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 (dyMRI) is used in most clinical trials with angiogenic 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 (RENSA) model. After intrarenal application of RENCA cells, mice developed a primary tumor and metastases to the lung and abdominal lymph nodes. In 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 dyMRI.

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 a 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 dilution of the remaining vessels. This dilution could influence the result of dyMRI such that no change in extravasation or 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 antiangiogenic strategies, may not lead to regression in tumor size but to stable disease. First clinical trials with antiangiogenic compounds demonstrated that information on early physiological changes is desperately needed to adjust the course, duration, and dose of therapy, based on their efficacy. 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, dyMRI 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. 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 angiogenic compounds and a study assessing changes in microcirculation in cervical cancer under chemotherapy in correlation with histomorphological tumor markers and clinical outcome.

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. 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. Its properties have been described in detail elsewhere.

To investigate novel therapeutic strategies such as antiangiogenic therapy, as well as evaluation of novel diagnostic strategies, the murine renal cell carcinoma (RENSA) is a particularly suitable animal model. 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 identified by the DNA-binding fluorescent dye H33342, and vessel blood flow velocity as measured by color Doppler imaging ultrasound. dyMRI was used to investigate the effect of the compound on vessel permeability, leakage space, and partial blood volume. 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 activity in all animal models investigated. A slight increase in efficacy could be shown for twice daily dosing of 50 mg/kg.4 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% (Versene) trypsin/EDTA, and viability was monitored using the cell analyzer system Casy 1 from Schärfe 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 Regierungspräsidium (Freiburg, Germany) and the Eidgenössische 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 0.5–1.5 volume percent isoflurane with an oxygen flow of 1.5 l/min. The injection of 10⁶ RENCA cells in syngeneic BALB/c mice induced progressive development of a primary tumor mass in the left kidney. One week after application, the primary tumor was macroscopically visible. At 10 days, spontaneous metastases developed in the regional lymph nodes, in the lung, the peritoneum, and the liver. The mean survival time of RENCA-bearing mice was ~32 days when 10⁶ RENCA cells were injected.

**Administration of Drug.** Therapy with PTK787/ZK 222584 or the vehicle was initiated 1 day after tumor cell inoculation. Mice received either PTK787/ZK 222584 (50 mg/kg, p.o. once daily) or vehicle (distilled water, p.o. once daily) until the animals were sacrificed. Fifteen animals were included in both groups. Oral applications were performed using a gastric tube. Animal weights were taken every second day.

**Administration of Fluorescent Dye H33342.** The DNA-binding fluorescent dye H33342 was obtained from Aldrich Chemicals Ltd. (Gillingham, United Kingdom). Solutions were prepared in sterile saline immediately before use. H33342 was injected via one of the lateral tail veins at a dose of 20 mg/kg. This dose was chosen because it was well below the toxic limit for this compound and allowed easy identification of labeled cells in histological sections (12). Mice were killed 1 min after injection.

**Evaluation of Tumors.** A previous publication (7) reports on kinetics of tumor growth and on investigations of antitumor and antiangiogenic activity on day 14 in comparison to day 21, showing a more significant efficacy on day 21. For this reason, day 21 for the detection by dyMRI, day 21 was chosen for the analysis. Therefore, after 2 weeks of treatment all mice were sacrificed for determination of weight and volume of primary tumors, lung weight, and number of lung metastases and the number of metastases in the abdominal lymph nodes. Volumes of primary tumors were evaluated macroscopically by measuring their extensions in three orthogonal dimensions. The number of metastases in the lung and abdominal lymph nodes were counted using a dissection microscope. In the abdominal cave, all visible lymph nodes were assessed for detection of metastasis, knowing that in healthy animals visible lymph nodes are usually absent. Lymph nodes were inspected randomly with a microscope to confirm tumor-bearing tissue.

**Immunohistochemistry.** For histological examination of the tumor vascularature, tumor tissues and lungs were frozen immediately in liquid nitrogen. Cryosections of tissue with a thickness of 5–10 µm were taken from both groups. For visualizing the blood vessels, immunohistochemical staining for CD31 (Pecam-1 and MEC13.3; Becton Dickinson GmbH) was performed, and vessels were counted macroscopically using a defined magnification (×200). Furthermore, immunohistochemical staining for VEGF-receptor 2 (anti-FK-1; PharMingen) was performed, and FLK-1-positive cells were counted using a defined magnification (×200).

**Additional sections of primary tumors were air-dried and studied under UV illumination using a Zeiss microscope (Carl Zeiss, Oberkochen, Germany) equipped with an epifluorescent source (×200). Blood vessel borders were identified by the surrounding halo of fluorescent H33342-labeled cells. For detecting vessel density, all recognized vessels were counted. The lumen enclosed by the halos was measured using a square scoring system. Vessels were characterized by their lumen size and assigned to one of the following groups: group 1, no detectable lumen; group 2, lumen less than one quadrant; group 3, lumen = one quadrant (= 62.5×62.5 µm²); and group 4, lumen more than one quadrant.

Currently, vascular density is investigated in “hot spots” only. This might lead to false high values of vessel density, e.g., in tumor tissues, because these data are not correlated to the number of “hot spots” found in the tissues. In our studies, a minimum of three slides from separate areas of each tumor were used for all analysis (CD-31 and dye H33342). Therefore, sections were representative for the whole tumor. Furthermore, the evaluation was performed by two persons who were blinded with respect to treatment of the animals.

**dyMRI.** The mouse tail vein was cannulated for contrast agents bolus application before placing the animal in the magnet. MRI experiments were carried out on a Bruker DBX 47/30 spectrometer (Bruker Medical, Fällanden, 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, Charm, 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 N A, 8; image matrix, 128×128, field-of-view F O V, 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, Zürich, 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

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Table 1 Effect of PTK787/ZK 222584 on different tyrosine kinases

<table>
<thead>
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<th>Kinase</th>
<th>Specification</th>
<th>IC₅₀ (nM)</th>
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<tr>
<td>KDR (VEGF-R2)</td>
<td>VEGF-R2</td>
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<tr>
<td>FLT-1 (VEGF-R1)</td>
<td>VEGF-R1</td>
<td>77 ± 12</td>
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<tr>
<td>Flk (mouse VEGF-R2)</td>
<td>Mouse VEGF-R2</td>
<td>270 ± 40</td>
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<tr>
<td>FLT-4 (VEGF-R3)</td>
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<td>664</td>
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<tr>
<td>c-Kit</td>
<td>730 ± 50</td>
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<tr>
<td>PDGF-R</td>
<td>580 ± 80</td>
<td></td>
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<tr>
<td>c-Met</td>
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<td></td>
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<tr>
<td>FGF-R1</td>
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<td>EGFR</td>
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<tr>
<td>v-Abl</td>
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4 Unpublished data.

**EFFECTS OF PTK787/ZK 222584 ON TUMOR VASCULAR BED**
Fig. 1. Effect of PTK787/ZK 222584 on vessel density in primary tumors of murine renal cell carcinoma (×200). Primary tumor tissues were quickly frozen in liquid nitrogen 21 days after subcapsular inoculation of the RENCA cells into the left kidney. Cryosections of the tissues were taken from both the control group (A) and treatment group (B) receiving 50 mg of PTK787/ZK 222584/kg daily. For the visualization of the blood vessels, immunohistochemical staining for CD31 was performed.
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 angiogenesis-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). Figure 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:

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

(compare methods). 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% ($P = 0.03$; 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 (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

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**Fig. 5.** Uptake of the contrast agent Endorem into the primary tumor tissue of RENCA mice at different time points. The Endorem infusion was started at image 10 (after 2 min). The curve shows the enhancement of Endorem versus time and reveals a significant increase in uptake of contrast agent for PTK787/ZK222584-treated animals ($n = 12$) compared with untreated animals ($n = 12$). The partial blood volume, represented by the maximal uptake of Endorem, is shown in the bar chart. PTK787/ZK222584-treated animals have a significant higher blood volume compared with the vehicle-treated animals. Values are means; bars, SE. $P$ values were calculated by comparing means of the treated group and means of the control group using the Mann-Whitney $t$ test (+, significant).

**Fig. 6.** Cryosections of primary tumors of the left kidney from RENCA mice ($\times 200$). Left, a representative section for the group treated with vehicle only; right, the group treated with PTK787/ZK222 584. One min before sacrifice, DNA-binding fluorescent dye Hoechst (H33342) was injected i.v in all animals. Vessels can be identified by the surrounding halo of fluorescent H33342-labeled cells, revealing reduced vessel density but enlarged vessel diameters in treated mice.
using a square scoring system. A significant decrease of the number of small vessels (diameter, <62.5 μm) by 76% ($P = 1.95 \times 10^{-5}$) 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.
Twenty-one days after treatment, corrosion cast technique was performed, and vessels of 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.

**DISCUSSION**

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/ZK222584, 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 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.
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