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# LY317615 decreases plasma VEGF levels in human tumor xenograft-bearing mice

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**Abstract** Angiogenesis plays an important role in tumor growth. Angiogenic growth factors may be useful as biomarkers of antiangiogenic activity since their plasma concentrations correlate with the efficacy of treatments directed toward angiogenic targets. SW2 small-cell lung carcinoma (SCLC), Caki-1 renal cell carcinoma and HCT-116 colon carcinoma tumors produce measurable plasma VEGF, bFGF and TGF$\beta$ in nude mice. Mice bearing these human tumor xenografts were treated orally twice daily with the PKC$\beta$ inhibitor, LY317615 (days 14–30 for SW2 and HCT116, and days 21–39 for Caki-1). Plasma was collected every 3 days from control and treated mice. LY317615 significantly decreased plasma VEGF levels in mice bearing SW2 SCLC and Caki-1 renal cell carcinoma compared to control plasma concentrations beginning 5–7 days after initiating therapy. VEGF plasma levels remained suppressed after termination of LY317615 treatment and for the duration of the study (an additional 2 to 3 weeks). Plasma VEGF levels in mice bearing HCT116 xenografts were not altered by LY317615 treatment and plasma bFGF and TGF-$\beta$ were not altered by LY317615 in any of the animals. As shown by CD31 immunohistochemical staining, LY317615 decreased intratumoral vessel density by nearly 40% in all three tumors. Only the Caki-1 tumor responded to single-agent LY317615 therapy with a measurable tumor growth delay. Thus, unexpectedly inhibition of PKC$\beta$ in vivo led to decreased VEGF production that persisted after therapy as well as to decreased intratumoral vessels. Plasma VEGF was a weak marker of response to LY317615, and plasma bFGF and TGF$\beta$ were not markers of LY317615 activity.

**Keywords** Protein kinase C · Antiangiogenesis · VEGF · Caki-1 renal cell cancer · LY317615 · SW2 SCLC

**Abbreviations** *bFGF* Basic fibroblast growth factor *PKC-$\beta$* Protein kinase C-beta · *SCLC* Small-cell lung cancer · *TGD* Tumor growth delay · *TGF-$\beta$* Transforming growth factor-beta · *VEGF* Vascular endothelial growth factor

## Introduction

As potential antiangiogenic compounds enter clinical trials, it has become apparent that it will be useful to identify surrogate markers of antiangiogenic activity [1]. Many studies have evaluated the clinical significance of increased angiogenic growth factor concentrations in patients with various cancers as surrogate markers to predict outcome after adjuvant therapy, extent of metastasis, tumor size, and tumor recurrence in asymptomatic patients [2, 3, 4, 5]. Many of these studies have focused on plasma or serum cytokine concentrations, while others have evaluated increases in tumor cytokine expression [6, 7, 8]. Clinical studies have shown that an elevated tumor cytosolic content of VEGF is a possible predictor of decreased disease-free intervals and post-relapse survival in patients with node-positive breast cancer [7]. Patients with increased tumor VEGF concentrations respond poorly to tamoxifen and chemotherapy [8]. In colorectal carcinoma patients, high serum

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VEGF has been found to correlate with advanced disease, high risk of early aggressive metastasis, a shorter survival period, response to adjuvant chemotherapy and poor outcome [9, 10, 11, 12].

VEGF and bFGF expression has been found to be higher in primary renal cell tumors of patients with metastasis than in primary tumors from patients who were disease-free at 48 months of follow-up [13]. Serum VEGF concentrations are significantly correlated with tumor stage and grade, and overall survival, but not with progressive disease in renal cell carcinoma patients [6]. VEGF expression correlated with poor prognosis in one of two studies of non-SCLC [14, 15]. Significant changes have been shown to occur in serum VEGF concentrations in lung cancer patients who respond to therapy compared to nonresponders [16]. Two studies have shown serum VEGF to be a predictor of survival in ovarian cancer patients [2, 17]. High tumor VEGF expression and high plasma VEGF have been found to correlate with lesser response to therapy in gastric carcinoma patients [18, 19]. In summary, higher plasma VEGF concentrations have been found to be associated with poor treatment outcome.

Protein kinase C isoforms have been shown to be an important component of the signal transduction pathway of both VEGF and bFGF [20, 21, 22, 23]. LY317615 is a PKC-$\beta$-selective kinase inhibitor, which acts by interaction at the ATP binding site [24, 25, 26, 27]. Administration of LY317615 orally twice per day on days 1 through 10 after surgical implant of VEGF-impregnated filters has been shown to result in markedly decreased vascular growth in the cornea of Fisher 344 female rats. A dose of 10 mg/kg of LY317615 decreased vascular growth to about one-half of VEGF-stimulated controls, while a dose of 30 mg/kg of LY317615 decreased vascular growth to the level in unstimulated surgical controls [28]. Administration of LY317615 (30 mg/kg) orally twice per day on days 1 through 10 after surgical implantation of bFGF resulted in decreased vascular growth to a level of 26% that in the bFGF controls. Nude mice bearing human tumor subcutaneous xenografts were treated with LY317615 orally twice daily on days 4 through 14 or 14 through 30 after tumor cell implantation. The number of intratumoral vessels in animals treated with LY317615 (30 mg/kg) was decreased to one-half to one-quarter of that in controls [28, 29, 30, 31, 32]. Although some of the tumors responded to LY317615 as an antiangiogenic agent, in no case was angiogenesis completely blocked as in the corneal micropocket neoangiogenesis model. The tumor growth delay (TGD) in the tested tumors did not correlate with intratumoral vessel decrease [33, 34, 35]. LY317615 is an attractive agent to examine for antiangiogenic activity and has completed a phase I clinical trial [36].

Angiogenic growth factor production in the plasma of mice bearing human tumors has been evaluated [35, 37]. SW2 small-cell lung carcinoma (SCLC), Caki-1 renal cell carcinoma, and HCT116 colon carcinoma tumors in nude mice secrete VEGF and bFGF that is measurable in the plasma of the animals [35, 37]. We evaluated the changes that occurred in plasma VEGF, bFGF and TGF$\beta$ levels of mice bearing the human SW2 SCLC, Caki-1 renal cell carcinoma, or HCT116 colon carcinoma tumors after treatment with the PKC-$\beta$ inhibitor LY317615. The predictive value of plasma concentrations of angiogenic growth factors in evaluating the activity LY317615 in human tumor xenograft models in terms of intratumoral vessel counts and tumor response was assessed.

## Materials and methods

### Tumor implantation and treatment

Male nude mice were purchased from Charles River Laboratories (Wilmington, Mass.) at 5 to 6 weeks of age. When the animals were 7 to 8 weeks of age they were exposed to 4.5 Gray total body radiation delivered using a GammaCell 40 irradiator (Nordion, Ottawa, Ontario). After 24 h, human SW2 SCLC cells, human Caki-1 renal cell carcinoma cells or human HCT116 colon carcinoma cells ($5\times10^6$) prepared from a brie of several donor tumors were implanted subcutaneously in a 1:1 mixture of RPMI tissue culture medium and Matrigel (Collaborative Biomedical Products, Bedford, Mass.) into a hind-leg of the animals.

LY317615 dihydrochloride was prepared as a 10 mg/ml suspension in USP saline. The LY317615 was administered by oral gavage at 100 mg/kg twice daily on days 14 through day 30 or days 21 through 39. Experiments for each tumor were performed in triplicate.

### Tumor volume and body weight measurements

The progress of each tumor was measured twice per week until it reached a volume of 4000 $mm^3$. TGD was calculated as the time taken by each individual tumor to reach 1000 $mm^3$ compared with the time in the untreated controls. Each treatment group included three animals per measurement and each experiment was performed three times. TGD (expressed in days) are the means $\pm$ SE for the treatment group compared with those for the control group [38]. Body weights of the animals were determined using an electronic balance each time the tumor volumes were measured.

### Plasma collection

Plasma samples were obtained every 3 days starting on day 7 after tumor cell implantation. Samples from three individual mice were obtained from the control group and the treatment group at each time point, in three independent experiments. To obtain plasma, mice were killed with $CO_2$ and blood was collected by cardiac puncture and placed into EDTA tubes on ice. The blood samples were centrifuged at 800 rpm for 30 min at 4°C, followed by 10 min at 3000 rpm. The plasma was collected and stored at −80°C until analysis.

### Multiplex assay

Multiplex kits were purchased from R&D Systems (Minneapolis, Minn.) and the assays were carried out as previously reported [34, 35]. Each 96-well filter plate (Millipore) was blocked with 100 µl blocking buffer for 30 min, and filtered through a vacuum at 2 psi. The 25× bead mix was vortexed for 1 min and sonicated for 30 s prior to diluting. The bead mix was diluted in wash buffer, and 50 µl was immediately added to each well. Wash buffer was

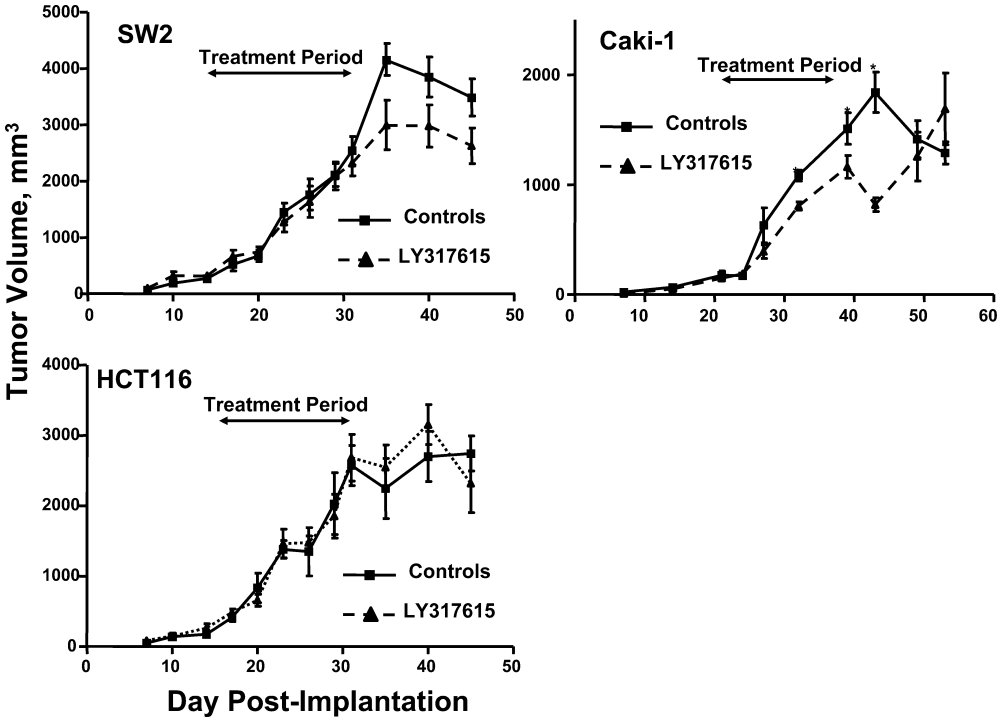


**Fig. 1** Tumor volumes in nude mice bearing human SW2 SCLC, human Caki-1 renal cell carcinoma or human HCT116 colon carcinoma, either untreated controls or treated with LY317615 orally twice daily days 14–30 (days 21–39 for Caki-1-bearing mice). The data represent the average results from three experiments, with each point being the average from nine individual tumors. *Bars* represent SEM. $^{*}P < 0.05$

vacuum-filtered. The standard was dissolved in the medium supplied in the kit. The standards and samples were added to the filter plates containing bead mix in 50-µl aliquots in triplicate (plasma samples were diluted 1:2 with the diluent provided in the kit). To measure TGF-$\beta$ in plasma, a 10-µl sample was acidified with 10 µl 2.5 *N* acetic acid/10 *M* HEPES and incubated for 10 min, then neutralized with 10 µl 2.7 *N* NaOH/1 *M* HEPES, and diluted with 170 µl RD6 medium for a 1:20 dilution. The bead mix is light sensitive and was thus stored in the dark. The plates were placed on a shaker at 4°C overnight to allow binding of growth factors to antibody-bound beads. The following day, the medium was vacuum-filtered, and 50 µl of detection antibody was added to each well. The wells were washed four times with 100 µl wash buffer. The plates were incubated on a shaker at room temperature for 1 h in the dark. The plates were washed four times with 100 µl wash buffer. To each well was added 50 µl streptavidin-PE. The plate was shaken for 15 min at room temperature in the dark. The wells were washed four times with 100 µl wash buffer. The beads were resuspended in 150 µl wash buffer for analysis. Immediately prior to analysis the plates were shaken to resuspend the beads. Plates were read on a FACScan Luminex 100 (Luminex Corporation, Austin, Tx.). Standard curves for each cytokine have been described previously [34]. Data for 100 beads per cytokine were collected for each standard and sample dilution. The lower limit for the assay was 5.6 pg/ml.

### Immunohistochemistry

Immunohistochemistry was performed to investigate the expression of CD31 on selected paraffin-embedded tissues. Paraffin sections of thickness 5 µm were deparaffinized in xylene and rehydrated through a graded series of alcohol into distilled water. Antigen retrieval was performed by heating the sections immersed in Target Retrieval Solution (Dako, Carpinteria, Calif.) to 96°C in a water bath for 20 min. Immunostaining was carried out using the labeled streptavidin-biotin technique. Endogenous peroxidase activity was first blocked with 3% (v/v) hydrogen peroxide in distilled water for 10 min at room temperature. All remaining steps were also performed at room temperature. Following a 30-min treatment with 10% normal horse serum diluted in phosphate-buffered saline (PBS), the sections were subsequently incubated overnight with goat anti-CD31 (RDI) (1:100 dilution in 1% rabbit serum). Sections were exposed to the secondary antibody for 10 min followed by a 10-min incubation with a peroxidase-conjugated streptavidin (Dako). For TGF$\beta$1, following addition of the primary antibody, peroxidase-conjugated streptavidin was added for 10 min, then Biotinyl Tyramide (BT; NEN Life Science Products, Boston, Mass.) for 10 min, followed again by streptavidin-peroxidase for 10 min. All signals were visualized using the Dako liquid DAB substrate-chromogen system. Negative controls included substitution of the primary antibody with PBS/1% normal horse serum.

### Intratumoral vessel counts

Blood vessels were counted as described previously [38]. The most vascular area of tumors was identified on low power (×100) and vessels were counted in ten high-power fields (×200). The data are presented as the means ± SEM for ten high-power fields [38].

### Statistical analysis

Associations between the variables were tested by Student's *t*-test. All differences were deemed significant at $P$ values $< 0.05$.

## Results

We examined the effect of twice-daily oral administration of the PKC-$\beta$ inhibitor LY317615 on tumor growth, angiogenic growth factor plasma levels and intratumoral vessel counts in nude mice bearing human SW2 SCLC, Caki-1 renal cell carcinoma and HCT116 colon carcinoma xenografts. For the faster growing SW2 SCLC and the HCT116 colon carcinoma xenografts, treatment was initiated on day 14 after tumor cell

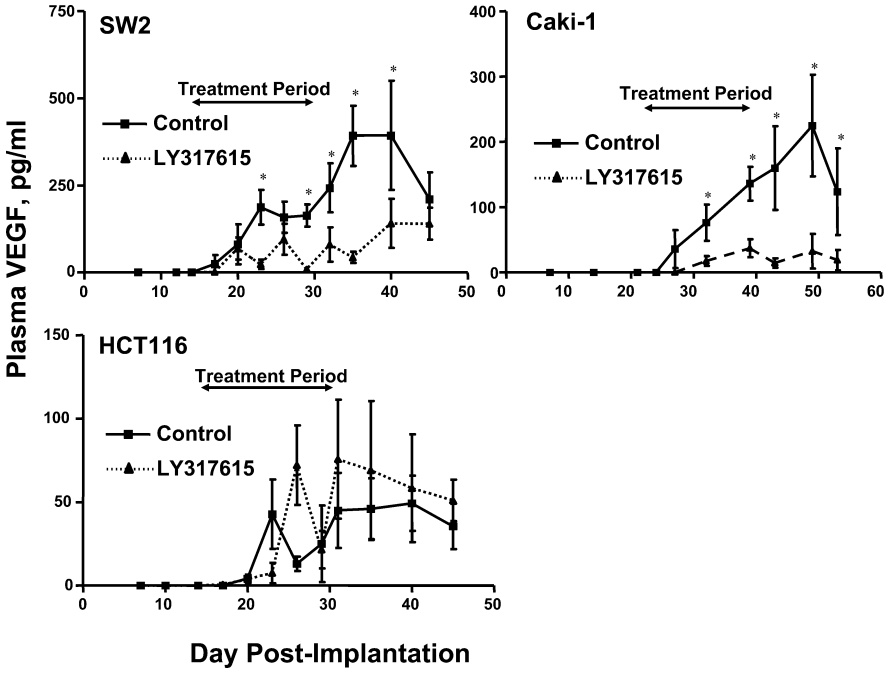


**Fig. 2** Plasma VEGF concentrations in nude mice bearing human SW2 SCLC, human Caki-1 renal cell carcinoma or human HCT116 colon carcinoma, either untreated controls or treated with LY317615 orally twice daily days 14–30 (days 21–39 for Caki-1-bearing mice). The data represent the average results from three experiments, with each point being the average from nine individual tumor-bearing mice. *Bars* represent SEM. $*P < 0.05$

implantation and was continued through day 30. For the slower growing Caki-1 renal cell carcinoma xenograft, LY317615 treatment was initiated on day 21 and was continued through day 39 (Fig. 1). There was no observable toxicity to the mice from the LY317615 treatment as determined by body weight changes.

Tumor dimensions were measured twice weekly to assess the tumor response to the single-agent LY317615 treatment. Because the blood draws for plasma collection were terminal events, it was not possible to follow the progress of individual tumors over the course of the experiment. Tumor dimensions were measured from the three animals that were bled for each time-point. Therefore, each data point for tumor volume and angiogenic factor level is the mean of nine animals. With this experimental design, single-agent LY317615 administration produced no TGD in animals bearing the SW2 SCLC or the HCT116 tumor. However, a TGD of about 15 days was observed in animals bearing the Caki-1 renal cell carcinoma (Fig. 1).

The plasma concentrations of $VEGF_{165}$, bFGF and TGF-$\beta$ in mice bearing the human SW2 SCLC, HCT116 colon carcinoma or Caki-1 renal cell carcinoma treated with LY317615 and in untreated controls were measured by the Luminex assay over the time-course of tumor growth. In the SW2 tumor experiments, the plasma $VEGF_{165}$ concentrations in the SW2 tumor control group continued to increase throughout the experiments reaching 400 pg/ml on day 40 after tumor implantation, while plasma $VEGF_{165}$ concentrations in the treated SW2 small-cell carcinoma-bearing animals had reached 75 pg/ml by day 20 and remained at this concentration for the duration of treatment. Upon completion of the treatment regimen on day 30, plasma $VEGF_{165}$ concentrations in the LY317615-treated mice remained markedly decreased compared to the concentrations in the untreated control mice.

The plasma $VEGF_{165}$ concentrations in the Caki-1 tumor-bearing control animals continued to increase throughout the experiment and peaked at 225 pg/ml on day 49 after tumor cell implantation. In the LY317615-treated group, the $VEGF_{165}$ plasma concentrations remained $< 30$ pg/ml throughout the treatment period (days 21–39) until the termination of the experiment on day 53. Plasma $VEGF_{165}$ concentrations in animals bearing HCT116 colon carcinoma were lower than those measured in the plasma of SW2- or Caki-1-bearing mice. The plasma $VEGF_{165}$ concentrations in the untreated HCT116 colon carcinoma-bearing control mice reached 50 pg/ml and remained at this level for the duration of the experiment. However, treatment with LY317615 did not result in a decrease in plasma $VEGF_{165}$ concentrations in HCT116 colon xenograft-bearing animals (Fig. 2). These results contrast with those obtained with the SW2 and Caki-1 tumor-bearing mice.

TGF-$\beta$ is detectable in the plasma of non-tumor-bearing normal mice. TGF-$\beta$ was detectable in the plasma at each time-point throughout the experiments and ranged from 25 to 55 ng/ml in mice bearing each of the three xenograft tumors. There was no effect on plasma TGF-$\beta$ concentrations of LY317615 treatment throughout the experiments in animals bearing any of the xenograft tumors (Fig. 3).

Plasma bFGF concentrations were not dependent on tumor volume in any of the tumor groups. Values were very unpredictable in each of the tumor groups, with average values in the range 0–100 pg/ml (Fig. 4). There were no differences in plasma bFGF concentrations between control and LY317615- treated groups in any of the tumor lines.

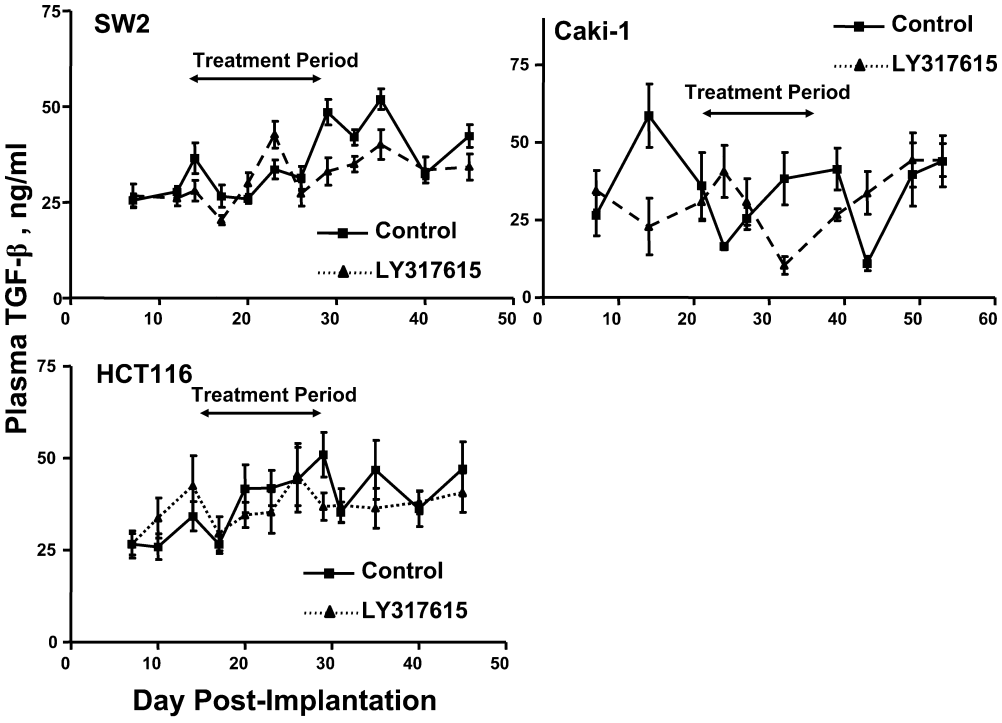


**Fig. 3** Plasma TGF-$\beta$ concentrations in nude mice bearing human SW2 SCLC, human Caki-1 renal cell carcinoma or human HCT116 colon carcinoma, either untreated controls or treated with LY317615 orally twice daily days 14–30 (days 21–39 for Caki-1-bearing mice). The data represent the average results from three experiments, with each point being the average from nine individual tumor-bearing mice. *Bars* represent SEM. $^*P < 0.05$

Intratumoral vessel density was determined by anti-CD31 staining in LY317615-treated and control tumors collected after 9 days of LY317615 treatment. The control Caki-1 renal cell carcinoma tumors had the highest vessel density with an average of 36 countable vessels per high-power field (Table 1). The control SW2 SCLC tumors had an average of 20.4 countable vessels per high-power field, while the control HCT116 colon carcinoma tumors had the lowest vessel density of 14.7 countable vessels per high-power field. Nine days after beginning LY317615 treatment, there was a near 40% reduction in intratumoral vessel density in the LY317615-treated groups compared to controls in mice bearing SW2 SCLC, HCT116 colon carcinoma or Caki-1 renal cell carcinomas ($P < 0.01$).

## Discussion

Tumor angiogenesis is considered an essential step in tumor growth and metastasis, and agents targeted toward angiogenesis are of great interest for cancer treatment [39, 40]. Many studies have evaluated the clinical significance of increased plasma or serum angiogenic growth factor concentrations in patients with various cancers as a predictor of outcome after adjuvant therapy, metastasis, tumor size, and recurrence in asymptomatic patients [2, 3, 4, 5, 6, 13, 14, 15, 16, 17, 18, 19]. Some of these studies have shown positive correlations between VEGF levels or VEGF level changes and tumor response or treatment outcome and some studies have shown no correlation.

The human tumor cell lines, SW2 SCLC, Caki-1 renal cell carcinoma and HCT116 colon carcinoma, secrete VEGF into the medium when grown in culture [34, 35]. In a study including 12 human tumor cell lines grown in cell culture and as xenograft tumors in nude mice, there was no correlation between secretion of VEGF in cell culture and plasma VEGF levels in mice bearing the same tumor cell grown as subcutaneous nodules [35]. In cell culture, Caki-1 cells secreted relatively high amounts of VEGF, HCT116 cells secreted moderate amounts of VEGF and SW2 cells secreted relatively low amounts of VEGF. When grown as xenograft tumors, both Caki-1 tumors and SW2 tumors produced relatively high VEGF plasma levels while HCT116 tumors produced moderate VEGF plasma levels [35]. When Caki-1 cells and SW2 cells were exposed to LY317615 in cell culture for 72 h, the 50% growth inhibitory concentrations ($IC_{50}$) were 50 $\mu M$ and 15 $\mu M$, respectively. These same concentrations of LY317615 resulted in a 50% decrease in the secretion of VEGF by the cultures [34]. Treatment with LY317615 produced a three- to sevenfold decrease in plasma $VEGF_{165}$ concentrations in mice bearing both the SW2 and Caki-1 tumors, but not in mice bearing HCT116 colon tumors after treatment with LY317615. The decrease in plasma VEGF concentrations relative to controls was evident 5 to 7 days after initiation of twice-daily oral administration of LY317615. This decrease continued over the course of the treatment period and persisted over the following 2 to 3 weeks after discontinuing treatment when the animals were killed. There were significantly decreased numbers of countable intratumoral vessels in all three tumors after LY317615 therapy.

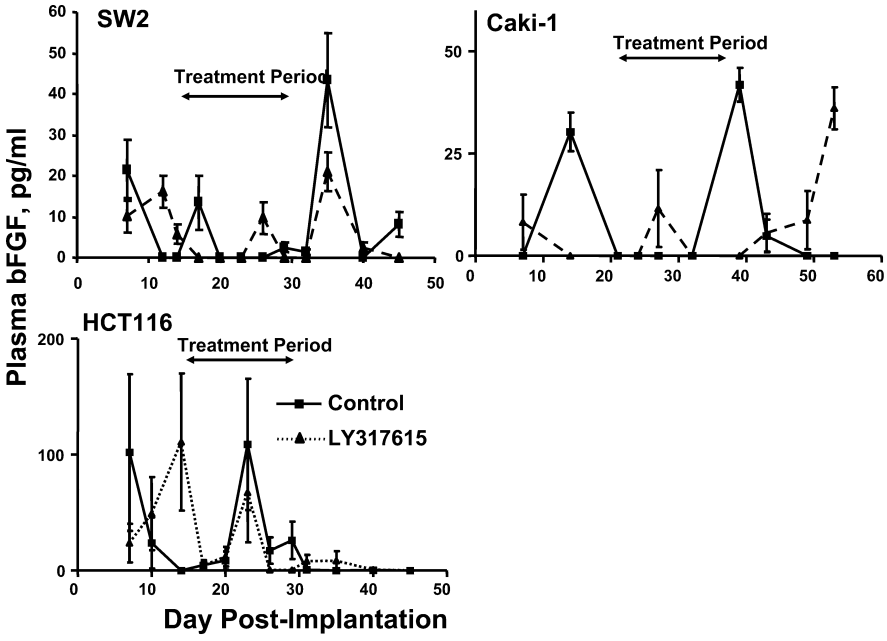


**Fig. 4** Plasma bFGF concentrations in nude mice bearing human SW2 SCLC, human Caki-1 renal cell carcinoma or human HCT116 colon carcinoma, either untreated controls or treated with LY317615 orally twice daily days 14–30 (days 21–39 for Caki-1-bearing mice). The data represent the average results from three experiments, with each point being the average from nine individual tumor-bearing mice. *Bars* represent SEM. $*P < 0.05$

**Table 1** Tumor vessel counts from untreated mice or mice treated with LY317615 for 9 days prior to analysis in terms of CD31 staining of intratumor endothelial cells

| | Tumor | | |
|---|---|---|---|
| | SW2 | HCT116 | CaKi-1 |
| Untreated | 20.4 ± 8.3 | 14.7 ± 6.5 | 36 ± 9.8 |
| Treated | 12.2 ± 8.6* | 9.2 ± 3.5* | 23.1 ± 11.3* |

*P < 0.01 vs untreated mice

Therefore, two measurements, decreased plasma VEGF and decreased intratumoral vessels, both indicated that a biologically active therapy had been administered to animals bearing the SW2 SCLC or the Caki-1 renal cell carcinoma. However, there was no TGD in the SW2 SCLC but the Caki-1 renal cell carcinoma responded to single-agent LY317615 with a TGD of 15 days. Similar results have been obtained in the KB human oral squamous cell carcinoma tumor where there was decreased intratumoral vessel density and decreased tumor VEGF mRNA expression in tumor-bearing mice treated with thalidomide, although there was no significant tumor growth-inhibitory effect [41]. In the same tumor, paclitaxel produced significant tumor growth inhibition but little change in intratumoral vessel density or tumor VEGF mRNA expression [41]. Guba et al. [42] have recently reported similar findings using rapamycin in CT-26 colon adenocarcinoma-bearing Balb/c mice. Both in cell culture and in serum from CT-26 tumor-bearing animals treated with rapamycin, VEGF secretion was decreased and intratumoral vessel density was decreased compared to controls. Rapamycin inhibited metastatic tumor growth and controlled the growth of established CT-26 tumors.

The Caki-1 renal cell carcinoma-bearing animals had high plasma concentrations of VEGF and the largest differential in VEGF levels between LY317615-treated animals and controls. The Caki-1 tumors had the highest number of intratumoral vessels with a mean of 36 per field in the control tumors. The HCT116 colon carcinoma-bearing animals had lower plasma VEGF concentrations in the untreated control group then the other two tumor control groups; however, the VEGF plasma levels in the LY317615-treated animals bearing the HCT116 colon carcinoma were similar to those of the treated groups bearing the other two tumors. The HCT116 tumors had the lowest number of intratumoral vessels with a mean of 14.7 in the control tumors. Both the Caki-1 tumors and the HCT116 tumors responded to LY317615 treatment as determined by intratumoral vessel counts with a 40% reduction in countable vessel numbers. However, only the Caki-1 tumors responded to the single-agent LY317615 treatment with a measurable TGD during the observation time of the experiment. Thus, one conclusion might be that the Caki-1 renal cell carcinoma is more dependent on VEGF-induced vascularization than is the HCT116 colon carcinoma. This effect may be indirect and may reflect the ability of the malignant cells to survive hypoxic stress. There is some indication that tumors with higher VEGF expression are more sensitive to antiangiogenic therapy than those with lower VEGF expression [43, 44, 45].

The kinetics of response to the antiangiogenic effects of LY317615 appeared to be slow. That is, although the decrease in plasma VEGF was evident shortly after administration of the compound began, slowed tumor growth in the Caki-1 tumor-bearing animals was not evident until 1 week later. It is possible that the faster growing SW2 SCLC tumors were beginning to respond

to the LY317615 when the experiment was terminated. Thus, the slower growth rate of the Caki-1 tumors may have made detection of a response to the single-agent LY317615 therapy more likely.

Given the variation in intrinsic and extrinsic conditions, the expression of angiogenic factors by individual tumors is likely to vary. Tumor angiogenic factor profiles are likely to differ among tumor types, and even among individual tumors of the same type. As tumors affect the host endothelial cell phenotype, various tumors implanted at the same anatomical site may differ in their response to antiangiogenic therapy. Other studies have shown that inhibition of tumor growth can range widely. SU5416 treatment produced from 0% to 85% inhibition in ten different tumor lines after treatment with the VEGFR2 inhibitor [46].

We report here the results of a study in which the value of plasma concentrations of angiogenic growth factors and intratumoral vessel counts as biomarkers for the antiangiogenic agent LY317615 was evaluated. Unexpectedly, the selective PKC-$\beta$ inhibitor LY317615 as a single-agent treatment significantly decreased plasma VEGF concentrations in two out of three subcutaneously growing human tumor xenografts in a prolonged manner that persisted well after termination of administration of the compound. Single-agent LY317615 therapy also resulted in significantly decreased intratumoral vessel density in three out of three subcutaneously growing human tumor xenografts. The nonstandard experimental design of this study allowed observation of significant tumor response to single-agent LY317615 therapy in one out of three subcutaneously growing human tumor xenografts. Thus, plasma VEGF was a weak marker of tumor response and plasma bFGF and TGF$\beta$ were not markers of response to the selective PKC-$\beta$ inhibitor LY317615 in three human tumor xenograft models.

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