PTK787/ZK 222584, a Specific Vascular Endothelial Growth Factor-Receptor Tyrosine Kinase Inhibitor, Affects the Anatomy of the Tumor Vascular Bed and the Functional Vascular Properties as Detected by Dynamic Enhanced Magnetic Resonance Imaging

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ABSTRACT

Antiangiogenic therapy is a promising new strategy of inhibiting tumor growth and formation of metastases. Recently, a number of compounds with different effects on tumor endothelial cells have entered clinical trials and revealed the need for diagnostic methods to detect their biological activity. Dynamic enhanced magnetic resonance imaging (adyMRI) is used in most clinical trials with antiangiogenic active compounds. We evaluated this method by using PTK787/ZK 222584, a specific inhibitor of the VEGF-receptor tyrosine kinases, which showed antitumoral and antiangiogenic activity in a murine renal cell carcinoma (RENCA) model. After intrarenal application of RENCA cells, mice developed a primary tumor and metastases to the lung and abdominal lymph nodes. After daily oral therapy for 21 days with either PTK787/ZK 222584 at a dose of 50 mg/kg or vehicle, primary tumors of all animals were analyzed by adyMRI. Gadolinium-DOTA (Dotarem) was used as a contrast agent to detect vessel permeability and contrast agent extravasation, whereas intravascular iron oxide nanoparticles (Endorem) were used to detect partial tumor blood volume. Additionally, vessel density, architecture, diameter, and blood flow velocity were investigated by appropriate methods.

Surprisingly, no changes in extravasation occurred under treatment with PTK787/ZK 222584 as compared with the control group, whereas a significant decrease in vessel permeability occurred. Furthermore, an increase in partial blood volume was found in the PTK787/ZK 222584-treated group, although vessel density was reduced as seen by histology. Using the corrosion cast technique, reduction in vessel density was significant but not very pronounced and predominantly attributable to the loss of microvessels only. This finding correlated with a shift to large vessel diameters in the primary tumors of PTK787/ZK 222584-treated animals and with reduction of blood flow velocity in the tumor feeding renal artery. From these findings, we conclude that the treatment with PTK787/ZK 222584 primarily reduces the number of tumor microvessels, accompanied by a hemodynamic dilatation of the remaining vessels. This dilatation could influence the result of adyMRI such that no change in extravasation or even an increase in partial tumor blood volume could be observed.

INTRODUCTION

Cytotoxic and cytostatic tumor therapy targets the tumor cells, resulting in inhibition of proliferation and induction of apoptosis. Standard noninvasive imaging techniques such as ultrasonography, computed tomography, and MRI are used to detect the success of these therapies, i.e., the reduction in tumor size. New anticancer therapies, such as antiantiogenic strategies, may not lead to regression in tumor size but to stable disease. First clinical trials with antiantiogenic compounds demonstrated that information on early physiological changes is desperately needed to adjust the course, duration, and dose of therapy, based on their efficacy (1). Furthermore, diagnostic methods other than measurement of tumor size are needed to detect the patient’s tumor response to the individual therapy at an early time point. This could avoid premature discontinuation of an effective therapy because of lack of tumor size regression or continuation of an ineffective therapy. Immunohistochemical analysis of vessel density in tumor biopsies is an invasive method that requires easy and repeatedly accessible tissue. At present, adyMRI is used in clinical trials with antiangiogenic agents to assess early changes in tumor-associated vasculature. This technique involves rapid administration of a small, paramagnetic contrast agent, followed by rapid measurement of signal intensity changes in tumor tissue. For diagnostic purposes, differences in enhancement patterns seem to be reliable predictors of the malignancy of breast lesions (2). However, interpretation of the clinical data is still difficult because of the high variability in the correlation with microvessel density, as shown in different Phase I trials with antiangiogenic compounds and a study assessing changes in microcirculation in cervical cancer under chemotherapy in correlation with histomorphological tumor markers and clinical outcome (3).

Various angiogenic factors produced by solid tumors have been identified, and their roles in the formation of new blood vessels have been elucidated. The VEGF is one of the most potent endothelial cell-specific angiogenic factors with a key role in tumor angiogenesis (4). VEGF is also known to induce vascular hyperpermeability. Its specificity for endothelial cells is explained by the enhanced expression of its two receptors, VEGF-R1 and VEGF-R2, on endothelial cells of tumor vessels. Therefore, VEGF-receptors are promising targets for the inhibition of angiogenesis in growing tumors. PTK787/ZK 222584 has been developed as a potent inhibitor of all three VEGF-R-tyrosine kinases (including the lymph-angiogenic VEGF-R3) in a joint effort by Novartis Pharma AG, Schering AG, and the Institute of Molecular Oncology at the Tumor Biology Center, Freiburg. Table 1 gives the IC50 of all affected tyrosine kinases. The compound has already entered clinical trials (5). Its properties have been described in detail elsewhere (6–8). To investigate novel therapeutic strategies such as antiantiogenic therapy, as well as evaluation of novel diagnostic strategies, the murine renal cell carcinoma (RENCA) is a particularly suitable animal model (7, 9, 10). In this model, primary kidney tumors are...
induced by subcapsular renal injection of RENCA cells. Subsequently, metastases in the lung, lymph nodes, and spleen develop (11).

The aim of our study was to evaluate the effect of antiangiogenic therapy on vessel density as detected by immunohistochemistry, vessel architecture as analyzed by corrosion casting, vessel diameter as assessed by light microscopy, vessel permeability as assessed by gradient recalled echo imaging, and partial tumor blood volume as assessed by double inversion recovery imaging. Lumen architecture was analyzed by corrosion casting, whereas intravascular iron oxide nanoparticles (Endorem) were applied to detect partial tumor blood volume. Intravascular gadolinium diethylenetriamine pentaacetic acid (Gd-DOTA) (Dotarem) was used as contrast agent to detect extravasation, whereas intravascular iron oxide nanoparticles (Endorem) were applied to detect partial tumor blood volume.

MATERIALS AND METHODS

Compounds. PTK787/ZK 222584 (1-[4-chloroanilino]-4-[4-pyridylmethyl]) was provided by Novartis Pharma AG (Basel, Switzerland) and Schering AG (Berlin, Germany). The dose of PTK787/ZK 222584 at 50 mg/kg is known to be the optimal dose from previous studies (6, 7). Higher single doses did not increase efficacy in all animal models investigated. A slight increase in efficacy could be shown for twice daily dosing of 50 mg/kg. We did not use the twice daily schedule in these studies to avoid loss of comparability with earlier trials.

Cell Culture. RENCA cells were originally obtained from a tumor that arose spontaneously in the kidney of BALB/c mice. Monolayers of murine RENCA cells were grown in RPMI 1640 with phenol red supplemented with 10% FCS, 2 mM l-glutamine, 100 units of penicillin/mL, and 100 μg of streptomycin/mL. RENCA cells were cultured in a humidified atmosphere of 95% air and 5% carbon dioxide at 37°C. Media were routinely changed every 3 days. Cells were released from the tissue flasks by treatment with 0.05% trypsin/EDTA, and viability was monitored using the cell analyzer Casy 1 from Scha¨rfe System (Reutlingen, Germany). For the experiments, cells were collected during logarithmic growth phase.

Animal Experiments. All experiments were carried out according to the guidelines of the Ethical Committee of the Regierungspr¨asidium (Freiburg, Germany) and the Eiden¨ogenssische Tierschutzverordnung approved by the Veterinary Office Basle. Female BALB/c mice were used at 6–8 weeks of age (approximate weight, 20 g). The injection of 10⁶ RENCA cells in 0.2-ml aliquots into the subcapsular space of the left kidney was performed through a flank incision after the animals were anesthetized with 0.5% isoflurane (Abbott, Cham, Switzerland) at 4.7 T equipped with a self-shielded 20-cm bore gradient system 21 days after tumor cell application. A resonator probe with inner diameter of 40 mm has been used. During the MRI experiments, the animals were anesthetized with 1.5% isoflurane (Abbott, Cham, Switzerland) in a 1:2 mixture of O₂:N₂O administered with a face mask. They were positioned on a cradle in supine position inside the resonator. The exact position of the animal was assessed by a scout imaging sequence.

Gradient recalled echo imaging sequences have been used for determination of blood vessel permeability. Acquisition parameters were: repetition delay TR, 20 ms; echo delay TE, 3.1 ms; number of averages NA, 8; image matrix, 128³, field-of-view FOV, 3.5 × 3.5 cm; slice thickness, 1.5 mm. Sixty-four sequential images with a time resolution of 20 s have been recorded. After the 10th repetitive image, a bolus application of 30 μl Dotarem (Guerbet AG, Z¨urich, Switzerland) was given manually by the tail vein during ~20 s.

Partial tumor blood volume was assessed by gradient recalled echo imaging sequence with the following measuring parameters: repetition delay TR, 10.7
ms; echo delay TE, 3.1 ms; number of averages NA, 2; image matrix, 128 × 64; field-of-view FOV, 3.5 × 3.5 cm; slice thickness, 2 mm. Forty sequential images with a time resolution of 1 s have been recorded. Sixty all of Endorem were injected into the tail vein during acquisition of image 1 using a spectrometer-controlled infusion pump. Heart rate or respiration was not monitored, and accordingly no triggering of the imaging sequence was applied.

The theoretical background for the measurement of vascular permeability and tracer leakage space has been described by Tofts (13) and Rudin et al. (14). The tracer uptake curves derived from the experimental signal enhancement were analyzed by least square fitting (SigmaPlot) using the bi-exponential model function:

\[ \frac{S(t)}{S_0 - 1} = a^* (1 - \exp(-k_a^*(t - t_0))) + b^* (1 - \exp(-k_b^*(t - t_0))) \]

\( S \) is the signal intensity at time point \( t \), \( S_0 \) is the reference signal intensity without contrast agent, \( k_a^* \) is the tracer uptake coefficient, \( t_0 \) is the relative tracer concentration at time point \( t \) is computed by \( \frac{S(t)}{S_0 - 1} \). From the fitting results, the initial slope of the uptake curve (see Fig. 2) and the final tracer uptake were computed as \( \frac{d(S/S_0 - 1)}{dt} \text{ at } t = 0 \) and \( (S/S_0 - 1)_{\text{final}} = a + b \), respectively. Tumor blood volume \( (TBV) \) was estimated as described previously (15, 16) according to \( TBV = -\ln(S/S_0) \).

**Color Doppler Imaging Studies.** Animals, still anesthetized from dyeMRI examination, underwent color Doppler imaging ultrasound as described previously (7). Primary tumors were located in all animals using a sonograph (Kranzbühler GE, Logic 700, Munich, Germany) having a direct-contact, high-frequency ultrasound transducer. The left renal artery was detected by color Doppler imaging, and measurements of systolic and diastolic blood flow velocity and resistance index were performed. To rule out a systemic effect of PTK787/ZK 222584, blood flow velocity was additionally determined in the abdominal aorta. Heart rate was also assessed.

**Corrosion Casting.** BALB/c mice received either PTK787/ZK 222584 (50 mg/kg, p.o. once daily) or vehicle (distilled water) one day after inoculation of RENCA cells as described above. After 21 days of treatment, the animals were thoracotomized in deep anesthesia, an olive-tipped cannula was inserted into the ascending aorta, and up to 30 ml of body warm saline were perfused to rinse the vascular system. Vessels were fixed by a 2–5 min infusion of 25 ml of body-warm glutaraldehyde solution (2.5% phosphate-buffered glutaraldehyde, pH 7.4; 400 milliosmoles). The final casting was performed by the application of 5–15 ml of Mercox CL 2B diluted with methylmetacrylate (4:1).

After having sacrificed the animal, polymerization of casting medium took place in a water bath (40–50°C). Primary tumors were taken out, and tissue was dissolved in a 7% KOH solution for 7–9 days. Casts of the primary tumors were coated with gold for electron microscopy. Microvascular corrosion casting is an established method to investigate vascular patterns of almost all organs. In the past, evaluation of these specimens using scanning electron microscope pictures had not allowed for quantitation, because depth could not be taken into account. However, scanning electron stereo images pairs allowed for quantitative three-dimensional measurements. Therefore, three-dimensionally reconstructed scanning electron microscope images (Stereoscan 180, Cambridge instruments) were used for quantitative assessment of vessel diameters, intervessel distances, and interbranch distances. This method was described in detail elsewhere (17, 18).

**Statistical Analysis.** All values reported were expressed as mean ± SE. Mann-Whitney \( t \) test was applied to all statistical analyses, except for corrosion cast results. Here, the Mann-Whitney rank sum test was used. \( P < 0.05 \) was considered statistically significant.

### RESULTS

**Antitumoral Activity of PTK787/ZK 222584.** PTK787/ZK 222584 was well tolerated by the mice in all experiments and had no significant effects on body weight or general well-being of the animals (data not shown). No animal died under treatment with the compound.

The primary tumor volumes in the left kidney of the PTK787/ZK 222584-treated group was compared with the control group. Treatment with PTK787/ZK 222584 for 21 days resulted in a 24% inhibition of primary tumor growth. The effect of PTK787/ZK 222584 on lung metastases was assessed by comparing the number of lung metastases in treated animals with those in untreated animals. Treatments with PTK787/ZK 222584 were well tolerated by the mice in all experiments and had no significant effects on body weight or general well-being of the animals (data not shown). No animal died under treatment with the compound.
ment resulted in 30% decrease of the number of lung metastases. Visible lymph nodes were reduced to 44.21% (data not shown).

In contrast to previous experiments, the reduction in tumor size by PTK787/ZK 222584 was not significant for all tumor sites, although vessel density detected by immunohistochemistry was dramatically reduced in all tumor tissues. This finding indicates a partial angio-genesis-independent growth of the fast-growing RENCA tumor. This hypothesis was supported by earlier findings (7), where vessel density declined in the period between 2 and 3 weeks after inoculation without slowing down tumor growth, indicating that the proliferation rate of tumor cells was higher than that of endothelial cells. Furthermore, tumors may have the possibility to infiltrate vessels for blood supply in murine models.

**Effect of PTK787/ZK 222584 on Vessel Density.** For histological examination of tumor vasculature, primary tumor tissues of all groups were stained for CD31. Initial examination of all tissue sections at low magnification showed homogeneous vessel density with lack of hot spots (Fig. 1). Comparison of vessel density in primary tumors of untreated animals with those of PTK787/ZK 222584-treated animals revealed a significant decrease of vessel density by 48% ($P = 0.00031$) under treatment. The reduction in vessel density did not lead to the heterogeneous appearance of vessel distribution.

**Effect of PTK787/ZK 222584 on FLK-1.** For histological examination of FLK-1-positive cells, primary tumor tissues of all groups were stained with anti-FLK-1. PTK787/ZK 222584-treated animals had a significant reduction of FLK-1-positive cells by 32% ($P = 0.0015$) compared with untreated animals (Fig. 2).

**Effect of PTK787/ZK 222584 on Vessel Permeability and Gd-DOTA Extravasation.** Twenty-one days after tumor cell inoculation, primary tumors could be located by high-resolution MRI technique (Fig. 3) in all animals ($n = 30$). Vessel permeability and extravasation were measured in 13 of the 15 animals of the control group and in 14 of the 15 animals in the PTK787/ZK 222584-treated group by using contrast agent Dotarem. In the 3 remaining animals, the application of contrast agent could not be performed, probably because of the small-diameter tail veins.

Relative GdDOTA concentration in tumor tissue is calculated from the MR images as $[\text{GdDOTA}] = (S/S_0 - 1)$ (compare methods). Fig. 4 shows the time course of contrast agent extravasation. Vessel permeability, which is known to correlate with the maximal slope, was significantly decreased in the PTK787/ZK 222584-treated group (48%; $P = 0.033$). Astonishingly, no significant difference between both groups could be detected with regard to extravasation, represented by the maximal uptake of the contrast agent.

**Effect of PTK787/ZK 222584 on Partial Tumor Blood Volume.** In an additional MRI experiment, tumor blood volume was assessed by dyMRI (Fig. 3). In these experiments, all animals received a bolus infusion of the intravascular contrast agent Endorem during MRI. Tumor blood volume was assessed in 12 of the 15 animals of the
control group and in 12 of the 15 animals in the PTK787/ZK 222584 treatment group.

The time course of Endorem uptake into the tumor is shown in Fig. 5. Relative tumor blood volume was estimated by:

\[ TBV = -\ln\left(\frac{S}{S_0}\right) \]

Comparing maximal Endorem uptake in primary tumors of untreated animals with PTK787/ZK 222584-treated animals, a significant increase in treated animals by 44% \((P = 0.038)\) becomes apparent, reflecting an increased partial blood volume under therapy.

**Effect of PTK787/ZK 222584 on Blood Flow Velocity.** Blood flow velocity in afferent tumor vessels was assessed by color Doppler ultrasound imaging technique. Twenty-one days after tumor cell inoculation, primary tumors could be located by sonography in all animals \((n = 30)\). Color Doppler imaging was used to identify the left renal artery, which could be detected in all animals. Blood flow velocity and resistance index in the left renal artery were quantified by Doppler technique. Direct comparison of systolic blood flow velocity in the left renal artery of PTK787/ZK 222584-treated and vehicle-treated animals revealed no difference. However, taking the values from the control vessel (abdominal aorta) into account, a previously described reduction of systolic blood flow velocity in the PTK787/ZK 222584-treated animals could be confirmed, showing a reduction in systolic blood flow velocity by 44% \((P = 0.03; \text{data not shown})\).

Previously, we anesthetized the animals by an i.p. application of Rompun/Ketanest, whereas in this experiment, the animals were anesthetized by an isoflurane anesthesia. It is known that gas anesthesia does not influence the vessel activity as strongly as systemic anesthesia \((19)\). This fact may have caused a stronger variability in blood flow velocity, influencing parameters such as blood pressure and heart rate in the animals anesthetized with isoflurane.

**Effect of PTK787/ZK 222584 on Vessel Diameter.** The effects of PTK787/ZK 222584 treatment on the volume of primary tumor vessels were detected by the use of H33342. Vessels could be identified in all histological sections by the surrounding halo of fluorescent H33342-labeled cells (Fig. 6). Tissues showed homogeneous vessel density distribution as already described by immunohistochemistry technique. Significant reduction of vessel density in PTK787/ZK 222584-treated animals by 58% \((P = 5.93 \times 10^{-6})\) shown by immunohistochemical technique could be confirmed \((\text{data not shown})\). Sections of primary tumors from treated animals exhibited a shift of vessel diameters toward larger lumen as compared with control animals (Fig. 7). Vascular lumen enclosed by the halos was quantified.
using a square scoring system. A significant decrease of the number of small vessels (diameter, <62.5 μm) by 76% (P = 1.95 × 10⁻⁵) occurred under therapy, whereas significant increases of the number of large vessels (diameter, ≥62.5 μm) by 307% (P = 0.043) and by 582% (P = 0.015) were observed, respectively.

Effect of PTK787/ZK 222584 on Vessel Architecture. Changes in vessel anatomy by PTK787/ZK 222584 treatment was assessed by the microvascular corrosion casting technique. Low-power magnifications revealed the characteristic features of tumor vascularity in all primary RENCA tumors, which was in striking contrast to the well-organized vascularity in kidneys of healthy mice (Fig. 8). Primary tumors showed a low or missing vessel hierarchy, heterogeneous vascular density, and the presence of evasates and of blind-ending vessels (Fig. 8). Comparison of corrosion casts of the control group (Fig. 8, Ab and Bb) and the PTK787/ZK 222584-treated group (Fig. 8, Ac and Bc) revealed a significant difference for vessel diameters and intervessel distances (Fig. 9). The vessels in the PTK787/ZK 222584-treated group were found to have 26% (P = 0.0001) larger diameter than the vessels of the controls. Mean intervessel distance was increased by 20% (P = 0.0001) in the PTK787/ZK 222584-treated animals. The reduction of mean interbranch distance in treated animals was not significant (data not shown). In summary, the vessels in primary tumors of the PTK787/ZK 222584-treated group were smaller in number but larger in diameter as compared with the control group.

Fig. 7. Primary tumors were quickly frozen in liquid nitrogen after i.v. injection of DNA-binding fluorescent dye Hoechst (H33342). Cryosections of the tissues were taken from both the control group (n = 10) and treatment group receiving 50 mg of PTK787/ZK222 584/kg daily (n = 10). Vessels were identified by the surrounding halo of fluorescent H33342-labeled cells. Vascular volume fraction enclosed by the halos was measured through a square ocular. Using a square scoring system, vessels were allocated to one of the shown groups. Values are means; bars, SE. Ps were calculated by comparing means of the treated group and means of the control group using the Mann-Whitney t test (*, significant; ***, P < 0.001).

Fig. 8. Vessels of right healthy kidney (a), untreated left tumor kidney (b), and PTK787/ZK222 584-treated left tumor kidney (c) of BALB/c mice fixed by corrosion cast technique under low-power (A) and high-power (B) magnification scanning electron microscopy. The treatment group received p.o. 50 mg of PTK787/ZK222 584/day (n = 5), whereas the control group received vehicle only (n = 5). R, cortex vessels; M, medulla vessels; G, glomerulum; SP, subcapsular plexus; N, vascular areas; □, avascular areas; ●, tumor vessels.
EFFECTS OF PTK787/ZK 222584 ON TUMOR VASCULAR BED

New anticancer therapies are currently being developed targeting the vasculature and inhibiting tumor angiogenesis. Likewise, new diagnostic methods such as dyMRI are under development to detect early changes of the targeted vasculature upon therapy. The aim of our work was to evaluate this method by the use of a specific inhibitor of the VEGF receptor tyrosine kinases, PTK787/ZK222 584, in the reliable RENCA model.

The use of the small molecular contrast agent GdDOTA (Dotarem), which rapidly leaks to the interstitial space, allows detection of vessel permeability and leakage space. Comparison of the rate of contrast agent uptake into tumor tissue, indicating tumor vessel permeability, between control and treated animals revealed a significant decrease of vessel permeability in the treated animals. Because, however, the rate of GdDOTA leakage is also dependent on tumor perfusion, the observed reduction in blood flow velocity might have slightly enhanced the observed vascular permeability decrease. Furthermore, changes in tumor perfusion by the treatment might influence our permeability measure. Thus, this parameter is rather an index of perfusion and permeability. However, the GdDOTA extravasation curve is to slow to be heavily influenced by perfusion changes.

Leakage space, detected by the maximum GdDOTA uptake, was not affected by PTK787/ZK 222584. Furthermore, the use of the large molecular contrast agent Endorem, which is expected to remain intravascular during the experimental time of 30 s, allows detection of total tumor blood volume. Surprisingly, the maximal uptake of Endorem, giving the partial blood volume in the primary tumors, was significantly higher for treated animals as compared with untreated animals. This result was in striking contrast to the significantly reduced vessel density and the reduction in FLK-1-positive cells as seen by histology.

The increase in tumor blood volume detected by dyMRI could be explained by the shift to larger blood vessel diameters in tumor tissues of treated animals as seen by DNA-binding fluorescent dye histology and the corrosion cast set up. The observed dilation of the remaining vessels most probably occurs to compensate for the decrease of tumor blood volume because of small vessel loss. The observed reduction of blood flow velocity in the tumor feeding renal artery in the treated animals, assessed by Color Doppler imaging sonography, supports this hypothesis. Tumor blood volume measurements by dyMRI should therefore be interpreted with care unless distribution of vessel size is assessed in parallel. In literature a method for noninvasive determination of mean vessel size index (20) is described, which, however, was not applied in the present study.

Tumor perfusion is dependent on the morphology and three-dimensional architecture of the vascular network. The latter can be studied by scanning electron microscopy of vascular corrosion casts. Qualitative comparison of primary tumors and healthy kidney of a BALB/c mouse revealed loss of the hierarchy and loss of the organ-specific vasculature in primary tumors. Quantitative analysis of casts from PTK787/ZK 222584-treated animals revealed significant differences of intervessel distance and vessel diameter as compared with control mice. Intervessel distance in primary tumors of PTK787/ZK 222584-treated animals estimated by evaluating the three-dimensionally reconstructed vascular network was significantly increased as compared with vehicle-treated mice. This is the first time that the antiangiogenic activity of PTK787/ZK 222584 is detected by three-dimensional vessel bed analysis.

Vessel diameter was found to vary between 10 and 100 μm in primary tumors of the orthotopic RENCA model, whereas the diameter of vessels with capillary wall structure range from 6 to 55 μm in human primary tumors (renal clear cell carcinoma, basalioma) and from 5 to 80 μm in xenografted tumors (sarcomas and colon carcinoma; Ref. 21). In primary tumors of PTK787/ZK 222584-treated animals, vessel diameters were significantly increased as compared with vehicle-treated mice, indicating primary reduction of microvessels. Studies with other VEGF-receptor tyrosine kinase inhibitors such as SU5416 and SU6668 revealed, in line with our study, reduction of microvessel density (22). Other investigators reported, in contrast to our results, a decrease of tumor vessel diameters by using a VEGF antibody (21, 22). The apparently contradictory results of our study demonstrate the importance of assessing different independent vascular parameters by complementary methods to get a clear picture of the mode of action of antiangiogenic drugs.

In conclusion, our results show that selective inhibition of VEGF-receptor activity by PTK787/ZK 222584 leads to antitumor effects without obvious side effects. Treatment with PTK787/ZK 222584 results in a significant reduction of tumor vessel density detected independently by three methods. In addition, we showed that the decrease of vessel density was primarily caused by a pronounced reduction of microvessel density, whereas the remaining vessels were found to be dilated. Furthermore, dyMRI revealed significant reduction of tumor vessel permeability without change of leakage space after PTK787/ZK 222584 treatment. Color Doppler revealed reduced blood flow velocity which, in combination with vessel dilation, may be the result of a possible organ-specific compensatory effect. The very sophisticated and subtle effect of VEGF-receptor tyrosine kinase inhibitors such as PTK787/ZK 222584 on the tumor vascular bed can only be evaluated by the combination of complementary in vivo analysis techniques such as dyMRI and Doppler ultrasound imaging.
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