Metronomic treatment with irinotecan (CPT-11) against malignant glioma xenografts inhibits angiogenesis and tumor growth

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Abstract

Purpose: Irinotecan (CPT-11) has shown emerging promise in the treatment of malignant gliomas. The mechanism of action has been considered that irinotecan sensitizes glioma cells to the cytotoxic actions of radiation therapy and alkylating agents. However, clinical trials using weekly or dosing of CPT-11 every 3 weeks have demonstrated imaging responses in only 10-15% of patients. In this study, we evaluated another mechanism of action, angiosuppression, of CPT-11 for ACNU-resistant gliomas using a metronomic administration schedule.

Methods: Two different types of treatment, 1) conventional and 2) metronomic were applied for the subcutaneous U87 model.

Results: We found that metronomic administration of CPT-11 significantly inhibited malignant glioma growth by inhibiting angiogenesis; this treatment protocol decreased the number of tumor vessels and the area of hypoxic lesions and decreased the expression of VEGF and HIF-1α, the most important angiogenic factors in gliomas. Metronomic treatment was superior compared to conventional treatment with regard to the severe systemic side effect of body weight loss. The

growth inhibitory effect was very similar at both the low and high doses of CPT-11.

Conclusions: These angiosuppressive effects of CPT-11 show promise for another usage of CPT-11 in metronomic and scheduled angiosuppressive chemotherapy with low dose and long-term administration for malignant gliomas without systemic side effects.

Introduction

Malignant gliomas have remained a major cause of death in adults and children despite therapeutic strategies that include maximal surgical resection followed by radiochemotherapy. Although there is great promise for future strategies involving anti-angiogenic agents, chemotherapy, or vaccines, it will likely be years before we see true therapeutic benefits from these newer modalities. Other than the nitrosoureas (BCNU, ACNU, CCNU) and temozolomide, no agents have consistently demonstrated clinically meaningful benefits for patients with gliomas.

Irinotecan has shown emerging promise in the treatment of malignant gliomas following many laboratory studies and phase I and II clinical trials [1, 2]. Currently, a phase II trial of BCNU and CPT-11 in adults with newly diagnosed or recurrent malignant gliomas and phase I and II trials of CPT-11 and temozolomide are ongoing. CPT-11 may hold importance for neuro-oncology for a number of reasons. CPT-11, topoisomerase I inhibitors, utilize a different mechanism of action compared with other glioma therapies, particularly the alkylating agents. Preclinical and clinical data indicate that irinotecan may sensitize glioma cells to the cytotoxic actions of radiation therapy and alkylating agents. In addition, CPT-11 is among a small group of cytotoxic agents that readily cross the blood-brain barrier. However, clinical trials using weekly or dosing of irinotecan every 3 weeks have demonstrated imaging responses in only 10-15% of patients. Recent data for CPT-11 and bevacizumab combination therapy suggests that there is a synergy between irinotecan and ant-VEGF therapies, with acceptable toxicity [3]. We have demonstrated the antiangiogenic action of CPT-11 *in vitro* [4], including the anti-VEGF and anti-HIF (hypoxia inducible factor) effects of CPT-11 itself. Moreover, metronomic dosing regimens- either continuous infusion or frequent administration without extended rest periods- could have targeting proliferating endothelial cells in the tumor [5]. In this study, we evaluated another mechanism of action, angiosuppression, of CPT-11 using a

metronomic schedule *in vivo* in the glioma model.

Materials and methods

Establishment of the ACNU-resistant clone: U87AR

Because U87-MG has been known to express faint O^6-methylguanine-DNA methyltransferase (MGMT) mRNA which is involved in the drug resistance of tumor cells to ACNU (Mineura *et al.*, 1996), we chose U87 MG for the establishment of the ACNU-resistant clone. U87-MG was obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in MEM supplemented with 10% FCS in a humidified atmosphere 5% CO_2 at 37°C. U87 MG was cultured in MEM supplemented with 10% FCS and 10 µM ACNU in a humidified atmosphere containing 5% CO_2 at 37°C, and the medium was changed 2 times per week for 5 weeks . Total RNA was extracted from glioma cells using the RNeasy Mini Kit (Qiagen GmbH, Germany) and we performed RT-PCR using the GeneAmp^{TM} RNA PCR Kit (Perkin–Elmer Cetus, Norwalk, CT), as described previously [6]. Briefly, 1 µg of total RNA was reverse transcribed by MuLV reverse transcriptase in the presence of random hexamers, followed by the indicated number of cycles of PCR reaction (95°C for 1 min, 55°C for 1 min, and 72°C for 1 min) in the presence of 2 µM MGMT specific primers (35 cycles), or the ß-actin specific primers (16 cycles) as a control. The MGMT primers included the reverse primer (5'-ATCCGATGCAGTGTTACACG -3' within exon 5) and the forward primer (5'-ACCGTTTGCGACTTGGTACT-3' within exon 1) [7]. The ß-actin primers included the reverse primer (5'-GGAGTTGAAGGTAGTTTCGTG-3') spanning bases 2429-2409 and the forward primer (5'-CGGGAAATCGTGCGTGACAT-3') spanning bases 2107-2126 [8]. The predicted sizes of the amplified MGMT and ß-actin DNA products were 923 bp and 214 bp, respectively. The quantification of these RT-PCR products levels was performed on a computer using the public domain NIH Image program (developed at the U.S. National Institute of Health). After repeated

exposure to ACNU, we obtained U87-MG cells that exhibited high expression of MGMT mRNA, which was referred to as U87AR.

U87-MG and U87AR glioma cell proliferation with chemotherapeutic agents (ACNU and SN38; active metabolite of CPT-11) was assessed using the MTT assays described previously [9]. ACNU was provided by Sankyo company (Tokyo). SN38 was provided by Daiichi Pharmaceutical Company (Tokyo). MGMT expression was markedly enhanced in U87AR compared to U87MG (data not shown). The calculated IC_{50} with ACNU was 80 µM for U87-MG and 988 µM for U87AR. In contrast, the IC_{50} with SN38 was 70 nM for U87-MG and 20 nM for U87AR. U87AR is ten times more resistant to ACNU compared with U87-MG. CPT-11 similarly inhibited both U87AR and U87-MG proliferation (data not shown).

U87AR SCID Mouse Subcutaneous Model

After the implantation of 1 x 10^5 U87AR cells in the flank of 6-week-old male SCID mice (Japan Clea, Japan), U87AR tumor tissue fragments were removed and then reimplanted into another SCID mouse flank. Harvested tumor fragments 1 mm^3 in size were simultaneously implanted into the flank of another SCID mouse. When the resulting tumors were palpable, the mice were allocated into each of the treatment groups. In the first set of studies, CPT-11 was administered to mice by conventional schedule on days 11-15 and 18-22 via intraperitoneal (i.p.) injection at 10 (low dose) and 40 (high dose) mg/kg in 10% dimethylsulfoxide (DMSO) in 0.9% saline. The mice were divided into three groups (6 per group), given: (a) CPT-11 low dose; (b) CPT-11 high dose; (c) saline. Body weight and xenograft tumor dimensions were recorded every each day. In the second set of studies, CPT-11 was administered to the mice by metronomic schedule on days 1 to 21 daily via i.p. injection at 1 (low dose) and 4 (high dose) mg/kg. The mice were divided into three groups (8 per group), and were treated as follows: (a) metronomic-CPT-11 low dose; (b) metronomic-CPT-11 high dose; (c) saline.

Body weight and xenograft tumor dimensions were recorded on each day. In the second experiment, 21 days after the implant, the tumor tissues were removed. In order to evaluate the hypoxic area in tumor sections, pimonidazole hydrochloride (Hypoxyprobe-1, Chemicon Temecula, CA) was administered at 120 mg/kg just prior to sacrifice. A portion of the tissues was immediately fixed in 10% phosphate buffered formalin for 48 hours, paraffin embedded, and used for routine pathological diagnosis and immunohistochemistry. Other portions of the tissues were immediately frozen in liquid nitrogen and stored at -70°C. In the third set of studies, half of the animals treated by the metronomic schedule resumed the treatment after 7 days cessation of treatment; the second cycle of metronomic administration occurred daily on days 29 to 50 via i.p. injection of CPT-11. The xenograft tumor dimensions were recorded every day. The protocols were in adherence with the institutional guidelines for animal welfare and experimental conduct. CPT-11 was provided by Daiichi Pharmaceutical Company (Tokyo).

Antibodies and immunohistochemistry

The Dako LSAB Kit for mouse and rabbit primary antibody (DAKO, Glostrup, Denmark) was used [9]. Tissue sections were deparaffined and incubated with 10% normal goat serum in PBS for 20 min. The sections were then incubated with a polyclonal anti-VEGF antibody, A-20 (Santa Cruz Biotech. Inc., CA) at a dilution of 1:100 (1 µg/ml IgG), a monoclonal MIB-1 antibody (Immunotech) and anti-HIF-1α antibody (Chemicon) at a dilution of 1:100 in PBS overnight at 4°C, and a monoclonal anti-mouse CD31 antibody (BD Pharmingen) at a dilution of 1:50 (10 µg/ml) and anti-pimonidazole antibody (Chemicon) at a dilution of 1:20 in PBS for 60 min at room temperature. Chromatographically purified mouse IgG and rabbit IgG (DAKO) at the same IgG concentration were used as negative controls. Sections were incubated with biotin-conjugated goat anti-mouse or anti-rabbit immunoglobulin for 10 min, followed by washing in PBS for 10 min. The sections were

then incubated with peroxidase-conjugated streptavidin solution for 5 min, followed by washing in PBS for 5 min. Sections were then stained with freshly prepared aminoethylcarbazole solution for 10 min, followed by washing for 5 min in tap water. The sections were then counterstained with hematoxylin and mounted with aqueous mounting media. The intracellular VEGF immunostaining was assessed for tumor cells using a semiquantitative scale (-, not detected; + moderate; ++. strong). Nuclei positive for MIB-1 were determined by counting at least 1000 tumor cells at homogenously stained area.

Tumor vascular density

Vascular density was scored using the vasoproliferative component of the microscopic angiogenesis grading system (MAGS) that has been used to quantify angiogenesis in a variety of tumors [10]. The number of vessels at 200x field (0.31 mm^2) was measured in microvessel "hot spots" (i.e., microscopic areas containing the most dense collections of microvessels, identified initially under low power magnification) with the use of an Olympus microscope, AHBT3 (Olympus, Tokyo, Japan) on CD34-stained tissue sections. Vascular density was defined by averaging the number of vessels in the three most vascularised areas.

Measurement of VEGF and HIF-1α levels in tumor extracts

Tumor extracts were prepared by homogenizing the tumors in a buffer [10]. VEGF levels were measured in tumor extract supernatants by Quantikine^{TM} Human VEGF Immunoassay (R&D systems, Minneapolis, MN). VEGF levels were normalized to total extract protein concentration as measured by DC protein assay and expressed as pg VEGF / mg total extract protein.

Total RNA from frozen tumor samples was collected using the RNeasy Mini Kit (Qiagen, Maryland, MD). Total RNA of 1 μg/μl or less was used to perform RT-PCR using the GeneAmp RNA PCR kit

(Applied Biosystems, Foster, CA). The RT-PCR conditions were as follows: 30 sec at 94℃, 30 sec at 55℃, 30 sec at 72℃. To visualize mRNA for human VEGF, HIF-1α and β-actin, RT-PCR was performed and the PCR products were visualized on agarose gels. The mRNA expression was then quantitated by densitometry. The VEGF primers consisted of the reverse primer (5'-CCTGGTGAGAGATCTGGTTC-3') and the forward primer (5'-TCGGGCCTCCGAAACCATGA-3') [11]. The HIF-1α primers included the reverse primer (5'-CCCTGCAGTAGGTTTCTGCT-3') and the forward primer (5'-CTCAAAGTCGGACAGCCTCA-3') [12].

Statistical analyses

Vascular density, MIB-1 positivities, tumor volumes, VEGF concentration and densitometric measurements are expressed as the mean ± SD. Statistically significant differences between the groups were determined using one-way analysis of variance and Tukey's test. All p-values are two-sided; values were considered statistically significant for $p < 0.05$.

Results

CPT-11 (conventional treatment) inhibits U87AR glioma growth but with systemic toxicity

CPT11 at low and high doses of conventional treatment significantly inhibited U87AR subcutaneous glioma growth more than 15 days after the tumor implantation (Figure 1A). The median survival time for each treatment was 7.5 weeks (control), 10.6 weeks (CPT11 low dose), and 11.5 weeks (CPT11 high dose). The effect against growth inhibition was very similar between the low and high doses of CPT11 treatments. However, the body weights of the mice treated with CPT11 were significantly lower 18 days after treatment compared to control mice, suggesting that conventional CPT11 treatment has systemic side effects even at a low dose of CPT11.

CPT-11 (metronomic treatment) inhibits U87AR glioma growth without systemic toxicity

Metronomic scheduling of CPT-11 inhibited U87AR glioma growth in a dose-dependent manner (Figure 1B). The total dose of CPT-11 administered was 21mg/kg and 84mg/kg with the low dose and high dose metronomic schedules, respectively, i.e., one by five dose with the conventional schedule. CPT-11 treatment significantly prolonged the survival of glioma bearing mice (data not shown). The body weights of the mice treated with CPT-11 by the metronomic schedule were very similar to those of control mice, suggesting that metronomic CPT-11 treatment exhibited no systemic side effects.

CPT-11 (metronomic schedule) inhibits U87AR angiogenesis and hypoxia

Metronomic CPT-11 treatment significantly decreased HIF-1α mRNA expression (Figure 2). VEGF165 mRNA expression was relatively low compared to controls, but was not significantly different. The U87AR proliferation rate was not different between the controls and the CPT-11 treatment groups (Figure 3A). However, the VEGF protein level was reduced in a dose-dependent manner following metronomic CPT-11 treatment (Figure 3B). Tumor angiogenesis was evaluated by vessel number and vessel area in the tissue sections. Metronomic CPT-11 treatment significantly decreased the vessel number and area (Figure 4).

Finally, HIF-1α expression and the hypoxic area were evaluated by immunohistochemistry. In the control groups, HIF-1α expression was observed strongly in the tumor cells around the site of necrosis. Metronomic CPT-11 treatment resulted in reduced necrosis and decreased HIF-1α expression (Figure 5 A, B, C). The hypoxic areas in tissue sections, which were positive for pimonidazole, were strongly observed around the necrosis in the control group. However, the pimonidazole positive area was decreased in the metronomic CPT-11 treatment groups, at both low and high doses (Figure 5 D, E, F).

CPT-11 (metronomic schedule) inhibition is cytostatic and exhibits resistance

Re-challenge of metronomic CPT-11 treatment occurred 7 days after the discontinuation of treatment. The tumor started to grow rapidly after the discontinuation of the treatment. The inhibitory effect on tumor growth was minimized by re-challenge metronomic treatment (Figure 6).

Discussion

We have previously demonstrated that SN-38, an active metabolite of CPT-11, selectively inhibited endothelial cell proliferation and significantly decreased both HIF-1α and VEGF expression in glioma cells in a dose- and time-dependent manner [4]. The study clearly showed CPT-11 exhibited a direct anti-angiogenic effect on endothelial cells and indirectly on glioma cells via down-regulation of HIF1α and VEGF. In this study, we demonstrated the effect of metronomic treatment with CPT-11 on glioma growth and angiogenesis. The most striking advantage of metronomic CPT-11 treatment compared to conventional high dose intermittent usage of CPT-11 is that it exhibits no systemic toxicity, such as loss of body weight. Another striking effect was inhibition of glioma angiogenesis through down-regulation of HIF-1α and VEGF, resulting in decreased vessel densities and areas in the treated groups. However, the limited effect of the re-challenge treatment suggests resistance against the angiosuppression for solid tumors, including gliomas. Furthermore, CPT-11 exhibited a dramatic growth inhibitory effect for the ACNU-resistant glioma clone. Because ACNU, as well as temozolomide, is commonly used in Japan as an alkylating agent for malignant gliomas [13 - 15], the finding that CPT-11 is effective for ACNU-resistant clone is clinically useful concerning the subsequent use of chemotherapy for recurrent cases.

Antiangiogenic activity of metronomic CPT-11 treatment

Most anticancer drugs cause DNA damage and inhibit tumor cell proliferation. They are usually administered at high doses to kill as many tumor cells as possible. However, standard

chemotherapeutic drugs, such as vincristine bleomycin, adriamycin, etoposide, 5-fluoruracil, carboplatin, paclitaxel, and cyclophosphamide, can target angiogenesis when the dose and frequency of administration are optimized. Among these chemotherapeutic agents, CPT-11 was more potent in inhibiting endothelial cell tube formation *in vitro* [4]. Therefore, we selected CPT-11 as a candidate metronomic agent for gliomas.

The antiangiogenic properties of the camptothecins; 9-AC, topotecan, gimatecan, and CPT-11 have been demonstrated in both *in vitro* and *in vivo* assays [16 – 22]. While the precise mechanism by which camptothecins affect angiogenesis remains unclear, these drugs may be valuable therapeutic agents for the treatment of angiogenic tumors.

Indeed, preclinical studies have indicated the ability of a number of established cytotoxic drugs, such as cyclophosphamide and vinblastine, to strongly inhibit the growth of sensitive and resistant tumors when administered according to an "anti-angiogenic schedule," i.e., continuous low-dose scheduling [23 – 25], and many antitumor chemotherapeutic agents, including CPT-11, have been tested clinically for their antiangiogenic potential in systemic cancer [26 – 28].

The continuous low-dose scheduling has been described as the most appropriate to exploit the antiangiogenic potential of cytotoxic drugs [5, 29]. The metronomic chemotherapy has been experimentally [30] and applied clinically for glioblastoma. Tuettenberg *et al.* [31] demonstrated that metronomic temozolomide in combination with the COX-2 inhibitor rofecoxib was feasible, safe, and maintained a good quality of life in 13 patients with glioblastoma, especially in those tumors that were characterized by a high angiogenic activity. Furthermore, Kong *et al.*[32] demonstrated that metronomic temozolomide treatment was effective for patients with recurrent temozolomide-refractory glioblastoma. We demonstrated that metronomic CPT-11 treatment decreased VEGF protein expression, which is the most potent and important angiogenic factor of gliomas and brain tumor stem cells [10, 33, 34]. HIF-1α mRNA expression was also strongly

inhibited, even with low dose metronomic CPT-11 treatment. In tissue sections, HIF-1α expression was decreased, and was associated with diminished necrosis and hypoxic areas detected by pimonidazole staining. The tumor cells within these hypoxic areas are likely important targets because they are resistant to chemotherapy and radiation therapy. Anti-angiogenic treatment, such as metronomic CPT-11 treatment may overcome this problem due to reduction of the hypoxic area. Metronomic CPT-11 treatment decreased the expression of VEGF protein. One of the mechanisms of CPT-11 inhibition of VEGF expression is suggested to be down-regulation of HIF-1α mRNA expression, i.e., directly upstream of the VEGF pathway [35]. Metronomic CPT-11 treatment decreased expression of VEGF protein, but not VEGF mRNA, in tumor tissues. The recently identified HIF-independent regulation of VEGF by the transcriptional coactivator PGC-1α may be related to the down regulation of VEGF protein [36]. One of the important mechanisms of anti-tumoral effect of anti-angiogenesis agents is normalization of tumor vasculature, resulting decrease of necrosis and hypoxic area in the tumor [37]. By contrast, overdose of anti-angiogenic agents could result excessive vascular regression associated with increased necrosis and hypoxic area. The therapeutic window of anti-angiogenic agents including CPT-11 is narrow and the administration schedule and the dose of anti-angiogenic agents is very important.

The recent combination therapy with CPT-11 and the angiogenesis inhibitor, TNP470 and thrombospondin-1 shows promise in future developments for the treatment of malignant gliomas as an alternative angiosuppressive treatment [38]. Moreover, dramatic recent advances in the treatment for malignant gliomas with bevacizumab (VEGF neutralizing antibody) combined with CPT-11 treatment[3] have suggested the usefulness of CPT-11 as an anti-angiogenic, cytostatic agent, independent of its cytotoxic anti-tumor effect. Taken together, these results raise the possibility of the novel clinical application of CPT-11 to target hypoxia-inducible factor and VEGF.

Resistance to anti-angiogenesis treatment.

In our study, the growth inhibitory effect of re-challenged metronomic CPT-11 treatment was limited. The lack of the effectiveness on the re-growing phase is similar to a previous study [39]. There are three reasons to explain this finding: 1) the metronomic treatment is not effective for relatively large tumors, as demonstrated with other antiangiogenesis strategies, 2) the resistance to metronomic treatment appeared with the first treatment, 3) the targeted tumor endothelial cells acquired resistance to CPT-11. The timing of the anti-angiogenic treatment is important. Several clinical studies with metronomic chemotherapy have not been effective for recurrent glioblastomas. Metronomic chemotherapy with methotrexate and cyclophosphamide was ineffective in relapsed, heavily-pretreated glioblastoma [40]. Although there were some responders, the oral metronomic regimens with four drugs, etoposide, cyclophosphamide, thalidomide and celecoxib did not significantly improve the overall survival in the heavily-pretreated group of malignant glioma patients who were generally ineligible for conventional protocols [41]. While metronomic chemotherapy may not be useful in patients with advanced disease, further studies using more potent angiogenic agents in patients with less advanced disease may be warranted. The mechanisms of resistance include revascularization as a consequences of upregulation of alternative pro-angiogenic signals; protection of the tumor vasculature either by recruiting pro-angiogenic inflammatory cells or by increasing protective coverage by pericytes; accentuated invasiveness of tumor cells into local tissues to co-opt the normal vasculature [42, 43]. Tumor endothelial cells are genetically distinct from normal endothelial cells. Tumor endothelial cell clones can acquire resistance to the chemotherapeutic used. To overcome resistance to antiangiogenesis treatment, the HIF regulatory network holds promise as a target, as with metronomic CPT-11 treatment, given its global effects on angiogenesis, invasion and stress-adaptive cell physiology.

In summary, metronomic CPT-11 treatment inhibited human glioma growth *in vivo* even in the ACNU-resistant clone. Metronomic CPT-11 treatment demonstrated an anti-angiogenic effect,

inhibition of HIF-1α and VEGF expression and decreased area of the hypoxic lesion. Clinically, CPT-11 may be a useful chemotherapeutic agent especially for ACNU-treated, recurrent malignant gliomas, in part through its angiosuppressive effect. In combination with strategies that exhibit resistance to antiangiogenic treatment, metronomic CPT-11 treatment presents an exciting future opportunity for improving and sustaining the benefits of malignant glioma therapy.

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Figure legends

Figure 1

The effect of CPT-11 on the growth of subcutaneous xenografts of U87 MG human malignant glioma cells in SCID mice. Mice were implanted in the flank with a tumor fragment. When the resulting tumors were palpable, mice were allocated into treatment groups of five animals each. A: Conventional treatment. CPT-11 (low: 10 mg/kg, high 40 mg/kg) was administered to the mice on days 11-15 and 18-22 via intraperitoneal injection. B: Metronomic treatment. CPT-11 (low: 1mg/kg, high: 4 mg/kg) was administered to mice on days 1-21 via intraperitoneal injection. Body weight and xenografts tumor dimensions were recorded twice weekly. The results are presented as the means±SD. $* p < 0.01$

Figure 2

HIF-1α and VEGF mRNA expression of U87 subcutaneous tumor tissue treated by metronomic schedule. A. HIF-1α expression was strongly observed in 4 of 8 control tumor tissues. HIF-1α expression were weak in tumor tissues with low and high dose metronomic treatment. B. Semiquantitation of mRNA expression using fluoroimager. HIF-1α / actin ratio were significantly lower in low and high dose metronomic treated group compared to control group. $*p < 0.01$

Figure 3

MIB-1 positivities and VEGF concentration measured by ELISA. A: MIB-1 positivities were similar between control and the CPT-11 treatment group. B: VEGF concentration in tumor tissues were significantly lower in low and high dose metronomic CPT-11 compared to control. $* p < 0.05$, $* p < 0.001$

Figure 4

Angiosuppressive effect of CPT-11. Immunohistochemical detection of tumor vessels stained by CD34. A: control, B: high dose metronomic treatment. Metronomic CPT-11 treatment significantly decreased the vessel number (C) and area (D).

Figure 5

Immunohistochemical detection of HIF-1α expression (A, B, C) and hypoxic area (D, E, F). In the control groups, HIF-1α expression was strongly observed in the tumor cells around the necrosis (A). Metronomic CPT-11 treatment resulted in a decrease of the necrotic area and decreased expression of HIF-1α (B; metronomic low dose, C; metronomic high dose). Hypoxic areas in tissue sections that were positive for pimonidazole were strongly observed around the necrosis in the control group (D). However, the pimonidazole-positive area was decreased in the metronomic CPT-11 treatment groups at both low (E) and high (F) dose.

Figure 6. CPT-11 metronomic treatment was cytostatic. The tumor growth was inhibited by first metronomic treatment (cycle 1). The tumor started to grow rapidly during the discontinuation of the treatment (rest). The inhibitory effect on tumor growth was minimized by re-challenge metronomic treatment (cycle 2).
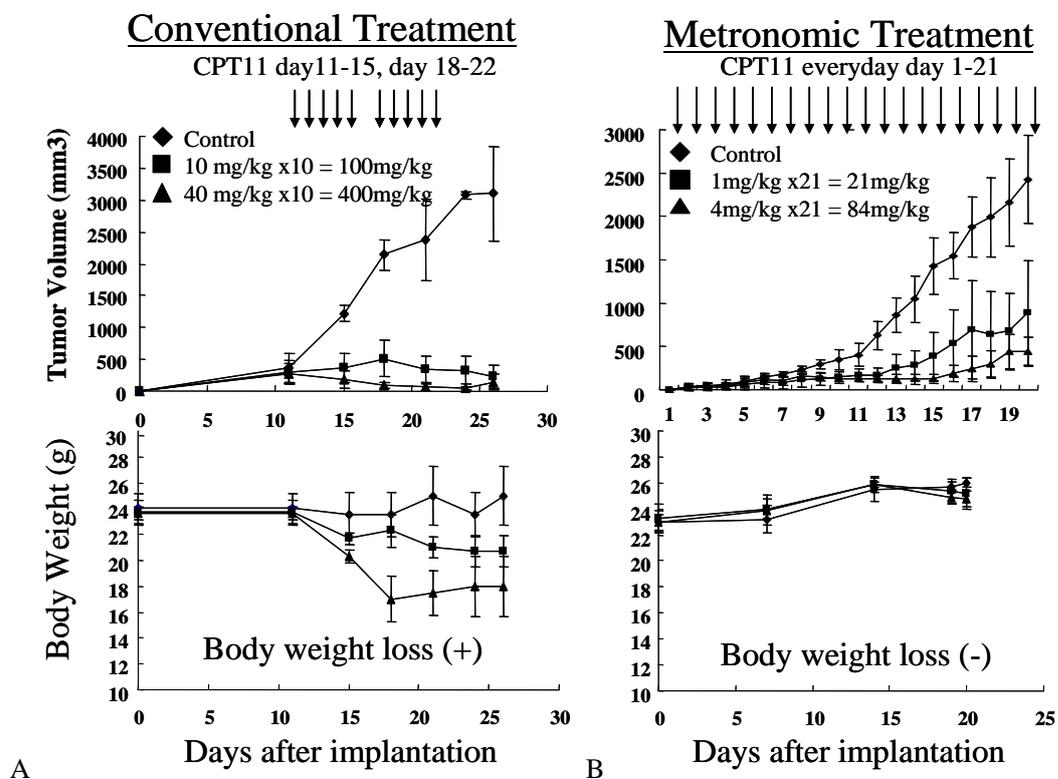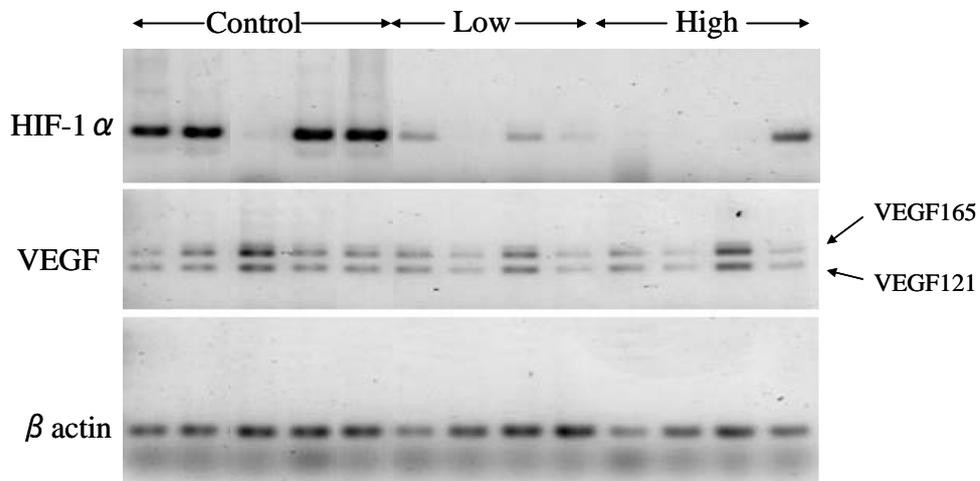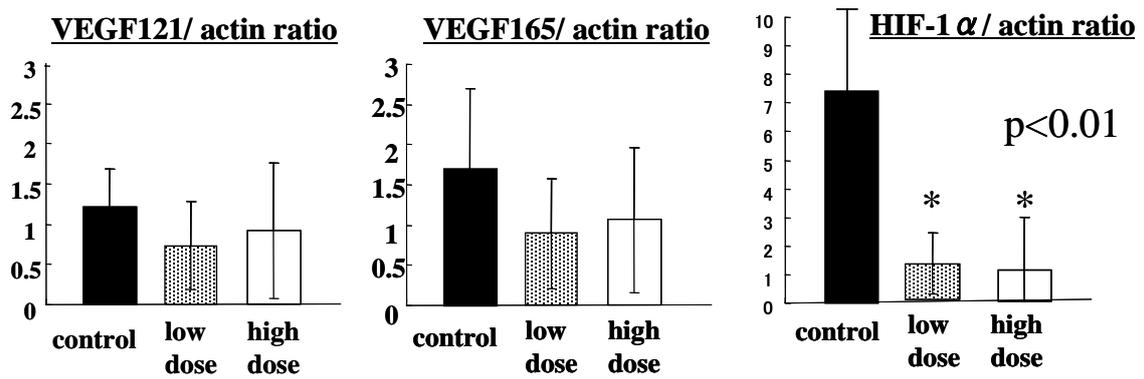
Figure 1

Conventional Treatment
CPT11 day11-15, day 18-22

↓↓↓↓↓ ↓↓↓↓↓

◆ Control
■ 10 mg/kg x10 = 100mg/kg
▲ 40 mg/kg x10 = 400mg/kg

Tumor Volume (mm3)

Body Weight (g)

Body weight loss (+)

Days after implantation

A

Metronomic Treatment
CPT11 everyday day 1-21

◆ Control
■ 1mg/kg x21 = 21mg/kg
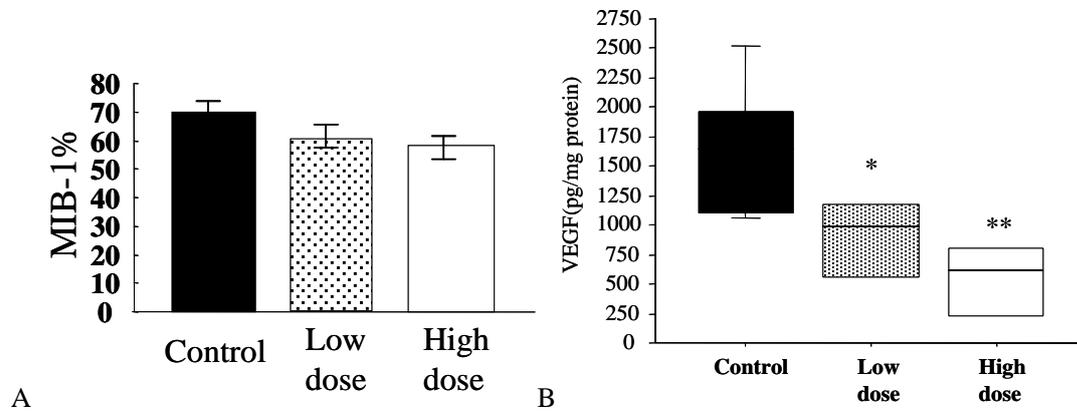▲ 4mg/kg x21 = 84mg/kg

Body weight loss (-)

Days after implantation

B

Figure 2

A

B

Figure 3

A

B

Figure 4

Figure 5

Figure 6