YM-359445, an Orally Bioavailable Vascular Endothelial Growth Factor Receptor-2 Tyrosine Kinase Inhibitor, Has Highly Potent Antitumor Activity against Established Tumors

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Abstract

Purpose: The vascular endothelial growth factor receptor-2 (VEGFR2) tyrosine kinase has been implicated in the pathologic angiogenesis associated with tumor growth. YM-359445 was a (3Z) -3-quinoxin-2(1H) -ylidene-1,3-dihydro-2H-indol-2-one derivative found while screening based on the inhibition of VEGFR2 tyrosine kinase. The aim of this study was to analyze the efficacy of this compound both in vitro and in vivo.

Experimental Design: We tested the effects of YM-359445 on VEGFR2 tyrosine kinase activity, cell proliferation, and angiogenesis. The antitumor activity of YM-359445 was also tested in nude mice bearing various established tumors and compared with other VEGFR2 tyrosine kinase inhibitors (ZD6474, CP-547632, CGP79787, SU11248, and AZD2171), a cytotoxic agent (paclitaxel), and an epidermal growth factor receptor tyrosine kinase inhibitor (gefitinib).

Results: The IC50 of YM-359445 for VEGFR2 tyrosine kinase was 0.0085 μmol/L. In human vascular endothelial cells, the compound inhibited VEGF-dependent proliferation, VEGFR2 auto-phosphorylation, and sprout formation at concentrations of 0.001 to 0.003 μmol/L. These concentrations had no direct cytotoxic effect on cancer cells. In mice bearing various established tumors, including paclitaxel-resistant tumors, once daily oral administration of YM-359445 at doses of 0.5 to 4 mg/kg not only inhibited tumor growth but also reduced its vasculature. YM-359445 had greater antitumor activity than other VEGFR2 tyrosine kinase inhibitors. Moreover, in human lung cancer A549 xenografts, YM-359445 markedly regressed the tumors (73%) at a dose of 4 mg/kg, whereas gefitinib caused no regression even at 100 mg/kg.

Conclusion: Our results show that YM-359445 is more potent than orally bioavailable VEGFR2 tyrosine kinase inhibitors, which leads to great expectations for clinical applicability.

Morbidity rate of cancer has been increasing remarkably in recent decades, and the development of diagnostic rapid capabilities has supported early detection and treatment. Although chemotherapy using anticancer drugs began >50 years ago, and the number of anticancer drugs has increased considerably, the prognosis of most patients has not yet been improved because of serious side effects or multidrug resistance (1). As a means of overcoming these problems, antiangiogenesis therapy targeting the vascular endothelial cells in tumors has attracted attention. This is because endothelial cells rarely acquire resistance due to their genetic stability, but cancer cells easily acquire resistance during chemotherapy through the use of cytotoxic agents (2, 3).

Angiogenesis is the complex process of forming new blood vessels from the preexisting vessels that occurs due to many physiologic and pathologic conditions (4, 5). The protrusion of endothelial cells allows local degradation of the basement membrane of the parent vessel; then, endothelial cells migrate outward in tandem to form a capillary sprout. The cells then proliferate followed by lumen formation with subsequent branching; however, the exact sequence of events involved and the regulation of angiogenesis remain unclear. In general, vascular proliferation occurs only during embryonic development and, with few exceptions (e.g., wound healing and in the female reproductive system), is a very slow process in the adult. In contrast, many pathologic conditions (e.g., cancer, atherosclerosis, and diabetic retinopathy) are characterized by persistent, unregulated angiogenesis (6). Therefore, experimental and clinical investigators continue to seek to identify medicinal agents capable of inhibiting the process of angiogenesis. One approach is to identify compounds that can retard vascular endothelial growth factor (VEGF) signaling cascade in vascular endothelial cells.

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VEGF is a key regulator of vascular functions and angiogenesis because it is a strong inducer of vascular permeability and a stimulator of endothelial cell migration and proliferation (7–10). VEGF seems to be important for the formation of ascites and pleural effusion in advanced cancer patients (11, 12). Moreover, VEGF expression is associated with the abnormal angiogenesis that occurs in diabetic retinopathy (13) as well as with several kinds of cancers, such as colorectal, gastric, pancreatic, breast, prostate, lung, and melanoma (14–20). In the clinical setting, bevacizumab, a recombinant humanized monoclonal antibody to VEGF with efficacy against colorectal and other malignancies, has already been approved for patients (21). The effects of VEGF are mediated by three endothelial cell receptor tyrosine kinases, VEGFR1 (Flt-1), VEGFR2 (KDR/Flik-1), and VEGFR3 (Flt-4). VEGFR2 seems to mediate the major growth and permeability actions of VEGF (22–25). Mice engineered to lack VEGFR2 fail to develop a vasculature and have very few endothelial cells that abnormally coalesce into disorganized vessels (26). Although targeting VEGFR2 has been considered appropriate for blocking VEGF signaling in the vascular endothelial cells of a tumor, high daily doses of each recent VEGFR2 tyrosine kinase inhibitor are needed for suppression of tumor growth in xenografted mice: 50 to 100 mg/kg (PTK787; refs. 27–31), 25 to 100 mg/kg (ZD6474; refs. 32, 33), 50 mg/kg (CP-547632; ref. 34), 200 mg/kg (SU6668; ref. 35), and 40 mg/kg (SU11248; ref. 36). To develop a VEGFR2 tyrosine kinase inhibitor with a more potent antitumor activity, we searched and found a (3Z)-3-quinolin-2(1H)-yldiene-1,3-dihydro-2H-indol-2-one derivative, YM-359445, through screening based on inhibition of VEGFR2 tyrosine kinase. Furthermore, we evaluated its antitumor efficacy both in vitro and in vivo and compared with other VEGFR2 tyrosine kinase inhibitors, including the most recently reported high-potency compound (AZD2171; ref. 37), and an epidermal growth factor receptor tyrosine kinase inhibitor (gefitinib). In addition, we show that YM-359445 is also effective in mice bearing cancer cells resistant to such cytotoxic agents as paclitaxel. In this study, YM-359445 is expected to exert a more potent effect than orally active, novel VEGFR2 tyrosine kinase inhibitors that have been used clinically.

Materials and Methods

Agents and cells. YM-359445, (3Z)-3-[(6-[[4-methylpiperazin-1-yl]methyl]quinolin-2(1H)-yldiene]-2-oxindoline-6-carbaldehyde O-[(1,3-thiazol-4-ylmethyl)oxime mono-l-tartrate (Fig. 1), and other tyrosine kinase inhibitors were synthesized at Yamanouchi Pharmaceutical Co. Ltd. (Tsukuba, Japan). Paclitaxel was purchased from Sigma (St. Louis, MO). Normal human endothelial cells [human umbilical vein endothelial cells (HUVECs)] were obtained from Clonetics (San Diego, CA) and cultured on gelatin-coated plates containing endothelial growth medium-2 (EGM bullet kit). Six human cancer cell lines, Colo205 and HCT-15 (colon cancer cells), A549 and NCI-H358 (lung cancer cells), PC-3 (prostate cancer cells), and A431 (human epidermoid carcinoma), were obtained from American Type Culture Collection (Rockville, MD). These cancer cell lines were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies, Grand Island, NY) and 1% antibiotic-antimycotic (Life Technologies). Multidrug-resistant MCF-7/ADR cells were cultured through the stepwise increase of concentrations of Adriamycin (38). When cells were able to survive at any given concentration of drug, they were passaged into medium with concentrations 1.5- to 2-fold higher. The cells subsequently obtained were able to survive in 10 μmol/L Adriamycin. All cell lines were cultured at 37°C in a humidified chamber containing 95% air and 5% CO2.

Inhibition of cell proliferation. The proliferation potency for endothelial cells was measured with bromodeoxyuridine proliferation assay (Roche, Basel, Switzerland). Briefly, HUVECs were placed on 96-well gelatin-coated plate at the density of 1 × 104 per well and then transferred into Medium 199 supplemented with 0.1% fetal bovine serum. After 24 hours, the cells were dosed with YM-359445 for 2 hours and stimulated by human recombinant VEGF (10 ng/mL; R&D Systems, Minneapolis, MN) for 18 hours. The cultures were pulsed with 10 μmol/L bromodeoxyuridine and reincubated for 4 hours. The cells were then fixed and measured for the bromodeoxyuridine incorporation. The incorporated bromodeoxyuridine was detected by ELISA method using a peroxidase-conjugated anti-bromodeoxyuridine antibody. The ELISA was developed by 3,3',5,5'-tetramethylbenzidine substrate, and the absorbance was measured at 392 nm. The proliferation potency for cancer cells was measured with the metabolic indicator Alamar Blue (Biosource International, Camarillo, CA), which was added 48 hours after drug treatment. The degree of proliferation was determined at 530 nm excitation and 590 nm emission.

VEGFR2 kinase domain expression and purification. The VEGFR2 cDNA-encoding amino acids 790 to 1,168 (catalytic domain) were obtained by performing reverse transcription-PCR using total RNA isolated from HUVECs. VEGFR2, nucleotide sequences encoding aFLAG epitope recognized by the M2 monoclonal antibody, were incorporated into the forward PCR primer. The cDNA was then subcloned into the pFastBac1 vector (Life Technologies). SF-9 cells that expressed recombinant VEGFR2 kinase domain via use of the Bac-to-Bac expression system were sonicated and centrifuged at 10,000 rpm for 30 minutes at 4°C. The supernatant was then collected. The VEGFR2 kinase domain was bound to M2-agarose (Sigma) and extracted with a FLAG epitope.

VEGFR2 kinase assay. We used a homogeneous time-resolved fluorescent assay format (39). The kinase reaction solution (25 μL) consisted of 100 ng VEGFR2 kinase domain in assay buffer [50 mmol/L HEPES (pH 7.5), 1 mmol/L MgCl2, 4 mmol/L MnCl2, 0.1% bovine serum albumin]. The kinase reaction was initiated by adding 25 μL of 2 μmol/L ATP in a black 96-well Optiplate (Perkin-Elmer, Wellesley, MA). After a 20-minute incubation at room temperature, 0.5 mol/L EDTA (10 μL) was added to terminate the reaction. Fifty microliters of homogeneous time-resolved fluorescent reagent mixture [6.5 ng cryptate-conjugated anti-phosphotyrosine antibody (PT66, Cis Bio International, Saclay, France) and 100 ng XL665-conjugated FLAG (M2) antibody (Cis Bio International) in quench buffer (50 mmol/L HEPES, pH 7.5, 0.1% bovine serum albumin, 0.5 mol/L KF)] was added to the reaction mixture. The quenched reaction was incubated for 2 hours at room temperature and then read using Discovery (Perkin-Elmer), a time-resolved fluorescence detector.

VEGFR-stimulated VEGFR2 autophosphorylation assay. HUVECs were incubated in gelatin-coated 12-well plates (1 × 105 per well) for 24 hours and then transferred into Medium 199 supplied with 0.1% fetal bovine serum. After 24 hours, the cells were dosed with YM-359445 for 2 hours and stimulated with human recombinant VEGF (50 ng/mL) for 5 minutes. Cells were lysed in TNE buffer containing 10 mmol/L Tris-HCl (pH 7.8), 1% NP40, 0.15 mol/L NaCl, 1 mmol/L EDTA, 10 μg/mL aprotinin, 1 mmol/L NaF, and 1 mmol/L Na3VO4. Cell lysates were detected using Western blot with anti-VEGFR2 (Hik-1 (A-3), Santa Cruz Biotechnology, Heidelberg, Germany), anti-phospho-VEGFR2 [p-Flk-1...
(Tyr204), Santa Cruz Biotechnology), and anti-phospho–mitogen-activated protein kinase (MAPK) antibody [phospho-p44/42 MAPK (Thr202/Tyr204)]; Cell Signaling Technology, Beverly, MA).

**Vessel sprout formation assay.** The angiogenesis kit (Kurabo, Osaka, Japan) was used according to the manufacturer's instructions. Briefly, the plate in which cells were already seeded and the medium for culture were contained in the kit. VEGF (10 ng/mL) was added in the medium to induce angiogenesis more strongly. The medium was replaced every 2 or 3 days. The test condition was medium control, anti-VEGF antibody (25 μg/mL; Sigma), or YM-359445 at a concentration of 0.003 μM. On day 13, the plates were fixed and stained with anti-CD31 antibody and goat anti-mouse IgG alkaline phosphatase conjugate/5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium.

**Determination of plasma concentrations after oral dosing in mice.** Because our aim was to develop a compound that would inhibit VEGF-induced angiogenesis after oral administration, we investigated whether YM-359445 would be absorbed after oral administration in male ICR mice (Japan SLC, Shizuoka, Japan). Plasma concentrations of YM-359445 were also measured after a single oral administration of 1 mg/kg. At the allotted times, three mice were sacrificed from each treatment group, and heart blood was collected into heparinized tubes. Plasma samples were deproteinized by adding 5 volumes of acetonitrile, and the supernatant was analyzed for YM-359445 content using liquid chromatography-tandem mass spectrometry. A standard curve was constructed from plasma added to known concentrations of the compound and processed and analyzed as described above. Concentrations down to 0.1 μmol/L (the lowest concentration in the standard curve) could be determined. Pharmacokinetic variables were determined using WinNonLin software.

In vivo VEGF-induced microvascular permeability models. Male ICR mice were treated with either 0.5 mg/kg YM-359445 or 0.5% methylcellulose as the vehicle control, delivered orally, and injected with 200 μL of 0.5% Evans blue solution into the tail vein. Thirty minutes later, in the ether-anesthetized mice, recombinant murine VEGF (30 ng/10 μL) was s.c. injected into the middle of one ear, and PBS was injected into the other ear. Thirty minutes after that, the ears were soaked in formamide for 2 days. The volume of Evans blue extracted was then quantified using an absorption detector set at 605 nm. The percent inhibition was calculated as follows: \( 1 - \frac{V_{\text{v}}}{V_{\text{p}}} \) of treated group / \( \frac{V_{\text{v}}}{V_{\text{p}}} \) of control group \( \times 100 \), where \( P_v \) is the value at the site injected with VEGF and \( P_p \) is the value at the site injected with PBS.

In vivo tumor xenograft models. Male nude mice were purchased from Charles River Japan, Inc. (Kanagawa, Japan). Female nude mice were used for MCF-7/ADR cells only. A single-cell suspension of each tumor cell line (2 \( \times 10^5 \) cells/mL) was s.c. injected into the flank of nude mice by s.c. injection. When the tumors reached volumes of 50 to 150 mm\(^3\), the mice were randomized into treatment groups of 5 to 6 per group; then, either tyrosine kinase inhibitor or its vehicle (0.5% methylcellulose) was given p.o. daily for >2 weeks. Either paclitaxel (20 mg/kg) or its vehicle (5% clemofole and 5% ethanol) \( \times 5 \) mL/kg. At the allotted times, three mice were sacrificed from each treatment group, and the volume of paclitaxel \( \times 5 \) mL/kg. At the allotted times, three mice were sacrificed from each treatment group, and the volume of paclitaxel extracted was then quantified using an absorption detector set at 605 nm. The percent inhibition was calculated as follows: \( 1 - \frac{V_{\text{v}}}{V_{\text{p}}} \) of treated group / \( \frac{V_{\text{v}}}{V_{\text{p}}} \) of control group \( \times 100 \), where \( P_v \) is the value at the site injected with VEGF and \( P_p \) is the value at the site injected with PBS.

Histologic analysis of Colo205 tumors. Each tumor was fixed in 10% buffered formalin for 24 hours before being preserved in paraffin wax. Sections were produced by standard histologic techniques. To visualize endothelial cells, CD31 was detected using an avidin-biotin method. Briefly, sections were incubated for 1 hour with monoclonal rat anti-mouse CD31 primary antibody (1:500 dilution) followed by a 30-minute incubation with mouse-adsorbed biotinylated rabbit anti-rat immunoglobulin (1:200 dilution) and a further 30-minute incubation with StreptABCComplex conjugated with horseradish peroxidase. Up to four randomly selected fields of view were counted for each section at the \( \times 10 \) objective lens magnification level to determine the CD31-positive area per 35 mm\(^2\).

**Statistical treatment.** Data are expressed as mean ± SE. The significance of differences between the two groups was determined using the unpaired Student's \( t \) test, and the comparison of more than two groups was determined using Dunnett's multiple range test. \( P \) values were calculated, and differences of \( P < 0.05 \) versus control were considered statistically significant.

**Results**

**Effect of YM-359445 on VEGFR2 kinase activity.** Inhibitors of VEGFR2 kinase activity are identified primarily by a time-resolved fluorescence readout. The effects of YM-359445 were also evaluated for ability to inhibit VEGFR2 kinase activity by using enzyme-based assays. The slope obtained for YM-359445 inhibition of human VEGFR2 kinase is shown in Fig. 2A. The IC\(_{50}\) was 0.0085 μmol/L, revealing that it was one of the most potent VEGFR2 tyrosine kinase inhibitors (Table 1).

**Effect of YM-359445 on VEGFR2 autophosphorylation induced by VEGF.** The ability of YM-359445 to inhibit VEGFR2 autophosphorylation by blocking VEGFR kinase activity in a dose-dependent manner was evaluated using cell-based assays. The ability of YM-359445 to inhibit the VEGF-induced phosphorylation of VEGFR2 was also monitored. The concentration of YM-359445 that achieved complete inhibition in this HUVEC.
assay was 0.001 μmol/L, which was also the concentration that inhibited MAPK phosphorylation (Fig. 2B). MAPK has been reported to be downstream of VEGF (40). YM-359445 also inhibited VEGFR2 autophosphorylation without decreasing total amount of VEGFR2 at a concentration of 0.03 μmol/L (Fig. 2C).

Effect of YM-359445 on cell proliferation. As illustrated in Table 2, YM-359445 potently inhibited VEGF-stimulated HUVEC proliferation (IC_{50}, 0.0015 μmol/L) at concentrations comparable with those observed in the inhibition of VEGFR2 autophosphorylation (Fig. 2B). The IC_{50}s for the proliferation of other various cancer cells caused by stimulation with fetal bovine serum were between 0.96 and 4.4 μmol/L, which was ~1,000 times greater than that for VEGF-stimulated HUVEC proliferation.

Effect of YM-359445 on angiogenesis in in vitro bioassays. The activity of YM-359445 was evaluated in in vitro angiogenesis bioassays. The endothelial cell sprout formation assay has been widely used in in vitro systems that effectively model the distinct temporal and spatial events underlying in vitro angiogenesis. The assay is also sensitive to the antiangiogenic effects of inhibitors (41). YM-359445 inhibited endothelial cell sprout formation at a concentration of 0.003 μmol/L as well as anti-VEGF antibody (Fig. 3).

Plasma concentrations of YM-359445 after oral administration to mice. Pharmacokinetic studies in mice showed that YM-359445 was detected in the plasma after p.o. administration. A dose-proportional plasma level of YM-359445 was observed

<table>
<thead>
<tr>
<th>Compound</th>
<th>VEGFR2 tyrosine kinase IC_{50} (μmol/L)</th>
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<tbody>
<tr>
<td>YM-359445</td>
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</tr>
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<td>AZD2171</td>
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<tr>
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<tr>
<td>CP79787</td>
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<tr>
<td>ZD6474</td>
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<tr>
<td>SU6668</td>
<td>6.0</td>
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</table>

* Determined using recombinant enzyme.

Table 2. Inhibitory effect of YM-359445 on cell proliferation

<table>
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<th>Origin</th>
<th>Cell line</th>
<th>Proliferation IC_{50} (μmol/L)* with YM-359445</th>
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</thead>
<tbody>
<tr>
<td>Human umbilical vein</td>
<td>HUVEC</td>
<td>0.0015</td>
</tr>
<tr>
<td>endothelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human colon cancer</td>
<td>Colo205</td>
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<tr>
<td></td>
<td>HCT-15</td>
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<td>1.6</td>
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<tr>
<td></td>
<td>NCI-H358</td>
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<td>PC-3</td>
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</tr>
<tr>
<td>Human epidermoid carcinoma</td>
<td>A431</td>
<td>3.5</td>
</tr>
<tr>
<td>Human breast cancer</td>
<td>MCF-7/ADR</td>
<td>0.96</td>
</tr>
</tbody>
</table>

*HUVECs were treated with YM-359445 after stimulation by 10 ng/mL human VEGF. Cancer cells were cultured with 10% fetal bovine serum and then treated with YM-359445.
when 1 mg/kg was given orally. A peak concentration of ~0.016 μmol/L was reached at 2 hours after oral administration. The effective plasma concentration for endothelial cells, HUVEC, was attained at ~24 hours after administration (Fig. 4). These concentrations did not reach up to the IC₅₀s of cancer cell proliferation, such as Colo205, shown in Table 2. Oral administration of YM-359445 produced good overall systemic exposure with a bioavailability of 23%. When 1 mg/kg was given i.v., total body clearance was 2,506 mL/h/kg, which was approximately half of the hepatic blood flow (5,000 mL/h/kg). Plasma elimination half-life was 4.9 hours.

**Effect of YM-359445 on VEGF-induced vascular permeability in vivo.** VEGF-induced vascular permeability in the ears of mice was assessed by injecting Evans blue solution into the tail. The extravasation of Evans blue at the site injected with VEGF was significantly greater than the site injected with PBS. YM-359445 showed a complete inhibition of vascular permeability induced by VEGF at the dose of 0.5 mg/kg (Fig. 5).

**Effect of YM-359445 on antitumor activity in vivo xenograft models.** Even when endothelial cells were treated with YM-359445 at a concentration of 0.0003 μmol/L for 2 hours and then incubated in YM-359445-free medium for 24 hours, VEGF-stimulated VEGFR2 autophosphorylation was still completely suppressed (data not shown). Therefore, it was expected that once daily repeated administration in vivo would be sufficient. In a preliminary study, YM-359445 caused body weight to decrease when given to normal mice at a rate of no less than 8 mg/kg for 14 days. All mice died at the dose of 64 mg/kg, several mice died at the dose of 32 mg/kg, and no mice died at the dose of 16 mg/kg. Therefore, it was determined that the maximum tolerated dose value of YM-359445 is 16 mg/kg. In contrast, YM-359445 did not cause significant myelosuppression, nor did it change the weight of the liver, spleen, or kidney at doses of up to 4 mg/kg. For this reason, we evaluated antitumor activity using tumor-xenografted mice with administration of up to 4 mg/kg. In the human colon cancer Colo205 xenograft model, treatment with 0.5, 1, 2, and 4 mg/kg YM-359445 for 14 days resulted in 57%, 80%, 98%, and >100% inhibition of tumor growth, respectively. The antitumor activity was more potent than the two other VEGFR2 tyrosine kinase inhibitors, SU11248 and ZD6474 (Fig. 6A). Another problem that occurred when treating with ZD6474 (50 and 100 mg/kg) was that it significantly decreased body weight after the initial administration (10% and 16%, respectively), whereas treatment with 0.5, 1, 2, and 4 mg/kg YM-359445 was generally well tolerated.
YM-359445 did not cause body weight to decrease (data not shown). Vascular endothelial cells in the tumors were found to have dose-dependently decreased the day after the final administration of YM-359445 (Fig. 7). Furthermore, in the human lung cancer A549 xenograft model, treatment with 2 and 4 mg/kg YM-359445 for 14 days resulted in tumor growth inhibition of >100% (tumor regression of 61% and 73%, respectively). Both the clinically approved epidermal growth factor receptor tyrosine kinase inhibitor gefitinib and the cytotoxic agent paclitaxel were significantly weaker than YM-359445 (Fig. 6B). The multidrug-resistant human colon cancer HCT-15 cell line that expresses MDR-1 (42) was observed to be preliminarily resistant to paclitaxel in its xenograft model. Therefore, in this study, the antitumor activity of YM-359445 and paclitaxel in the xenograft model was examined for 14 days. Whereas treatment with 20 mg/kg paclitaxel did not significantly inhibit tumor growth, treatment with 2 mg/kg YM-359445 resulted in a remarkable inhibition of tumor growth (97%; Fig. 8A and B). These results, together with the antitumor activity of YM-359445, other VEGFR2 tyrosine kinase inhibitors, paclitaxel, and gefitinib in other human various cancer xenograft models, are summarized in Table 3. Prolonged oral dosing of YM-359445 produced a significant and dose-dependent inhibition of tumor xenograft growth in all models. The efficacy of YM-359445 was more potent than any other compound, especially AZD2171, the most recently reported high-potency VEGFR2 tyrosine kinase inhibitor (37). At doses of 1, 2, and 4 mg/kg in Colo205 xenografted mice, YM-359445 tended to suppress tumor growth to a greater degree.

**Discussion**

Chemotherapy using cytotoxic agents aimed at killing cancer cells has historically been the main means of treating cancer patients. Recently, a novel chemotherapy method, involving the suppression of tumor growth or metastasis by targeting not cancer cells but vascular endothelial cells, has been explored. Antiangiogenesis agents would be expected to be effective in solid tumors that acquire resistance to cytotoxic agents. Recently, the usefulness of VEGF signaling blockade has attracted attention in the clinical field because VEGF signaling is an important part of vascular endothelial cell proliferation. Several orally active and low molecular compounds that use VEGFR2 tyrosine kinase inhibition as their mechanism of action are being aggressively developed as remedies for cancer (43–47).

YM-359445 is obtained by the structural modification of a basic compound that was found by randomly screening available compounds for the purpose of finding a novel VEGFR2 tyrosine kinase inhibitor. No (Z)-3-quinolin-2(1H)-ylidene-1,3-dihydro-2H-indol-2-one derivatives have been found that have a highly potent effect both in vitro and in vivo. In an enzyme assay for VEGFR2 tyrosine kinase activity, YM-359445 had an IC_{50} of 0.0085 μmol/L, which was extremely potent compared with other VEGFR2 tyrosine kinase inhibitors.
inhibitors. YM-359445 is not a nonspecific kinase inhibitor, because the IC_{50}s of protein kinase A, protein kinase C-α, phosphoinositide-dependent kinase-1, serum and glucocorticoid-inducible kinase-1, and c-Jun NH_{2}-terminal kinase-3 were >1 μmol/L (data not shown). At a concentration of 0.03 μmol/L, YM-359445 inhibited VEGFR2 tyrosine kinase activity completely. In a cell culture system, the IC_{50} of YM-359445 against HUVEC proliferation induced by VEGF was 0.0015 μmol/L, which was a remarkably selective and potent inhibitory effect compared with its efficacy against other various types of cancer cells. In addition, YM-359445 inhibited VEGF-stimulated autophosphorylation of VEGFR2 at a concentration of 0.0001 μmol/L. YM-359445 also inhibited sprout formation of endothelial cells at a concentration of 0.003 μmol/L, which indicates high cell permeability. In this pharmacokinetic study of p.o. single dosing at 1 mg/kg YM-359445 in mice, the bioavailability was 23%, and the maximum plasma concentration of the unchanged form of YM-359445 was 0.016 μmol/L, which indicated that the ADME values were also satisfactory. In addition, because YM-359445 inhibited VEGF-stimulated permeability after administration of a low dose to mice, investigation into the usefulness of the compound for the retention of both pleural effusion and ascites might be interesting.

YM-359445 also showed extremely potent antitumor activity in various human cancer xenografts. YM-359445 was effective in human colon cancer Colo205 xenografts without decreasing the body weight at doses of 0.5, 1, 2, and 4 mg/kg. Most recently, a highly potent AZD2171 that inhibits tumor growth at low doses of 0.75 to 6 mg/kg has been reported (37), but YM-359445 would be more potent. Although two other VEGFR2 tyrosine kinase inhibitors (SU11248 and ZD6474) dose-dependently suppressed tumor growth, the potencies were much weaker than YM-359445. When evaluating human lung

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Cell line</th>
<th>Compound</th>
<th>Route, schedule</th>
<th>Dose (mg/kg)</th>
<th>% Growth inhibition*</th>
<th>% Regression ¹</th>
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<tr>
<td>Colon</td>
<td>Colo205</td>
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<td>57 ¹</td>
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<td></td>
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<td></td>
<td></td>
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<td>25</td>
<td>64 ¹</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>CP-547632</td>
<td>p.o., daily</td>
<td>100</td>
<td>82 ¹</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>CGP7987</td>
<td>p.o., daily</td>
<td>30</td>
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<td></td>
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<td>50</td>
<td>89 ¹</td>
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<td></td>
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<td>HCT-15</td>
<td>p.o., daily</td>
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<td>NCI-H358</td>
<td>YM-359445</td>
<td>p.o., daily</td>
<td>0.5</td>
<td>77</td>
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<td>A375</td>
<td>YM-359445</td>
<td>p.o., daily</td>
<td>0.5</td>
<td>14</td>
<td>—</td>
</tr>
</tbody>
</table>

* Each value represents % inhibition of tumor growth versus control.
¹ Each value represents % tumor regression from day 0.
⁺ P < 0.01.
⁺⁺ P < 0.05.
⁺⁺⁺ P < 0.001.
YM-359445: A VEGFR2 Tyrosine Kinase Inhibitor


YM-359445, an Orally Bioavailable Vascular Endothelial Growth Factor Receptor-2 Tyrosine Kinase Inhibitor, Has Highly Potent Antitumor Activity against Established Tumors

Nobuaki Amino, Yukitaka Ideyama, Mayumi Yamano, et al.


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