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Evaluation of the angiogenesis inhibitor KR-31831 in SKOV-3 tumor-bearing mice using ^{64}Cu -DOTA-VEGF₁₂₁ and microPET[☆]

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Abstract

KR-31831 ((2R,3R,4S)-6-amino-4-[N-(4-chlorophenyl)-N-(1H-imidazol-2-ylmethyl)amino]-3-hydroxyl-2-methyl-2-dimethoxymethyl-3,4-dihydro-2H-1-benzopyran), an angiogenesis inhibitor, was evaluated in tumor-bearing mice using molecular imaging technology. Pre-treatment microPET images were acquired on SKOV-3 cell-implanted nude mice after injection with ^{64}Cu -DOTA-VEGF₁₂₁. KR-31831 (50 mg/kg) was then injected intraperitoneally into the treatment group ($n=3$), while injection vehicle was injected into the control ($n=4$) and blocking ($n=3$) groups. After injections occurred daily for 28 days, all groups of mice underwent post-treatment microPET imaging after injection with ^{64}Cu -DOTA-VEGF₁₂₁. The post-treatment images showed high tumor uptake in the control group and reduced tumor uptake in both the blocking and treatment groups. ROI analysis of the tumor images revealed $6.25\pm1.18\%$ ID/g at 1 h, $6.55\pm0.69\%$ ID/g at 2 h, and $4.68\pm0.63\%$ ID/g at 16 h in the control group; $3.87\pm0.45\%$ ID/g at 1 h, $4.50\pm0.44\%$ ID/g at 2 h, and $3.63\pm0.25\%$ ID/g at 16 h in the blocking group; and $4.03\pm0.74\%$ ID/g at 1 h, $4.37\pm0.67\%$ ID/g at 2 h, and $3.83\pm0.90\%$ ID/g at 16 h in the treatment group. Biodistribution obtained after the post-treatment microPET imaging also demonstrated high tumor uptake ($3.74\pm0.27\%$ ID/g) in the control group and reduced uptakes in both the blocking group ($2.69\pm0.73\%$ ID/g, $P<.05$) and the treatment group ($3.11\pm0.25\%$ ID/g, $P<.05$), which correlated well with microPET imaging data. Immunofluorescence analysis showed higher levels of VEGFR2 and CD31 expressions in tumor tissues of the control and blocking groups than in tumor tissues of the treatment group. These results suggest that the antiangiogenic activity of KR-31831 is mediated through VEGFR2 and microPET serves as a useful molecular imaging tool for evaluation of a newly developed angiogenesis inhibitor, KR-31831.

Keywords

KR-31831; ^{64}Cu -DOTA-VEGF₁₂₁; MicroPET; VEGFR2; Angiogenesis

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1. Introduction

Molecular imaging technology using positron emission tomography (PET) has recently proven useful in drug development. There are two methods used for this purpose [1]: the first involves labeling a new drug with a chemically identical radioisotope, and the second method uses a known radiotracer with the same mechanism of action as a new drug. With the first method, a new drug must be labeled with a radioisotope that is chemically identical to an atom already incorporated in the molecule. One example of this is labeling a novel anticancer agent, *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide with ^{11}C , in which ^{12}C was replaced with ^{11}C . This radiotracer was used to predict normal tissue toxicity and tumor pharmacokinetics using PET during the early stage of drug development [2–4]. This method requires a new drug which can be labeled with a chemically identical radioisotope and therefore has some limitations. The second method has been widely used to determine appropriate doses and to predict the efficacy of a new drug. An example of this is a study performed with NAD-299, a novel compound with high selectivity and affinity to 5-HT_{1A} receptors in vitro and in vivo. The 5-HT_{1A} receptor occupancy of NAD-299 was determined in cynomolgus monkeys using [*carbonyl*- ^{11}C]WAY-100635 and PET, which can then be used to determine appropriate doses for the initial human study [5].

KR-31831, (2R,3R,4S)-6-amino-4-[*N*-(4-chlorophenyl)-*N*-(1*H*-imidazol-2-ylmethyl)amino]-3-hydroxyl-2-methyl-2-dimethoxymethyl-3,4-dihydro-2*H*-1-benzopyran, is a newly developed antiangiogenesis inhibitor [6]. It inhibits the proliferation, migration, invasion and tube formation of endothelial cells in vitro and also inhibits in vivo angiogenic activity in mouse Matrigel plug assay. In addition, mRNA expression of VEGFR2 is shown to be suppressed by KR-31831 treatment [6,7].

Targeted VEGF or VEGFR molecular imaging enables diagnosis and monitoring of proliferation and development of angiogenic tumors. VEGF is essential for normal and abnormal blood-vessel angiogenesis, vasculogenesis, and endothelial cell growth under both physiological and pathological conditions [8]. All members of the VEGF family mediate angiogenic activity through specific binding to tyrosine kinase receptors, called VEGFRs. The VEGF family includes VEGF-A, VEGF-B, VEGF-C, and VEGF-D. VEGF-A binds to endothelial cell-specific VEGFR1 (Flk-1) and VEGFR2 (Flk-1, KDR), both of which are associated with advanced tumor growth and induction of tumor angiogenesis [9,10]. They are also shown to be over-expressed by tumor-associated vasculature. This over-expression occurs commonly in various human tumors and correlates with tumor growth rate, proliferation, and tumor metastatic potential [11]. The binding of VEGF-A to VEGFR2 causes dimerization of the receptor followed by activation through autophosphorylation [12]. This tyrosine kinase activity of VEGFR2 is more efficient than that of VEGFR1, and therefore, activation of VEGFR1 alone is not sufficient to induce the angiogenic activity of VEGF-A [13]. Human VEGF-A has several isoforms, VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₃, VEGF₁₈₉, and VEGF₂₀₆, which are generated by alternative mRNA splicing. Of the isoforms, VEGF₁₂₁ is a soluble form that does not bind to heparin and is active as a disulfide-linked homodimer [14]. Binding of VEGF₁₂₁ to VEGFR2 serves as an excellent candidate for molecular imaging [15]. In addition, in rabbit cornea assay and xenograft experiments, VEGF₁₂₁ is a more tumorigenic isoform than is VEGF₁₆₅ or VEGF₁₈₉ [16]. VEGF₁₂₁ has also been reported to be over-expressed by human glioma U87MG cells, which induced tumor-associated intracerebral hemorrhages by the rupture of VEGF-induced neovessels [17].

Directly measuring changes in VEGFR expression requires VEGFR-specific radiotracers for PET imaging. Radiotracers based on VEGF/ VEGFR have been developed for imaging of VEGFR expression in various disease models. Of these radiotracers, ^{64}Cu -DOTA-VEGF₁₂₁

has been used to successfully monitor VEGFR expression in U87MG tumor-bearing mice, in a murine model of hindlimb ischemia, and in a rat model of stroke [18–20].

In the present study, antiangiogenic activity of KR-31831 was evaluated using ^{64}Cu -DOTA-VEGF₁₂₁ and microPET in SKOV-3 tumor-bearing nude mice.

2. Materials and methods

2.1. Reagents and equipments

KR-31831 and $^{64}\text{CuCl}_2$ were provided by KRICT (Daejeon, Korea) and KIRAMS (Seoul, Korea), respectively, and VEGF₁₂₁ and DOTA-VEGF₁₂₁ were provided by NIBIB, NIH (Bethesda, MD, USA). High purity HCl (30%) was obtained from Merck Chemicals (Darmstadt, Germany), and all other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate buffer was treated with Chelex 100 resin before use for radiolabeling. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry was performed on a Voyager-DE STR Biospectrometry Workstation (Applied Biosystems Inc, CA, USA). Thin layer chromatography (TLC) was performed using a Bioscan radio-TLC scanner (Washington DC, USA). Radioactivity was measured in a dose calibrator (Biodex Medical Systems, Shirley, NY, USA) and tissue radioactivity was measured using a 2480 WIZARD² automatic gamma counter (PerkinElmer, Waltham, MA, USA). MicroPET images of the mice were acquired using an Inveon microPET/CT scanner (Siemens Medical Solutions, Malvern, PA, USA). All animal experiments were performed in compliance with the rules of the Samsung Medical Center Laboratory Animal Care.

2.2. Preparation of ^{64}Cu -DOTA-VEGF₁₂₁

DOTA-VEGF₁₂₁ was labeled with ^{64}Cu using a known method [18]. $^{64}\text{CuCl}_2$ (110–200 MBq) in 0.01 N HCl (50 μL) was added to DOTA-VEGF₁₂₁ (10 μg /37 MBq) in 0.1 M sodium acetate buffer (pH 6.5). The reaction mixture was diluted with the same buffer to a total volume of 300 μL and then incubated at 40 °C for 1 h with constant shaking (400 rpm) using a Thermomixer (Eppendorf, Hamburg, Germany). Reaction progress was determined by radio-TLC. At the end of the reaction, the product was diluted with 0.1 M sodium acetate buffer for injection into mice.

2.3. Cytotoxicity of KR-31831

Cytotoxicity of KR-31831 on SKOV-3 cells (human ovarian carcinoma cells) was determined using the XTT assay. SKOV-3 cells (1×10^3 cells/well) were grown for 24 h in 96-well plates. The cells were washed twice with PBS and then incubated in FBS-free RPMI 1640 media with various concentrations of KR-31831 (0, 100, 300, 500, 800, and 1000 μM) for 24 h. After the cells were rinsed twice with PBS, 100 μL of RPMI 1640 media containing XTT solution (10 μL , 5 mg/ml in PBS) was added and the cells were incubated at 37 °C for 4 h. Cell viability was measured by absorbance at a wavelength of 470 nm using a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA, USA).

2.4. Immunoblotting

VEGFR2 protein (recombinant human VEGFR2/KDR Fc Chimera, R&D Systems, Minneapolis, MN, USA) was treated with or without 30 μM of KR-31831 and incubated at room temperature for 1 h, which was then treated with VEGF for 1 h. The mixture was added to protein loading buffer (8% SDS, 0.4 M Tris-HCl (pH 8.0), 1 M sucrose, 10 mM EDTA, 0.02% bromophenol blue, and 4% β -mercaptoethanol), separated by 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane. The membrane was incubated with primary antibodies (Rabbit anti-VEGF, 1:1000 diluted, Cell Signaling Technology, Boston, MA, USA), and then with incubated

with horseradish peroxidase-conjugated secondary antibodies (Goat anti-rabbit IgG, 1:3000 diluted, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunoreactive protein was visualized using an enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ) and quantified using Image J software (NIH, Bethesda, MD, USA).

2.5. Animal model

The animal model was prepared by subcutaneously inoculating SKOV-3 cells (5×10^6) into the right flanks of six-week-old, male BALB/c nude mice.

2.6. MicroPET imaging

MicroPET imaging was performed on an Inveon microPET/CT scanner, which has 10 cm transaxial and 12.7 cm axial field of view and operates exclusively in 3D mode. After two weeks of inoculation with SKOV-3 cells, mice with a tumor volume of $115 \pm 25 \text{ mm}^3$ underwent pre-treatment microPET imaging ($n=10$). Two mice were anesthetized with isoflurane and placed in a cradle, equipped with mask for anesthesia gas supply and warm water pads at the tail veins for injection. The microPET images of these mice were acquired for 10 min at 1 h, 2 h, and 16 h after injection of ^{64}Cu -DOTA-VEGF₁₂₁ (7.4 MBq/2–3 μg protein/mouse). The next day, the mice were divided into control ($n=4$), blocking ($n=3$), and treatment groups ($n=3$). Mice in the treatment group were intraperitoneally injected daily with KR-31831 dissolved in a 10:10:80 mixture of Cremophor EL–ethanol–saline (50 mg/kg) for 28 days. Mice in the control and blocking groups ($n=7$) were injected with only the injection vehicle (Cremophor EL–ethanol–saline) during the same period and with the same frequency. On the final day of KR-31831 treatment, mice in both the control and treatment groups were injected with ^{64}Cu -DOTA-VEGF₁₂₁ (7.4 MBq/mouse) and underwent post-treatment microPET imaging. Mice in the blocking group were co-injected with ^{64}Cu -DOTA-VEGF₁₂₁ and VEGF₁₂₁ (100 μg /mouse) [2]. Static images were acquired for 10 min at 1 h, 2 h, and 16 h post-injection, and the images were reconstructed using 3D-ordered subset expectation maximization. The images were then processed using Siemens Inveon Research Workplace 4.0 (IRW 4.0). Regions of interest (ROIs) were manually drawn over the tumors, and the average signal level in the ROIs was measured. Tumor to background uptake ratios were calculated from the ratio of the average signal level of the tumor ROI to a background ROI over the contralateral side of the mice. During this period, tumor volumes in all groups of mice were measured every other day. In order to determine tumor volume, the longest longitudinal diameter (length) and the longest transverse diameter (width) were measured using a vernier caliper. Tumor volume was then calculated by multiplying length by width² by 1/2 [21,22].

2.7. Biodistribution studies

After post-treatment microPET imaging, the mice were sacrificed by cervical dislocation and tissues of interest (blood, muscle, heart, lung, liver, kidney, spleen, stomach, small and large intestines, femur, and tumor) were removed, weighed, and counted. Data are expressed as the percent injected dose per gram of tissue (% ID/g).

2.8. Immunofluorescence staining

After biodistribution, tumor tissues from control ($n=4$), blocking ($n=3$), and treatment groups ($n=3$) were fixed in 4% paraformaldehyde for 4 h. The specimens were then dehydrated in ethanol, embedded in paraffin and cut into 5- μm -thick sections on a Reichert microtome. The fixed sections were deparaffinized and hydrated, which were then rinsed in PBS and blocked with 3% BSA in PBS for 30 min. For VEGFR2 staining, the sections were incubated with rabbit anti-VEGFR2 antibody (1:20, Cell Signaling Technology) at 4 °C for 16–18 h and washed with PBS. The sections were then incubated with FITC-conjugated

anti-rabbit secondary antibody (1:100, Santa Cruz Biotechnology) at room temperature for 3 h. For CD31 staining, the sections were incubated with anti-CD31 antibody (1:200, BD Biosciences) at 4 °C for 1 h and rinsed in PBS. The sections were then incubated with Cy3-conjugated anti-rat secondary antibody (1:100, BD Biosciences) at room temperature for 2 h and rinsed three times in PBS for 2 min. All sections were mounted with 4',6-diamidino-2-phenylindole (DAPI) to localize the nuclei. For ROI selection, 5 areas in each tumor tissue section were selected and then 3 ROIs were drawn on each area. Quantification of VEGFR2 and CD31 expressions in the regions was processed using image analysis software (Metamorph, USA), and quantitative data were analyzed using the unpaired, two-tailed Student's *t* test. Differences at the 95% confidence level ($P<.05$) were considered significant.

3. Results and discussion

3.1. Preparation of ^{64}Cu -DOTA-VEGF₁₂₁

DOTA-VEGF₁₂₁ was identified by MALDI-TOF mass spectrometry, and the average number of DOTA conjugated to VEGF₁₂₁ was 3.2–3.6. After radiolabeling of DOTA-VEGF₁₂₁ with $^{64}\text{CuCl}_2$, the radiochemical purity of ^{64}Cu -DOTA-VEGF₁₂₁ was measured based on radio-TLC; ^{64}Cu -DOTA was not detected but a small amount of ^{64}Cu was present. Characterization of ^{64}Cu -DOTA-VEGF₁₂₁ in tumor-bearing animal model has been thoroughly investigated [18], and only tumor uptake levels after treatment with an angiogenesis inhibitor were measured in the present study. The specific activity was approximately 3.7 GBq/mg, and the decay-corrected radiochemical yield of ^{64}Cu -DOTA-VEGF₁₂₁ was 94%–96% and radiochemical purity was 87%–93%.

3.2. Cytotoxicity of KR-31831

Cytotoxicity of KR-31831 on SKOV-3 cells was evaluated using the XTT assay. Cell viability of SKOV-3 cells treated with KR-31831 decreased in a dose-dependent manner relative to that of control; 100%±4.16%, 71.37%±5.37%, 48.87%±4.41%, 38.15%±7.95%, 29.94%±3.93%, and 25.35%±1.31% at 0, 100, 300, 500, 800, and 1000 μM, respectively (Fig. 1). This result suggested that KR-31831 induced cell death, implicating its antitumor activity on SKOV-3 cells.

3.3. Immunoblotting

Inhibitory effect of KR-31831 on VEGFR2 was determined using the immunoblotting method. Strong band of VEGF binding to VEGFR2 was shown without KR-31831, whereas the band intensity was significantly reduced in the presence of KR-31831 (Fig. 2A). Quantitative data showed that VEGF binding to VEGFR2 was reduced by 60% in the presence of KR-31831 relative to that of control (Fig. 2B), demonstrating that KR-31831 may have inhibitory effect on VEGFR2.

3.4. MicroPET imaging

The tumor-bearing mice underwent pre-treatment microPET imaging at two weeks after inoculation. The images demonstrated high radioactivity accumulation in the liver and kidneys but low regional uptake in the tumors ($115\pm25\text{ mm}^3$, $n=10$) (Fig. 3A). ROI analysis of the tumors from the pre-treatment microPET images revealed 2.80%±0.61% ID/g at 1 h, 3.00%±0.36% ID/g at 2 h, and 2.40%±0.95% ID/g at 16 h post-injection (Fig. 3B). Tumor uptake levels were relatively low due to small size of tumors in pre-treatment mice; tumor to background uptake ratios were 3.52±1.34 at 1 h, 3.89±0.88 at 2 h, and 3.10±0.64 at 16 h in the control group and 2.78±0.08 at 1 h, 3.75±0.12 at 2 h, and 2.95±0.25 at 16 h in the treatment group.

On the 28th day of KR-31831 treatment, all three groups of mice underwent post-treatment microPET imaging (Fig. 3A). The images showed high tumor uptake in the control group and reduced tumor uptake in both the blocking and treatment groups. ROI analysis of the tumors revealed $6.25\% \pm 1.18\%$ ID/g at 1 h, $6.55\% \pm 0.69\%$ ID/g at 2 h, and $4.68\% \pm 0.63\%$ ID/g at 16 h in the control group; $3.87\% \pm 0.45\%$ ID/g at 1 h, $4.50\% \pm 0.44\%$ ID/g at 2 h, and $3.63\% \pm 0.25\%$ ID/g at 16 h in the blocking group; and $4.03\% \pm 0.74\%$ ID/g at 1 h, $4.37\% \pm 0.67\%$ ID/g at 2 h, and $3.83\% \pm 0.90\%$ ID/g at 16 h in the treatment group (Fig. 3B). Tumor uptake was blocked with VEGF₁₂₁ by 38% at 1 h, 31% at 2 h, and 22% at 16 h post-injection, which is similar to the results reported by Cai et al. [18]. Although 100 μ g of VEGF₁₂₁ was not sufficient to completely block the tumor uptake by ⁶⁴Cu-DOTA-VEGF₁₂₁, the tumor uptake levels in the blocking group were significantly lower than those in the control group, indicating that the radiotracer binds specifically to VEGFR. Similarly, tumor uptake after KR-31831 treatment was reduced by 36% at 1 h, 33% at 2 h, and 18% at 16 h post-injection. A moderate reduction of tumor uptake by KR-31831 suggested that the dose of KR-31831 used in this study might have not been sufficient to elicit full activity. Tumor to background uptake ratios were 7.02 ± 1.08 at 1 h, 6.81 ± 0.57 at 2 h, and 6.60 ± 2.01 at 16 h in the control group, and 3.19 ± 1.02 at 1 h, 4.87 ± 2.26 at 2 h, and 5.56 ± 2.04 at 16 h in the treatment group.

During the treatment period, the difference in tumor volume was not significant between control and treatment groups. The average tumor volume of all mice was approximately 115 ± 25 mm³ before KR-31831 treatment, whereas the average tumor volume increased to 365 ± 121 mm³ in the control and blocking groups and to 319 ± 168 mm³ in the treatment group.

Cai et al. reported that small tumors (tumor volume 64.9 ± 24.6 mm³) showed high VEGFR2 expression and thus high ⁶⁴Cu-DOTA-VEGF₁₂₁ uptake, whereas large tumors (1164.3 ± 179.6 mm³) had low VEGFR2 expression and low radioactivity uptake [18]. In the present study, however, SKOV-3 cell lines expressing both VEGF and VEGFR2 were used for inoculation into mice [23–25], and tumor uptakes were lower in small tumors of pre-treatment mice (115 ± 25 mm³) than those in large tumors of post-treatment mice (319 – 365 mm³). The discrepancy could be explained by the reasons that SKOV-3 cells may have different levels of VEGFR expression from those of U87MG cells and different methods of tumor volume measurement might have been used in both studies.

3.5. Biodistribution studies

Biodistribution obtained after the post-treatment microPET imaging demonstrated high uptake in the liver and kidneys, which is similar to the results of the post-treatment images (Fig. 4). While kidney uptake was much higher than liver uptake in the study using U87MG tumor-bearing mice (33% ID/g and 17% ID/g at 2 h), the reverse relationship was observed in the study using 4T1 tumor-bearing mice (13% ID/g and 18% ID/g at 1 h) [18,26]. This was also observed in our case using SKOV-3 tumors: uptake was 2.5 times higher in the liver than in the kidneys. The high liver uptake may be partly due to a small amount of ⁶⁴Cu impurity from the reaction.

In the control group, high levels of radioactivity also accumulated in tumor tissue ($3.74\% \pm 0.27\%$ ID/g), and the uptake levels were reduced in both the blocking group ($2.69\% \pm 0.73\%$ ID/g, $P < .05$) and treatment group ($3.11\% \pm 0.25\%$ ID/g, $P < .05$) (Fig. 4 Inset). Tumor uptake after KR-31831 treatment was reduced by 17%, which correlates with an 18% reduction in uptake observed from microPET ROI analysis of tumors at 16 h post-injection. In the blocking group, tumor uptake was reduced by approximately 28% with co-injection of VEGF₁₂₁, which is comparable to a 22% reduction at 16 h post-injection in microPET ROI

data and also a 30% reduction reported by Cai et al. [18]. The microPET and biodistribution data indicate therapeutic effect of KR-31831 on SKOV-3 tumors.

3.6. Immunofluorescence staining

Angiogenesis in SKOV-3 tumor tissues has been detected by immunohistochemical staining, and KR-31831 is known to have antiangiogenic activity through down-regulation of VEGFR2 expression [7]. Therefore, VEGFR2 and CD31 expressions were compared in the tumor tissues of mice in the control and treatment groups. Single immunohistochemical staining for VEGFR2 appeared to have the endothelial cell staining pattern. Therefore, two-color double immunofluorescence staining was performed in order to confirm that the cells that express VEGFR2 are endothelial cells. Tumor tissues in the control and blocking groups showed stronger VEGFR2 and CD31 staining than did tumor tissues in the treatment group (Fig. 5A). Although VEGFR2 and CD31 staining was performed using different sections from the same tumor tissues, they were shown to be co-localized on the tumor tissues. Quantitative data showed that VEGFR2 expression was reduced by 9.4% in the tumor tissues of the blocking group and by 33.3% in the treatment group, relative to the expression level in the control group (Fig. 5B). Similarly, CD31 expression was also reduced by 5.9% and 28.2% in the tumor tissues of blocking and treatment groups compared to the expression level in the control group (Fig. 5B). These results indicated that both VEGFR2 and CD31 expressions are reduced by the treatment of KR-31831 in the treatment group relative to the expression levels in the control and blocking groups. These data support the results of microPET and biodistribution studies, in that tumor uptake was reduced in the treatment groups compared to that of the control group. These results suggest that the antiangiogenic activity of KR-31831 is mediated through VEGFR2.

4. Conclusions

Antiangiogenic activity of KR-31831 was successfully evaluated using molecular imaging technology based on ^{64}Cu -DOTA-VEGF₁₂₁/VEGFR. MicroPET imaging and biodistribution data along with immunoblotting and immunofluorescence staining results suggest that the antiangiogenic activity of KR-31831 is mediated via VEGFR2. These results also demonstrate that microPET can be a useful tool in evaluating new drug candidates.

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Fig. 1.
Cytotoxicity of KR-31831 on SKOV-3 cells using the XTT assay.

Fig. 2.

Effects of KR-31831 on VEGFR2. (A) Immunoblot findings of VEGF binding to VEGFR2 with or without pre-incubation with 30 μ M of KR-31831. (B) Quantitative analysis of the band intensity with or without 30 μ M of KR-31831.

Fig. 3.

(A) MicroPET images of SKOV-3 tumor-bearing mice obtained at 1, 2, and 16 h after injection of ^{64}Cu -DOTA-VEGF₁₂₁. After the pretreatment microPET imaging was performed on day 0, the mice were divided into control, blocking, and treatment groups. Mice in the treatment group were intraperitoneally injected with KR-31831 daily for 28 days, whereas mice in the control and blocking groups were injected with only the injection vehicle during the same period. The post-treatment microPET imaging was performed on day 28, and mice in the blocking group were co-injected with ^{64}Cu -DOTA-VEGF₁₂₁ and VEGF₁₂₁ (100 $\mu\text{g}/\text{mouse}$). (B) The post-treatment microPET ROI analysis of tumor tissues. Control group: white solid; blocking group: grey solid; treatment group: black solid. * $P < .05$, ** $P < .01$ compared to control level.

Fig. 4. Biodistribution of ^{64}Cu -DOTA-VEGF₁₂₁ in mice obtained after the post-treatment microPET imaging. The data are represented as a mean $\pm$ SD of groups of 3–4 mice. Control group: white solid; blocking group: grey solid; treatment group: black solid. * $P < .05$ compared to control level. Inset is the radioactivity uptake levels in tumor tissues.

Fig. 5. (A) Immunofluorescence staining of VEGFR2 (green) and CD31 (red) in tumor tissues (control, blocking, and KR-31831 treatment). (B) Quantitative analysis of the expression levels of VEGFR2 and CD31. Control group: white solid; blocking group: grey solid; treatment group: black solid (magnification $\times$ 200).