

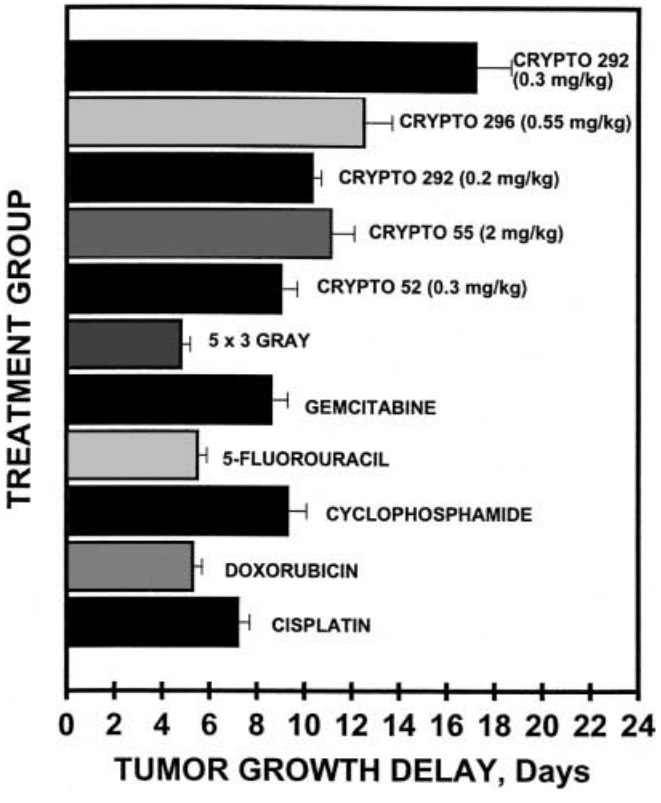


Fig. 3 Growth delay of the rat 13762 mammary carcinoma grown as a subcutaneous tumor in a hind leg of female Fischer 344 rats after treatment with: cisplatin (8 mg/kg) i.p., day 7; doxorubicin (1.5 mg/kg) i.p., days 7 through 11; cyclophosphamide (100 mg/kg) i.p., days 7, 9 and 11; 5-fluorouracil (30 mg/kg) i.p., days 7 through 11; gemcitabine (60 mg/kg) i.p., days 7, 10 and 13; fractionated radiation therapy (5 × 3 Gray) days 7 through 11; CRYPTO 52 (0.3 mg/kg) i.v., over 6 h, days 7, 9 and 11; CRYPTO 55 (2 mg/kg) i.v., over 6 h, days 7, 9 and 11; CRYPTO 292 (0.2 mg/kg) i.v., over 6 h, days 7, 9 and 11; CRYPTO 296 (0.55 mg/kg) i.v., over 6 h, days 7, 9 and 11; or CRYPTO 292 (0.3 mg/kg) i.v., over 6 h, days 7 and 9. Tumor growth delay was determined when the tumors reached a volume of 1000 mm^3. Columns represent the means of at least two independent experiments; bars are SEM

The MX-1 human breast carcinoma grown as an xenograft tumor in female nude mice was used as a model to examine cryptophycin 52 and cryptophycin 55 in combination chemotherapy regimens. For these initial studies, each chemotherapeutic agent was administered on a standard dosage regimen simultaneously with the cryptophycin. Administration of cryptophycin 52 or cryptophycin 55 could be added to treatment with doxorubicin, gemcitabine, paclitaxel or vinorelbine without additional toxicity to the animals as determined by weight loss. When administered as single agents, cryptophycin 52 and cryptophycin 55 produced tumor growth delays of 11.4 and 18.8 days, respectively, in the MX-1 breast carcinoma (Fig. 4). Doxorubicin produced a tumor growth delay of 6.1 days as a single agent in the MX-1 tumor. The regimens combining doxorubicin with each cryptophycin resulted in tumor growth delays of 16.2 days and 23.1 days for cryptophycin 52 and cryptophycin 55, respectively, thus indicating additivity of the two treatments. Paclitaxel administered as a single agent produced a tumor growth delay of 11.8 days in the MX-1 tumor. The combinations of paclitaxel and the cryptophycins resulted in tumor growth delays of 13.5 and 23.1 days for cryptophycin 52 and cryptophycin 55, respectively, thus producing a tumor response greater than either agent alone. Simultaneous treatment with gemcitabine or vinorelbine and the cryptophycins produced less favorable results in tumor response, which were increased to tumor growth delays compared with gemcitabine or vinorelbine alone but not as great as the cryptophycins administered as single agents.

Clinical treatment regimens for breast cancer often involve doxorubicin and 5-fluorouracil. Therefore a comparison was made of the efficacy of regimens combining doxorubicin, cryptophycin 52 or cryptophycin 55 simultaneously or sequentially with 5-fluorouracil in the MX-1 human breast carcinoma xenograft model. As single agents, the cryptophycins were more effective against the MX-1 tumor than was doxorubicin (Fig. 5). 5-Fluorouracil was administered on a daily times-five schedule, beginning either the same day as the other agent on days 7 through 11 (tumor growth delay, 7.4 days) or at or near completion of the administration of the other agent on days 12 through 16 (tumor growth delay, 6.2 days). Sequential administration of doxorubicin and 5-fluorouracil was a more effective treatment

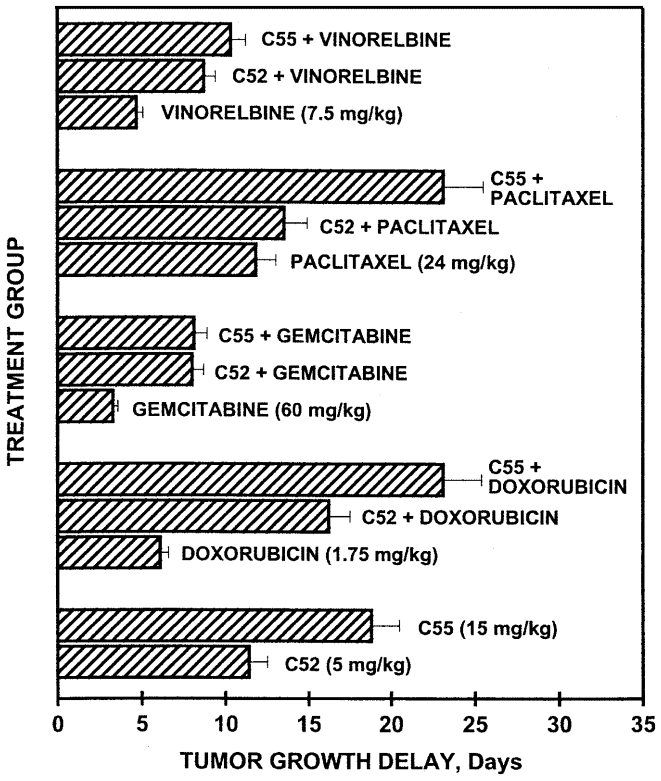


Fig. 4 Growth delay of the human MX-1 breast carcinoma xenograft grown subcutaneously in female nude mice after treatment with: CRYPTO 52 (5 mg/kg) i.v., days 7, 9, 11, 13 and 15; CRYPTO 55 (15 mg/kg) i.v., days 7, 9, 11, 13 and 15; doxorubicin (1.75 mg/kg) i.v., days 7 through 11; gemcitabine (60 mg/kg) i.p., days 7, 10, 13 and 16; paclitaxel (24 mg/kg) i.v., days 7, 9, 11 and 13; vinorelbine (7.5 mg/kg) i.v., days 7, 10 and 13 or combinations including CRYPTO 52 or CRYPTO 55. Columns are the means of two independent experiments; bars are SEM

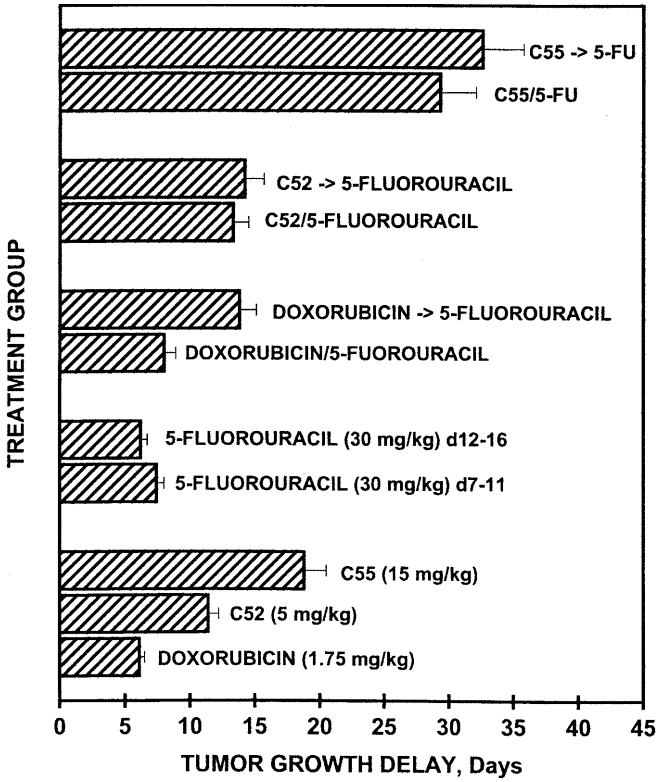


Fig. 5 Growth delay of the human MX-1 breast carcinoma xenograft grown subcutaneously in female nude mice after treatment with: doxorubicin (1.75 mg/kg) i.v., days 7 through 11; CRYPTO 52 (5 mg/kg) i.v., days 7, 9 ,11, 13 and 15; CRYPTO 55 (15 mg/kg) i.v., days 7, 9, 11, 13 and 15; 5-fluorouracil (30 mg/kg) i.p., days 7 through 11 or days 12 through 16 or combinations including 5-fluorouracil. Columns are the means of two independent experiments; bars are SEM

regimen, producing a tumor growth delay of 13.5 days, than simultaneous administration of the two agents which produced a tumor growth delay of 8.0 days. With the cryptophycins there was no significant difference in the tumor growth delays produced by the simultaneous or sequential combination regimens with 5-fluorouracil. However, with both cryptophycins the trend was toward the sequential regimen as being more effective. The tumor growth delays produced by the combination of cryptophycin 55 and 5-fluorouracil were 29.3 and 32.6 days, respectively, for the simultaneous and sequential regimens, thus indicating that these treatment combinations produce greater-than-additive tumor growth delays.

Combinations cryptophycin 52 and cryptophycin 55 and three antitumor platinum complexes, cisplatin, carboplatin and oxaliplatin, were studied in two small cell lung carcinoma xenografts, SW2 and H82, and one non-small cell lung carcinoma xenograft, Calu-6. Cryptophycin 52 and cryptophycin 55 were active antitumor agents against the SW2 human small cell lung cancer tumor, producing tumor growth delays of 6.8 days and 8.7 days, respectively [4, 5] (Fig. 6). Of the three antitumor platinum complexes, cisplatin was active against the SW2 tumor producing a tumor growth delay of 7.4 days, while carboplatin and oxaliplatin were marginally active against this tumor. The combination of cryptophycin 52 and cisplatin resulted in a tumor growth delay of 12.3 days, which was greater-than either agent alone and combinations of cryptophycin 52 with carboplatin and oxaliplatin resulted in tumor growth delays that did not significantly differ from the effect of cryptophycin 52 alone. On the other hand, the combination of cryptophycin 55 with each of the antitumor platinum complexes resulted in tumor growth delays that were greater-than-additive for the two independent agents. The tumor growth delays were 24.4 days, 34.9 days and 32.7 days for combinations of cryptophycin 55 and cisplatin, carboplatin and oxaliplatin, respectively.

As single agents, the cryptophycins were very active antitumor agents against the human H82 small cell lung carcinoma xenograft, producing tumor growth delays of 12.7 days and 20.9 days after treatment with cryptophycin 52 and cryptophycin 55, respectively (Fig. 7). Cisplatin was the most effective antitumor platinum complex producing a tumor growth delay of 7.6 days, while oxaliplatin produced a tumor growth delay of 3.7 days and carboplatin a tumor growth delay of

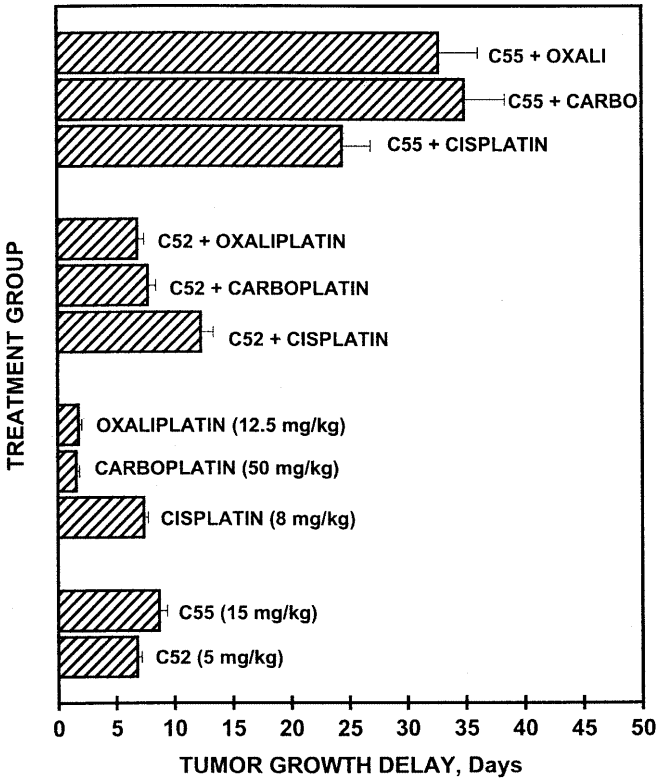


Fig. 6 Growth delay of the human SW-2 small cell lung carcinoma xenograft grown subcutaneously in male nude mice after treatment with: CRYPTO 52 (5 mg/kg) i.v., days 7, 9, 11, 14, 16 and 18; CRYPTO 55 (15 mg/kg) i.v., days 7, 9, 11, 14, 16 and 18; cisplatin (10 mg/kg) i.p., day 7; carboplatin (50 mg/kg) i.p., day 7; oxaliplatin (12.5 mg/kg) i.p., days 7 and 14 or combinations including a cryptophycin and an antitumor platinum complex. Columns are the means of two independent experiments; bars are SEM

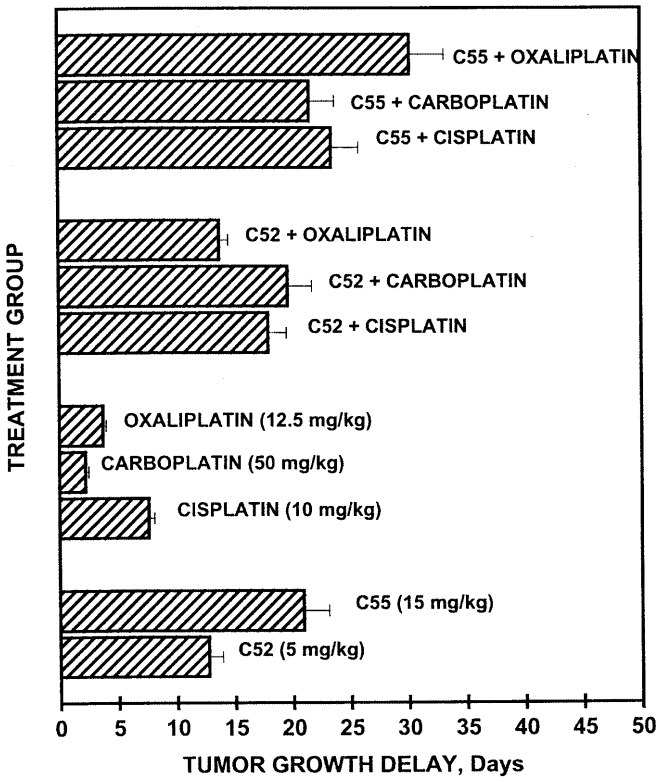


Fig. 7 Growth delay of the human H82 small cell lung carcinoma xenograft grown subcutaneously in male nude mice after treatment with: CRYPTO 52 (5 mg/kg) i.v., days 7, 9, 11, 14, 16 and 18; CRYPTO 55 (15 mg/kg) i.v., days 7, 9, 11, 14, 16 and 18; cisplatin (10 mg/kg) i.p., day 7; carboplatin (50 mg/kg) i.p., day 7; oxaliplatin (12.5 mg/kg) i.p., days 7 and 14 or combinations including a cryptophycin and a platinum complex. Columns are the means of two independent experiments; bars are SEM

2.2 days against the H82 tumor. The combinations of cryptophycin 52 with each of the antitumor platinum complexes resulted in tumor growth delays that were greater than either agent alone, with the combinations of cryptophycin 52 with cisplatin and carboplatin being the most effective and resulting in tumor growth delays of 17.9 and 19.6 days, respectively. The combinations of cryptophycin 55 with each of the antitumor platinum complexes also resulted in tumor growth delays that were greater than either of the treatment agents alone. The most effective treatment regimens were cryptophycin 55 with cisplatin and cryptophycin 55 with oxaliplatin, resulting in tumor growth delays of 23.4 days and 30.2 days, respectively.

Both cryptophycin 52 and cryptophycin 55 were active antitumor agents against the human Calu-6 non-small cell lung carcinoma xenograft, producing tumor growth delays of 14.9 days and 16.6 days, respectively (Fig. 8). Cisplatin was the most effective of the antitumor platinum complexes against this tumor, with a resultant tumor growth delays of 9.3 days. Oxaliplatin was also an active antitumor agent against the Calu-6 tumor, producing a tumor growth delay of 5.6 days, while carboplatin produced a tumor growth delay of 2.4 days against this tumor. The combination treatment regimens including cryptophycin 52 and each of the antitumor platinum complexes resulted in tumor growth delays that were greater than that of either agent administered alone. The most effective treatment regimens were cryptophycin 52 and carboplatin and cryptophycin 52 and oxaliplatin, resulting in tumor growth delays of 17.2 days and 18.3 days, respectively. The combination of cryptophycin 55 and cisplatin resulted in a highly effective treatment regimen with a tumor growth delay of 49.7 days, which was greater than additive for the two treatments. The combination of cryptophycin 55 and carboplatin was also highly effective, resulting in a tumor growth delay of 33.0 days, which was greater than additive for the two treatments. The combination of cryptophycin 55 and oxaliplatin produced a tumor growth delay of 23.2 days, reflecting additivity of the two individual treatment agents.

Discussion

Chemotherapy is one of the three major modalities used in the treatment of solid malignant tumors. Chemother-

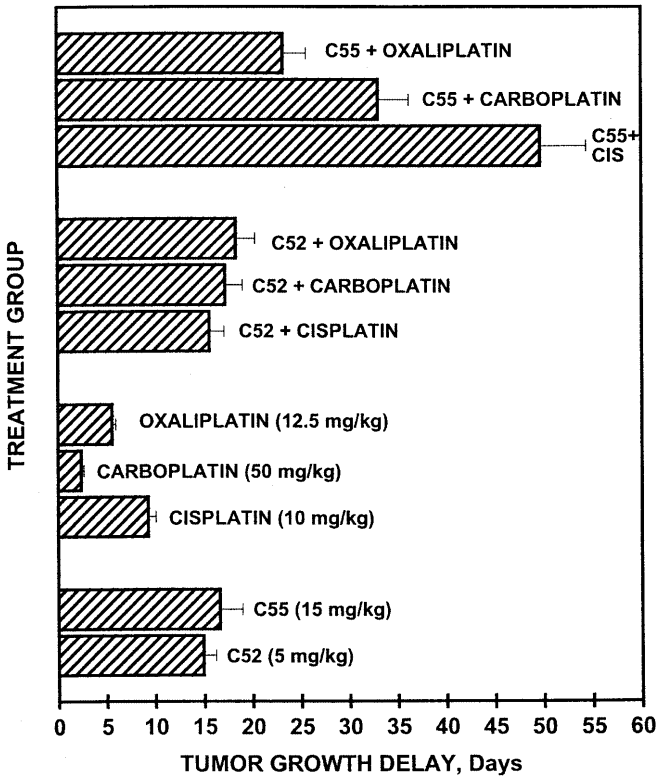


Fig. 8 Growth delay of the human Calu-6 non-small cell lung carcinoma xenograft grown subcutaneously in female nude mice after treatment with: CRYPTO 52 (5 mg/kg) i.v., days 7, 9, 11, 14, 16 and 18; CRYPTO 55 (15 mg/kg) i.v., days 7, 9, 11, 14, 16 and 18; cisplatin (10 mg/kg) i.p., day 7; carboplatin (50 mg/kg) i.p., day 7; oxaliplatin (12.5 mg/kg) i.p., days 7, 11, 15 and 19 or combinations including a cryptophycin and a platinum complex. Columns are the means of two independent experiments; bars are SEM

apeutic agents are rarely administered as single agents and are frequently combined because they have non-overlapping toxicities, different mechanisms of action and/or molecular targets that may be complementary in leading to cell death. As new chemotherapeutic agents enter the clinical armamentarium, it is important to define their potential for use in terms of dose response and in combination treatment regimens. The dose response relationship for many antitumor agents is steep both for therapeutic and toxic effects. Although increasing the duration of administration of the cryptophycins from an intravenous injection to a 6-h intravenous infusion improved the tumor response to the compounds somewhat, the largest increase in tumor response was obtained when the dose intensification of the regimen was increased by administering the same total dose of cryptophycin 292 in two doses via 6-h infusion rather than in three doses via 6-h infusion. Further studies are needed to determine whether the more dose intensive regimen can maintain acceptable toxicity.

The cryptophycins were active antitumor agents in all five tumors used in this study and have well-established activity in many other murine tumors and human tumor xenografts [2, 18, 34]. The antitumor effects of the cryptophycins compare very favorably with the antitumor activity of many currently used agents and the cryptophycins are active antitumor agents in multi-drug resistant tumor models [2].

Antitubulin agents are frequently incorporated into combination chemotherapy protocols, based not only on their lack of cross resistance with drugs that alkylate DNA, but also because of their unique mechanism of action. Several natural products have been discovered that bind to and disrupt microtubule function in cells leading to cell death [7, 13]. A number of these molecules or synthetic derivatives have been developed in antineoplastic agents. The *Vinca* alkaloids, vinblastine and vincristine, have been available for over 40 years [11]. Newer agents of this type include vindesine, a synthetic derivative of vinblastine, and vinorelbine, a semisynthetic derivative of vinblastine. Like the other *Vinca* alkaloids, these compounds block polymerization of microtubules, leading to impaired formation of the mitotic spindles [8, 10, 12]. The taxanes, paclitaxel and docetaxel, have found the widest clinical use of the microtubule agents [19]. The taxanes promote the assembly of microtubules and stabilize them against depolymerization. Both cryptophycin 52 and cryptophycin 55 were able to combine with each of the other anticancer drugs tested at full doses of both agents. The use of antitubulin agents in combination therapy extends beyond the spectrum of cancers for which definitive activity has been demonstrated. Vincristine is approved as a component of combination therapy for use in Hodgkin's lymphoma, non-Hodgkin's lymphomas, rhabdomyosarcoma of childhood, neuroblastoma and Wilm's tumor [8, 9, 10]. Vinblastine has a similar spectrum of activity and, in addition, mycosis fungoides, advanced carcinoma of testis, Kaposi's sarcoma and histiocytosis X. Vindesine has also shown activity against resistant hematological malignancies, breast carcinoma, malignant melanoma and adenocarcinoma of the lung. Paclitaxel has demonstrated significant activity in ovarian carcinoma, breast carcinoma, melanoma, non-small cell lung carcinoma and small cell lung carcinoma. Cryptophycin 52 and cryptophycin 55 were active here against breast cancer, non-small cell lung cancer and small cell lung cancer. The overall most active combinations were a cryptophycin together with doxorubicin in the MX-1 human breast cancer, cryptophycin 55 together with 5-fluorouracil in the MX-1 human breast cancer, and, in general, cryptophycin 55 together with each of the three antitumor platinum complexes in the human SW-2, H82 and Calu-6 lung cancers. Thus, the cryptophycins demonstrated the ability to combine favorably with a topoisomerase II inhibitor, an antimetabolite and DNA damaging agents. The combination of cryptophycin 55 with paclitaxel produced a tumor growth delay in the MX-1 breast carcinoma that was greater than either agent alone. Interestingly, however, the combination of cryptophycin 52 or cryptophycin 55 with vinorelbine resulted in tumor growth delays that were decreased compared with the cryptophycins as single agents.

Clinical experience has taught that pharmacokinetics and pharmacodynamics are important determinants of antitubulin agent clinical utility and that small changes in chemical structure can lead to differences in dose-limiting toxicities and spectrum of antitumor activity. The cryptophycins represent an exciting new family of antitubulin agents. Cryptophycin 52 is currently undergoing early clinical testing [24]. While the optimal doses and schedules for cryptophycin 52 in the clinic remain to be determined, the findings of these xenograft studies indicate that cryptophycin 52 should be a very useful chemotherapeutic agent in combination treatment regimens.

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