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A novel angiogenesis inhibitor, Ki23057, is useful for preventing the progression of colon cancer and the spreading of cancer cells to the liver

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ABSTRACT

Ki23057 is a new, small synthetic tyrosine kinase inhibitor that blocks autophosphorylation of the VEGF receptor2 (VEGFR2). To determine the effect of Ki23057 as an anti-angiogenic agent, we studied the effect of Ki23057 for colon cancer and vascular endothelial cells *in vitro* and *in vivo*. Ki23057 inhibited VEGF-induced proliferation of human umbilical vein endothelial cells (HUVECs), whereas no inhibitory effect of Ki23057 on the proliferation of three colon cancer cells (LM-H3, LoVo and LS174T) was observed by means of the cell count assay. Ki23057 inhibited tube formation of HUVECs. Immunoprecipitation demonstrated that Ki23057 inhibited tyrosine phosphorylation of VEGFR2 in HUVECs. Ki23057 exhibited a significant inhibitory effect on the growth of the xenografted LM-H3 tumours and the spreading of cancer cells to the liver. Anti-CD31 antibody stained significantly fewer microvessels in the xenografted tumours treated with Ki23057 compared with controls. Ki23057 may be a promising new antiangiogenic agent for colon cancer.

1. Introduction

Although new drugs for colon cancer have been developed and survival is improving, the prognosis of patients with colorectal cancer is poor. Novel treatment approaches and new drugs are necessary. Recent investigations of the proliferation, progression and metastasis of colon cancer have elucidated the molecular mechanism, and molecular targeting therapy for cancer has begun to draw attention. Several molecular targeting agents have been developed and efficacy of these agents has been reported.[1–3] Molecular targeting agents, such as imatinib and bevacizumab, have been examined in clinical trials.

Imatinib mesylate, an inhibitor of the tyrosine phosphorylation of bcr-abl and c-kit, has been reported to have a strong effect on chronic myeloid leukaemia and gastrointestinal stromal tumours (GIST).[4,5] Bevacizumab is a humanised monoclonal antibody directed against VEGF-A. Hurwitz and colleagues reported that treatment with bevacizumab in addition to irinotecan, 5-fluorouracil and leucovorin resulted in a significantly longer survival time than chemotherapy alone in colorectal cancer.[6] Thus, molecular targeting therapy is anticipated to be promising for colon cancer.

Angiogenesis is essential to provide both oxygen and nutrition for tumours beyond 1 mm in size.[7] Colon cancer requires angiogenesis for progression and metastasis.[8] One of

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the significant angiogenic factors, VEGF, is produced by several cancers, including colon cancer.[9,10] VEGFR2 exists primarily on endothelial cells and is associated with proliferation, migration and tube formation of endothelial cells.[10] VEGFR2 has an extracellular VEGF-binding domain, a single membrane-spanning domain, and an intracellular split tyrosine kinase domain. Binding of VEGF and VEGFR2 induces receptor tyrosine kinase phosphorylation and stimulates the phospholipase Cγ-protein kinase C-mitogen-activated protein (MAP) kinase /extracellular single-regulated kinase (ERK) pathway, as well as the phosphatidylinositol 3′-kinase/AKT pathway. These signals are associated with the proliferation of HUVECs.[11,12] Inhibition of angiogenesis can be a promising target for colon cancer therapy.[13] In colorectal cancer, VEGF levels are elevated and correlate with a poor clinical outcome.[9] Some studies have indicated VEGF expression as an independent factor in predicting patients' prognosis.[14,15]

The novel compound, Ki23057, is a small synthetic molecule that interrupts proliferation-related receptor tyrosine kinase (RTK) pathways, as an autophosphorylation inhibitor. Ki23057 exhibits considerable inhibitory activity against RTK receptors, including autophosphorylation of VEGFR, platelet-derived growth factor receptor β (PDGFRβ), fibroblast growth factor receptor2 (FGFR2) and c-Kit, but not the EGFR, insulin like growth factor-1 receptor (IGF1R), or c-met.[16]

In this study, we examined the effect of Ki23057 on progression of colon cancer to elucidate whether Ki23057 is a promising angiogenesis inhibitor.

2. Materials and methods

2.1. Compound

Ki23057 (molecular weight 538.1) was synthesised by Kirin Brewery Co., Ltd. (Gunma, Japan).[16] The chemical structure is shown in Fig. 1. Ki23057 was dissolved in distilled water, stored in a light-shielded container at 4 °C, and used within 5 days. For *in vivo* experiments, the agent was dissolved in distilled water and administered orally. For *in vitro* experiments, the diluted Ki23057 was mixed at various concentrations with Dulbecco,s modified Eagle medium (DMEM; Bioproducts, Walkersville, MD, USA).

2.2. Cells and culture conditions

LM-H3 is a human colon cancer cell line that was established from a liver metastasis in our laboratory.[17] LM-H3, LoVo and LS174T, which are colon cancer cell lines, were cultured in DMEM supplemented with 10% foetal bovine serum (FBS; Gibco, Grand Island, NY), 2% penicillin (ICN Biomedicals, Ohio, USA) and 0.5 nM sodium pyruvate (Cambrex Bio Science, Walkersville, MD). Human umbilical vein endothelial cells (HUVECs) were purchased from Kurabo (Osaka, Japan). HUVECs were cultured in endothelial cell growth medium 2 (HuMedia-EB2, Kurabo, Osaka, Japan). The HUVEC culture medium is composed of 2% FBS, EGF (epidermal growth factor) 10 ng/ml, hydrocortisone 1 μg/ml, gentamicin 50 μg/ml, amphotericin B 50 ng/ml, b-FGF (basic fibroblast growth factor) 5 ng/ml, and heparin 10 μl/ml. These cells were incubated at 37 °C in a humidified atmosphere with 5% carbon dioxide.

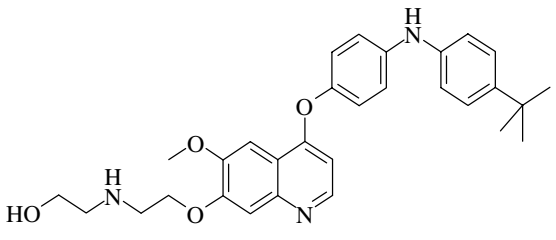

Fig. 1 – Chemical structure of Ki23057.

2.3. Effect of Ki23057 on proliferation of colon cancer

The proliferation of human colon cancer cell lines, LM-H3, LoVo, and LS174T, in the presence and absence of VEGF +/− Ki23057 was evaluated using cell count assay. The number of cancer cells was calculated using a Coulter Z2 (Beckman Coulter, Fullerton, CA) after culture in medium including Ki23057 at various concentrations (0, 10, or 300 nM). LM-H3 (7×10^3 cells), LoVo (7×10^3 cells) and LS174T (10^3 cells) were seeded into 96-well microplates (Falcon) with 150 μl DMEM containing 2% FBS. VEGF (100 ng/mL) was subsequently added to each well and the plate was incubated for 72 h. Then the number of cancer cells was counted using the Coulter Z2.

2.4. Effect of Ki23057 on proliferation of HUVECs

The proliferation of HUVECs in the presence and absence of VEGF was evaluated using cell count assay. HUVECs were seeded at 2.5×10^3 cells per well on a 96 well-plate in HuMedia-EB2 medium. Each well was treated with Ki23057 at various concentrations (0, 10, 30, 100, and 300 ng/mL). VEGF (10 ng/mL) was subsequently added to each well and the plate was incubated for 72 h at 37 °C in 5% CO_2. The number of cells was calculated using the Coulter Z2.

2.5. Effect of Ki23057 on apoptosis induction of HUVECs

Apoptosis was detected using flow cytometry by staining cells with annexin V-FITC and propidium iodide (PI; BD Pharmingen, San Diego, CA) labelling. HUVECs were seeded at a density of 3.0×10^4 cells/mL in a 6-well plate. The plates were incubated for 72 h at 37 °C with or without VEGF (10 ng/ml), and with Ki23057 (0, 10, 30, 100, 300 nM). Cells were stained with 2.5 μl of Annexin V-FITC and/or 2.5 μl of propidium iodide (50 ng/mL) according to the instructions of the manufacturer, incubated for 15 min at room temperature in the dark, and immediately analysed by flow cytometry using a Becton Dickinson FACScan. Viable cells were not stained with either dye, apoptotic cells with only annexin V-FITC, and secondary necrotic cells with both annexin V-FITC and PI.

2.6. Effect of Ki23057 on HUVEC tube formation

Capillary tube formation of endothelial cells was measured by coculture with normal human fibroblasts using an Angiogenesis kit (Kurabo, Osaka Japan). Normal human fibroblasts in EB2 were plated in 24-well plates and cultured with HUVECs. After 24 h, Ki23057 was added at a concentration of 0, 30 or

300 nM, followed by VEGF (10 ng/mL). The cells were cocultured for 11 days, fixed at room temperature in 70% ethanol and stained with anti-CD31 antibody. The number of vessels was counted as follows. The areas to be counted were determined at the beginning, which comprised twelve sections including four sections in the centre and eight sections near the margin, and were observed at 40 magnification. The numbers of vessels intersecting randomly placed 40 dots were counted, at 12 sections per well. The average was determined as the number of vessels.

2.7. Effect of Ki23057 on VEGFR2 phosphorylation of HUVECs

The effect of Ki23057 on receptor phosphorylation was examined as follows. HUVECs were plated at a density of 2×10^6cells and cultured for 24 h in Humedia-EB2 with 5% FBS. The supernatant was subsequently discarded and the cells were rinsed with Humedia-EB2 with 0.5% FBS. Cells were incubated in medium with Ki23057 (1–10^3 nM) for 90 min. VEGF (50 ng/mL) was added to stimulate the cells for 5 min. 600 μl Lysis buffer was added to the incubated culture for 2 h at 4 °C. Cells were isolated by centrifugation at 4 °C at 15000 rpm for 10 min. The protein concentration of each sample was adjusted and lysates were incubated with Flk-1 antibody: sc-504; 1:50 (Santa Cruz, Delaware, CA) for immunoprecipitation. Immunocomplexes were purified by incubation with protein G-sepharose 4 Fast Flow (20 μl) (Amersham Biosciences, Uppsala, Sweden) for 1 h at 4 °C. Electrophoresis was conducted using 3–8% Tris-Acetate gels (Invitrogen life tech, Carlsbad, CA) at 120 V for 1.5 h. The electrophoresed protein bands were transferred to Immobilon-P Transfer membrane (Millipore, Bedford, MA). The membrane was incubated in PBS-T (10 mM PBS and 0.05% Tween 20) supplemented with 5% bovine albumin (Sigma, St. Louis, MO) for 1 h at room temperature. To detect phosphorylated VEGFR2, the membrane was placed in PBS-T solution containing primary antibody:p-Tyr (PY20); 1:2000 (BD Biosciences Pharma CA) and incubated for 1 h at room temperature. The membrane was washed three times with PBS-T for 10 min, and incubated overnight at 4 °C with peroxidase-labelled secondary antibody: GAM-HRP; 1:2000 (Amersham, Aylesbury, UK). The membrane was placed in PBS-T solution, incubated for 1 h at room temperature, and then washed. Bands were detected using an enhanced chemiluminescence system (Amersham Bioscience, UK).

2.8. Effect of Ki23057 on tumour growth

LM-H3 (10^7 cells) was inoculated by subcutaneous injection into the backs of nude mice. After 10 days, mice were randomly divided into two groups: controls and Ki23057 (25 mg/kg/mice). Accordingly, 25 mg/kg/day of Ki23057 was administered for 5 days per week for 2 weeks ($n = 7$), except in control ($n = 7$). The mice were weighed twice weekly. The tumour volume was calculated by the formula: 0.5× (longest diameter)×(shortest diameter)^2. All of the control mice ($n = 7$) and the treatment mice ($n = 7$) were sacrificed at the 14th day after the Ki23057 treatment. We measured the size of each subcutaneous tumour and calculated the mean volume of the tumours to compare the two groups. Tumours were stored at −80 °C. The xenografts were fixed in 10% neutral buffered formalin solution. Microvessels in xenografted tumours from the mice treated with Ki23057 and control mice were stained immunohistochemically using anti-mouse CD31 (PECAM-1, BD Pharmingen, USA) antibody by the avidin-biotin-peroxidase complex technique. Tissues were incubated with CD31 antibody for 30 min. After incubation with the secondary antibody, sections were washed in PBS and incubated in streptavindin-peroxidase reagent for 5 min at room temperature. Finally, specimens were incubated in PBS containing diaminobenzidine and 1% hydrogen peroxidase for 10 min (Histofine SAB-PO kit, Nichirei, Tokyo, Japan), followed by Mayer's Haematoxylin. We have chosen five views in the tumour, which are the centre, upper, lower, left, and right side. Those which were CD31 positive and had tubular formation were determined as new formed vessels and the mean of the five views were determined as the numbers of new vessels.

2.9. Effect of Ki23057 on the spreading of cancer cells to the liver

BALB/c nude mice were used ($n = 14$). The models of liver metastasis underwent laparotomy from the left side of the abdomen, ethanol anaesthetised, and the spleen was drawn outside the body. Injection of 5×10^5LM-H3 cells/0.1mL PBS into the lower side of the spleen was performed by a 26G needle. One minute after injection, splenectomy was performed after the splenic artery and vein were ligated. The wound had single closure by nylon yarn. After 2 weeks, mice were randomly divided into two groups: controls ($n = 7$) and Ki23057 treatment groups (25 mg/kg/mice; $n = 7$). Ki23057 (25 mg/kg/day) was administered orally for 5 days and had a 2 day interval. This was repeated for another week. At the end of treatment, All of the control mice ($n = 7$) and the treatment mice ($n = 7$) were sacrificed. The liver was removed and weighed to compare the two groups.

2.10. Statistical analysis

Results were expressed as the mean±SD. Student's *t*-test was used for statistical analysis and significance was defined at $p < 0.05$.

3. Results

3.1. Effect of Ki23057 on proliferation of HUVECs and colon cancer cells

VEGF significantly ($p < 0.05$) increased the proliferation of HUVECs, compared with the control. Ki23057 significantly inhibited growth-stimulating effect of VEGF on HUVECs in a dose dependent manner. The mean cell numbers of HUVECs were 19090, 16995, 14855, and 6600 in a presence of Ki23057 at concentrations 10 nM, 30 nM, 100 nM, and 300 nM, respectively (Fig. 2a). HUVEC was significantly suppressed by 10 nM or more of Ki23057 compared with the control. On the other hand, no growth-effect of VEGF was found in human colon cancer cells (LM-H3, LoVo, and LS174T). Ki23057 (10–300 nM)

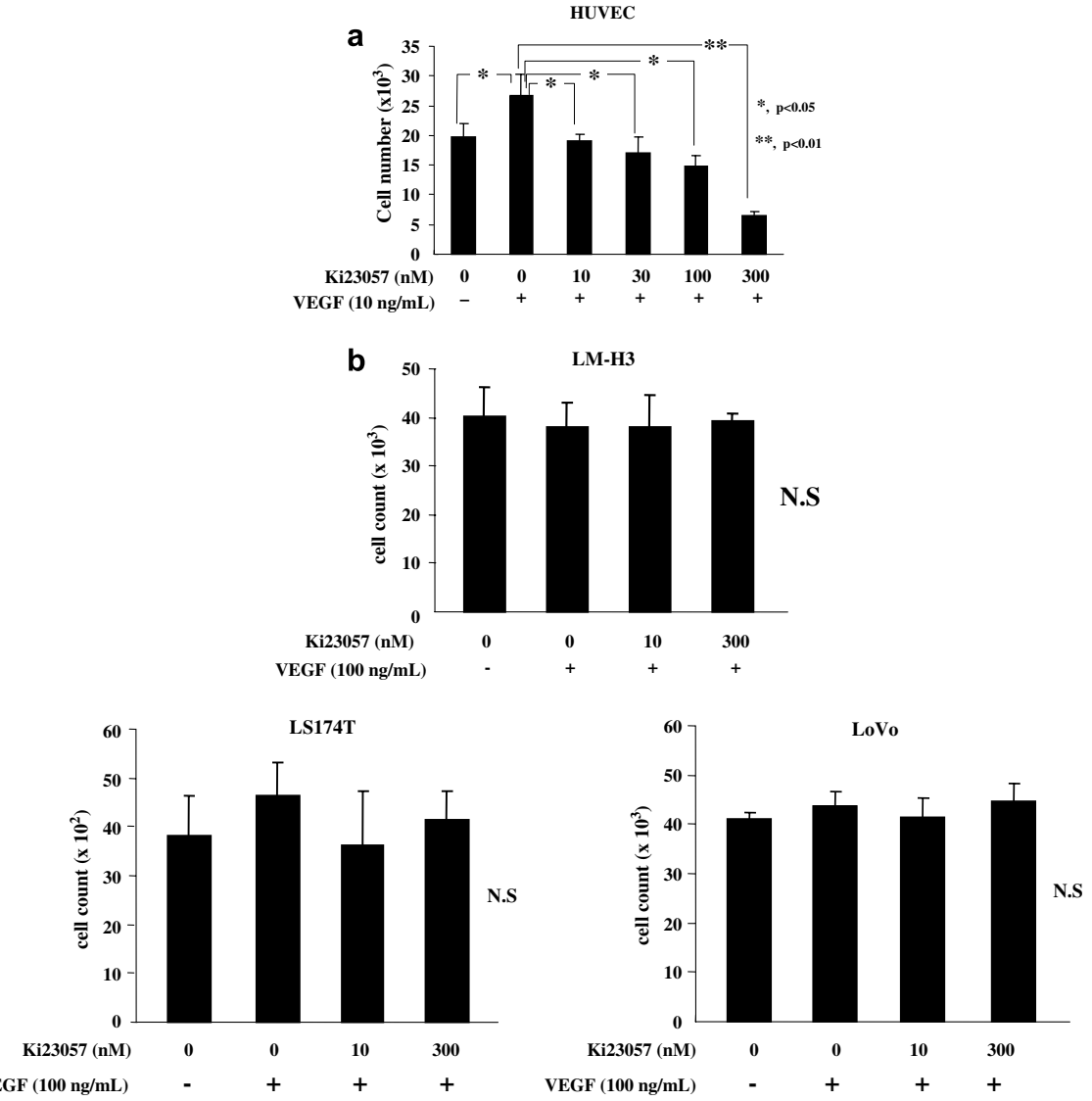

Fig. 2 – (a) The growth inhibitory effect of Ki23057 on endothelial cells. The cell viability of endothelial cells was inhibited by 24.8% at concentrations of 300 nM compared with that in the absence of Ki23057. *, $p < 0.05$ **, $p < 0.01$. (b) **The growth inhibitory effect of Ki23057 on three cancer cell lines. The cell viability of LM-H3, LoVo and LS174T was not inhibited by Ki23057 at 10–300 nM. (N.S: not significant).** (c) **Apoptosis induction by Ki23057. Double staining of cells with Annexin V (FITC) and PI was performed to clarify the induction of apoptosis during the growth suppression of HUVECs by Ki23057 with or without 10 ng/mL VEGF. Cells staining annexin V positive, PI negative were considered to be apoptotic. Ki23057 was added to the HUVECs cultures at a concentration of 0–300 nM. In HUVECs, Ki23057 at 10 nM induced apoptosis at a rate of 12.1%. The apoptosis rates induced by Ki23057 at 30, 100, 300 nM were 14.0%, 16.7% and 26.3%, respectively. Ki23057 at a concentration of 10–300 nM increased apoptosis induced in HUVECs.**

did not affect the growth of these colon cancer cell lines (LM-H3, LoVo, and LS174T) (Fig. 2b).

3.2. *Effect of Ki23057 on apoptosis induction of HUVECs*

Fig. 2c shows the rates of apoptosis induced by Ki23057 in HUVECs. Ki23057 was added to HUVECs cultures at the concentration of 0–300 nM with or without 10 ng/mL VEGF. In HUVECs, Ki23057 at 0 nM without VEGF induced apoptosis at a rate of 12.3%. Ki23057 at 0 nM with 10 ng/mL VEGF induced apoptosis at a rate of 9.8%. The apoptosis rates induced by Ki23057 at 10, 30, 100, 300 nM with 10 ng/mL VEGF were 12.1%, 14.0%, 16.7% and 26.3%, respectively. Ki23057 at concentration of 10–300 nM induced apoptosis in HUVECs.

3.3. *Effect of Ki23057 on tube formation of HUVECs*

Tube formation was inhibited in HUVECs treated with Ki23057 compared with controls (Fig. 3a). The average numbers of vessels were 4.50±0.71/HPF (without addition of VEGF and Ki23057), 7.94±1.33 /HPF (10 ng/mL VEGF, without Ki23057), 1.47±0.37/HPF (10 ng/mL VEGF and 30 nM Ki23057) and 0.19±0.16/HPF (10 ng/mL VEGF and 300 nM Ki23057) (Fig. 3b). The average number of vessels was significantly decreased

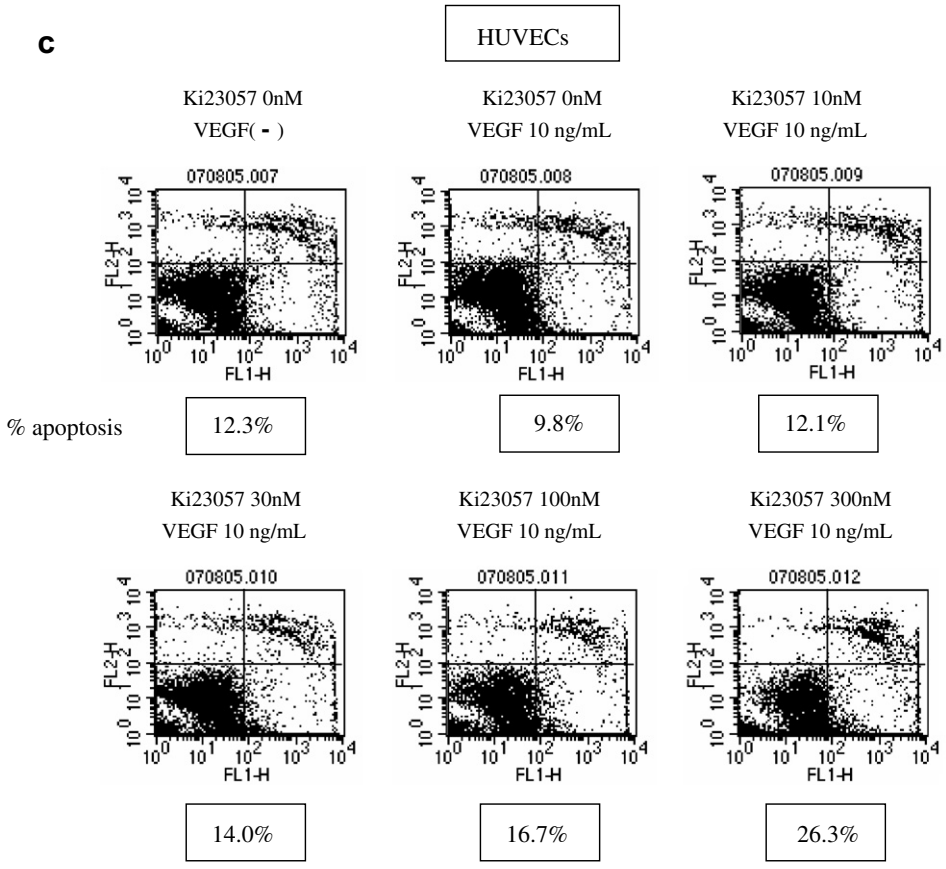

Fig. 2 (*continued*)

in HUVECs treated with Ki23057 (30 nM and 300 nM), compared with VEGF 10 ng/mL alone ($p < 0.01$).

3.4. *Effect of Ki23057 on VEGFR2 phosphorylation in HUVECs*

Phosphorylated VEGFR2 was not detected in HUVECs, in the absence of VEGF (50 ng/mL). When VEGF was added to the condition medium, phosphorylated VEGFR2 was detected. The phosphorylation of VEGFR2 was inhibited by Ki23057 in a dose-dependent manner (Fig. 4).

3.5. *Effect of Ki23057 on tumor growth in vivo*

Fig. 5a shows the representative macroscopic findings of one of seven mice. Fig. 5b shows the mean volume of tumours in each group. Ki23057 exhibited a significant inhibitory effect on the growth of xenografted LM-H3 tumours in mice (Fig. 5b). From the 10th day after tumour inoculation, Ki23057 administration started and this day was determined as day 0. Ki23057 was administered at 25 mg/kg/day, for 5 days and had a 2 day interval. This schedule was repeated for another week. The average volume of tumours at day 0 was 284±56 mm^3 in control group and 307±89 mm^3 in the treatment group. At day 3, the average volume of tumours was 174±49 mm^3 in the treatment group and 277±61 mm^3 in the control group ($p < 0.01$). At day 14, the average volume of the tumours was 203±59 mm^3 in the treatment group and 549±187 mm^3 in the control group (Fig. 5b) ($p < 0.01$). After day 3, the size of tumour was significantly reduced in the treatment group compared with the controls. None of the mice died during the experiment.

3.6. *Effect of Ki23057 on vessel counts in xenografted tumours*

Microvessel density in xenografted tumours treated with Ki23057 was less than that in controls (Fig. 6). The average number of vessels was 8.29 ± 2.40/HPF in the control group. By contrast, that in the treatment group was 4.50±1.02 /HPF. The average number of vessels decreased significantly in xenografted tumours treated with Ki23057, compared to controls ($p = 0.0023$).

3.7. *Effect of Ki23057 on the spreading of cancer cells to the liver*

As there were a multitude of blended nodules in the livers (Fig. 7a), liver weight was compared. Fig. 7a shows the representative macroscopic findings of one of seven mice. Fig. 7b shows the mean volume of tumours in each group. The weight of the liver was 3.73±1.00 g in the control group ($n = 7$) and 2.43±0.52 g in the treatment group ($n = 7$). The liver weights in the control group were significantly greater than the treatment group ($p = 0.034$) (Fig. 7b). Compared with the control group, the treatment group exhibited significant

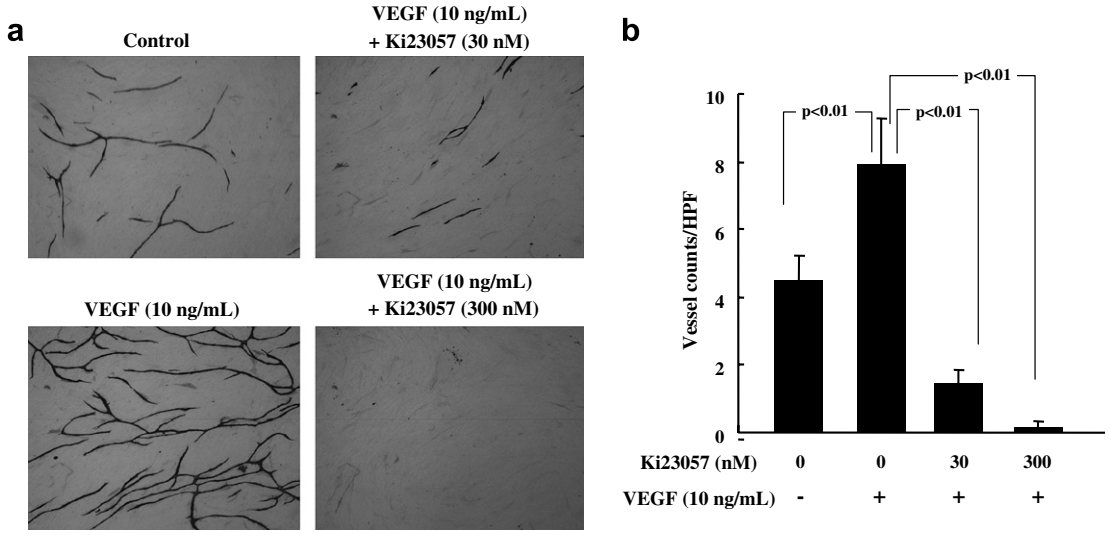

Fig. 3 – (a) Microscopic findings of endothelial cell tube formation. Tube formation was enhanced in the presence of VEGF. Ki23057 remarkably suppressed tube formation in a dose-dependent manner. (b) Effect of Ki23057 on endothelial cell tube formation induced by VEGF. Tube formation was remarkably increased in HUVECs with VEGF compared with that without VEGF. Tube formation was more strongly inhibited in HUVECs treated with Ki23057 (30 nM and 300 nM) compared with that without Ki23057. HUVEC were cocultured with fibroblasts for 11 days. The average numbers of vessels were 4.50±0.71/HPF (without VEGF or Ki23057), 7.94±1.33 /HPF (10 ng/mL VEGF without Ki23057), 1.47±0.37/HPF (10 ng/mL VEGF and 30 nM Ki23057) and 0.19±0.16/HPF (10 ng/mL VEGF and 300 nM Ki23057). The average number of vessels was significantly decreased in HUVECs treated with Ki23057 (30 nM and 300 nM), compared with VEGF alone (10 ng/mL), ($p < 0.01$).

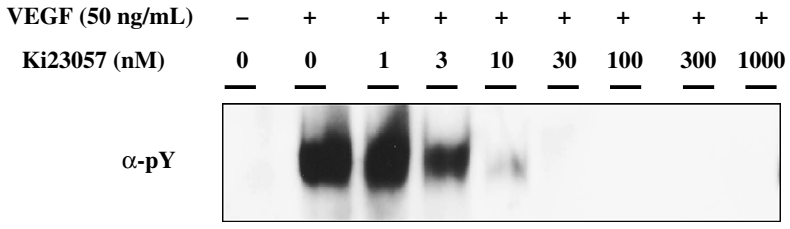

Fig. 4 – Effect of Ki23057 on VEGF-induced VEGFR2 autophosphorylation in HUVECs. No phosphorylated VEGFR2 was observed in the absence of VEGF (50 ng/mL). The addition of VEGF induced phosphorylation of VEGFR2. Ki23057 inhibited the phosphorylation of VEGFR2 in a dose-dependent manner.

inhibition of the spreading of cancer cells to liver. No significant body weight loss was observed in either of the two groups throughout the experiment. None of the mice died during the experiment.

4. Discussion

The small synthetic molecule, Ki23057, is an autophosphorylation inhibitor that interrupts the proliferation-related receptor tyrosine kinase (RTK) pathways. This compound exhibits considerable inhibitory activity against autophosphorylation of VEGFR, PDGFR, FGFR2 and c-kit. It was previously reported that Ki23057 inhibited receptor tyrosine kinase phosphorylation in cell lines that express RTKs, which is very important for proliferation.[16] In our previous study, Ki23057 inhibited the tyrosine phosphorylation of K-samII/FGFR2, VEGFR1, VEGFR2, PDGFRβ and c-kit with IC_{50} values of 88 nM, 69 nM, 83 nM, 100 nM and 480 nM, respectively.[18] There were no significant inhibitory effects on EGFR and IGF1R. Pharmacokinetic parameters of orally administered Ki23057 were reported previously as follows. The area under the plasma concentration curve (AUC) was 41 mg/ml; maximum plasma concentration (C_{max}), 2μg/ml; time point showing maximum concentration (t_{max}), 1.5 h; and elimination half-life ($t_{1/2}$), 93 h.[16] Upon oral administration, a stable blood concentration is maintained for a long period; thus, oral administration of Ki23057 may contribute to efficacy.

In the present study, Ki23057 inhibited the proliferation and tube formation of endothelial cells by suppression of VEGFR2 phosphorylation, but exhibited no inhibitory effect on the proliferation of colon cancer cells. VEGFR2 phosphorylation was inhibited by Ki23057 at 3 nM or more. The proliferation of HUVECs was significantly inhibited by Ki23057 at 10 nM or more. These findings suggested that higher concentration of Ki23057 might be necessary for inhibition of cell proliferation than for VEGFR2 phosphorylation in HUVECs. We

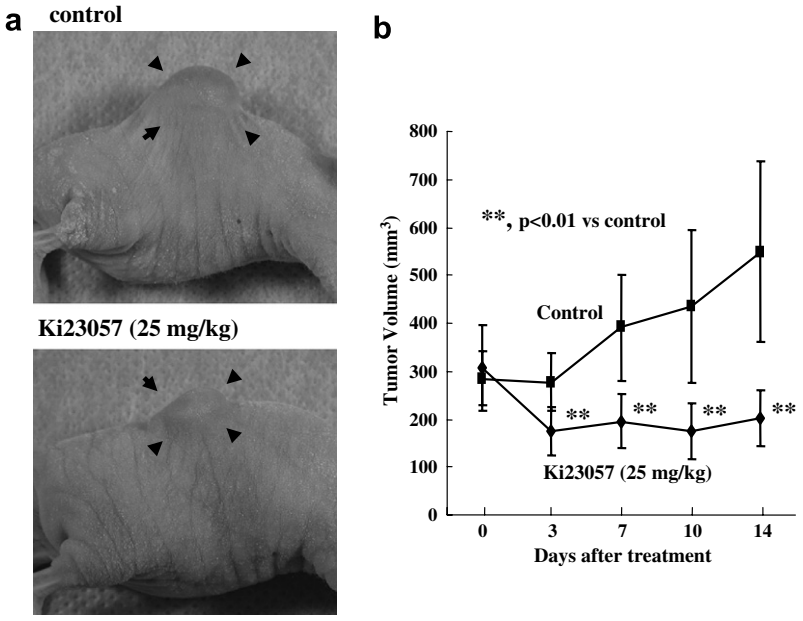

Fig. 5 – (a) Macroscopic findings of xenografted LM-H3 tumours in mice. The representative macroscopic findings of one of seven mice are shown. All of the control mice ($n = 7$) and the treatment mice ($n = 7$) were sacrificed at the 14th day after the Ki23057 treatment. (b) Effect of Ki23057 on subcutaneous LM-H3 tumour growth in mice. From the 10th day after tumour inoculation, Ki23057 administration started and this day was determined as day 0. Ki23057 was administered at 25 mg/kg/day, for 5 days and had a 2 day interval. This schedule was repeated for another week. From day 3, tumour size in the Ki23057 group was significantly ($p < 0.01$) inhibited compared with the control group. No mice died during the investigation. No significant changes were observed in the body weight of the mice. **, $p < 0.01$.

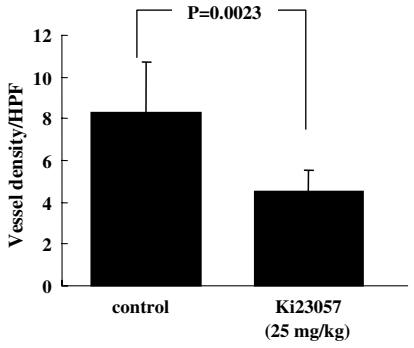

Fig. 6 – Effect of Ki23057 on tumour microvessel density. Microvessels stained by anti-CD31 antibody in the xenografted tumours of the treated group were significantly fewer than those of control group ($p = 0.0023$). The average vessel counts were 8.29 ± 2.40/HPF in the control group and 4.50 ± 1.03/HPF in the Ki23057 treated group. Angiogenesis was more strongly inhibited in the xenografted tumours treated with Ki23057 compared with the control tumours.

previously reported that LM-H3 produced high levels of VEGF and strongly induced angiogenesis.[19] To determine the inhibitory effect of Ki23057 on angiogenesis, LM-H3 was used as an *in vivo* model. Ki23057 inhibited the progression of colon cancer*in vivo*. Ki23057 suppressed vessel density in xenografted tumours. Ki23057 also inhibited the spreading of cancer cells to the liver. Our data indicated that Ki23057 exhibited a strong inhibitory effect on angiogenesis and inhibited the progression and liver metastasis of colon cancer.

Some VEGFR2 tyrosine kinase inhibitors have been developed and clinical trials are ongoing. For example, PTK787/ZK222584 is an angiogenesis inhibitor targeting VEGFR tyrosine kinases. SU11248 selectively inhibits VEGFR2 and PDGFRβ phosphorylation. AEE788 is an inhibitor of EGFR and VEGFR. However, there are no VEGFR2 tyrosine kinase inhibitors in phaseIII investigations that can significantly prolong the overall survival of the patients with metastatic colorectal cancer. One possible explanation of this result could be the short half-life of the tyrosine kinase inhibitors. In view of this short half-life, the tyrosine kinase inhibitors are theoretically disadvantageous in maintaining constant drug levels above a therapeutic threshold, compared with bevacizumab, which has a half-life of about 20 days. For example, the elimination half-lives ($t_{1/2}$) of the tyrosine kinase inhibitors PTK787/ZK222584, ZD6474, SU11248, BAY43-9006 and AEE788 are ~6 h, ~15 h, ~40 h, ~37 h and ~24 h, respectively.[3,20–23] The pharmacokinetic parameters of orally administered Ki23057 demonstrated that the $t_{1/2}$ is >93 h.[16] From the viewpoint of the pharmacokinetics, Ki23057 may be superior to the other tyrosine kinase inhibitors.

Recently, the gold standard of front line treatment of metastatic colorectal cancer has been the combinations of oxaliplatin with the infusional 5-FU regimens (FOLFOX). Also, it is well known that the targeted therapy, inhibition of angiogenesis by blocking Vascular Endothelial Growth Factor (VEGF) using the monoclonal antibody bevacizumab (Avastin) enhances the activity of the co-administered chemotherapy

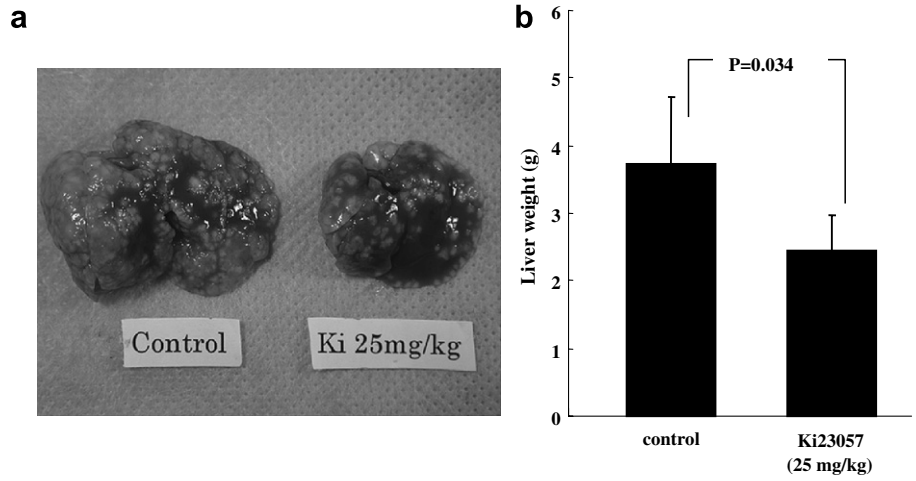

Fig. 7 – (a) Macroscopic findings of the spreading of cancer cells to liver in mice. The representative macroscopic findings of one of seven mice are shown. At the end of treatment, all of the control mice ($n = 7$) and the treatment mice ($n = 7$) were sacrificed. More blended nodules were seen in the control group compared to the Ki23057 treatment group. (b) Effect of Ki23057 on the spreading of cancer cells to liver in mice. The weight of the liver was 3.73±1.00 g in the control group and 2.43 ± 0.52 g in the treatment group ($p = 0.034$). Compared with the control group, the treatment group exhibited significant inhibition of effect of Ki23057 on the spreading of cancer cells to the liver. Body weight loss was not observed in either of the groups throughout the experiments.

regimens that have been studied as first or second line treatment of patients with metastatic colorectal cancer.[24] Indeed, the combination of FOLFOX and bevacizumab reported to be feasible and highly effective, and merits further evaluation in patients with metastatsic colorectal cancer.[25] On the other hand, there was no information concerning the tolerance of the combination of FOLFOX plus bevacizumab. Ki23057, a small synthetic molecule that interrupts VEGFR2 pathways as a phosphorylation inhibitor and has different inhibitory mechanisms for HUVEC from VEGF monoclonal antibody of bevacizumab, which indicates that Ki23057 might be useful for the tumours with tolerance for bevacizumab. Ki23057 can be considered as an Avastin substitute regarding the cost of the antibody for medical insurances and society because Ki23057 is a small synthetic molecule with cost benefits.

Recently, oral anti-cancer drug therapy for unresectable advanced colorectal cancer has drawn attention. The oral fluoropyryzine drugs, UFT, S-1 and capecitabine, are useful for unresectable, metastatic colorectal cancer.[26,27]Oral administration of UFT/LV achieved similar overall survival to venous administration of 5-FU/LV and there is no difference in toxicity between either therapy group. As oral administration is more convenient for patients, we believe that oral administration may become a standard in the chemotherapy for unresectable colorectal cancer. Since angiogenesis inhibitors are used with a combination of cytotoxic agents in cancer therapy, oral administration of such inhibitors would be desirable. As Ki23057 is administered orally, Ki23057 may be superior to bevacizumab from the viewpoint of the patients' convenience.

In this study, we examined Ki23057 in the context of inhibition of angiogenesis. However, it is considered that Ki23057 may exhibit direct inhibitory effects towards certain cancers in which proliferation is related strongly to the expression of VEGFR, PDGFR, FGFR and c-kit. It has been reported that FGF plays an important role in the proliferation of scirrhous gastric cancer, which can be inhibited by Ki23057 through suppression of FGFR2 phosphorylation.[18,28] Ki23057 also inhibits autophosphorylation of c-kit, which contributes to the proliferation of GIST. As GIST considered a hypervascular tumour, it is possible that Ki23057 may have an inhibitory effect on proliferation of GIST by suppressing both c-kit and VEGFR2. Ki23057, therefore, may be suitable for treatment of a wide range of solid tumours.

In conclusion, a novel VEGFR2 phosphorylation inhibitor, Ki23057, inhibited the proliferation and liver metastasis of colon cancer through suppression of angiogenesis. Ki23057 appears therapeutically promising as an antiangiogenic agent against colon cancer.

Conflict of interest statement

None declared.

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