Evaluation of anti-angiogenic effects of a new synthetic candidate drug KR-31831 on xenografted ovarian carcinoma using dynamic contrast-enhanced MRI

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Introduction

Angiogenesis is the process of the development of new blood vessels from existing micro vasculature and it is essential in solid tumor growth and metastasis. The targeting of blood vessels is considered a potentially useful anticancer strategy because endothelial cells are more accessible than other cells to pharmacological agents delivered via the blood, and they are also thought to be genetically stable compared with tumor cells. Recent studies demonstrated that dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) technique provides noninvasive characterization of anti-angiogenic response of tumor due to its ability to detect morphologic and functional characteristics of tumor vasculature by the differential distribution of contrast agents in tissues (1). KR-31831 (2R,3R,4S)-6-amino-4-[n-(4-chlorophenyl)-N-(1H-imidazon-2ylmethyl)amino]-3-hydroxyl-2-methyl-2dimenhoxymethyl-3,4-dihydro-2H-1-benzopran, is a newly developed anti-angiogenic candidate drug in our co-worker group. They already reported that KR-31831 plays a role as a novel anti-angiogenic agent in bovine aortic endothelial cells (BAECs) and also down regulated VEGF-induced tumor formation and proliferation of HUVECs by inhibiting intra cellular Ca^{2+} release and Erk1/2 activation (2). In this study, therefore, we evaluated anti-angiogenic effects of a new synthetic agent KR-31831 on xenografted human ovarian carcinoma model using DEC-MRI on a micro 7.0 Tesla MR system.

Methods

Subcutaneous xenograft tumor model and treatment: The human ovarian carcinoma SKOV3 cell line was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and plus 1% ampicillin and streptomycin. The anti-angiogenic efficacy of KR-31831 was tested in male BALB/c nu/nu mice (7 weeks old). Xenografted ovarian tumors were established by subcutaneous injection of 2×10^6 SKOV3 cells in a total volume of 0.1 mL of a serum-free medium containing 50% Matrigel (BD Bioscience) into the right thigh under isoflurane anesthesia. As the tumors became palpable, the mice were randomized into 2 groups. KR-31831 was prepared as suspension in vehicle (10% cremophor and 10% absolute ethyl alcohol in normal saline) for intra peritoneal injection. In the treated group (n = 6), mice were daily treated with KR-31831 at 50 mg/kg of body weight. In the control group (n = 6), mice were also daily treated with vehicle alone intraperitoneally for 21 days. All the mice were sacrificed on day 21 after treatment and the tumor tissues were excised from the mice for the subsequent histological analysis.

Immunohistochemistry of CD31 for microvessel density: Formalin-fixed, paraffin-embedded sections were sliced into 5 μm thickness corresponding to the DCE-MRI sections for evaluation of microvessel density (MVD) with CD31 staining by the immunohistochemical ABC methods. Tissue slides were incubated with rat anti-mouse CD31 (Abcam, 1/150) for 30 min followed by HRP-conjugated goat anti-rat IgG (Dako) for 30 min. The color reaction was developed using the chromagen 3,3-diaminobenzidine (DAB, Dako) and counterstaining with Mayer's heamtoxylin.

In vivo dynamic contrast-enhanced MRI: All in vivo MRI were carried on a micro 7.0 Tesla MRI System (Bruker-Biospin) equipped with a 20 cm gradient set capable of supplying up to 400mT/m in 100 μsec rise-time. A birdcage coil (70 mm i.d., Bruker-Biospin) was used for excitation, and an actively decoupled phased array coil was used for receiving the signal. Mice were anesthetized with 1.5% isoflurane in 70% N_2O and 30% O_2 administered via an MR-compatible mobile inhalation anesthesia system. The tail vein was cannulated before placing mice in the magnet. DCE-MRI was performed using a FLASH sequence (TR = 67 ms, TE = 3 ms, flip angle = 70°, FOV = 30×30 mm, imaging matrix = 128×128, slice thickness = 2.5 mm, 120 dynamics): five baseline scans were acquired with different flip angles (5°, 15°, 35°, 60°, and 70°), then mice were injected via the tail vein with 0.1 mmol/kg Dotarem (Guerbet). DCE-MR images were acquired prior to initiating treatment with KR-31831 and again on day 3 and 21 after treatment. The permeability parameter (K^{trans}) was calculated using Toft's pharmacokinetic model (3).

Results

Figure 1 shows that there are different changes in permeability between control and treatment subject. For quantification of this change, the K^{trans} values were averaged across the whole tumor region for each subject and compared between control group and KR-31831 treated group (Fig. 2). The non-parametric statistical analysis (Wilcoxon signed-rank test) shows decreasing K^{trans} values on day 21 compared to those on day 3 in KR-31831 treated group ($P < 0.05$), whereas no significant in control group ($P = 0.84$). As shown in figure 3, tumor microvessels, immunohistochemically analyzed with CD31 antibody reactivity in vascular tumor areas without necrosis in correspondence to the MRI sections, were also markedly reduced in KR-31831 treated tumor tissues (B) as compared to control tumors (A) on 21 day after treatment.

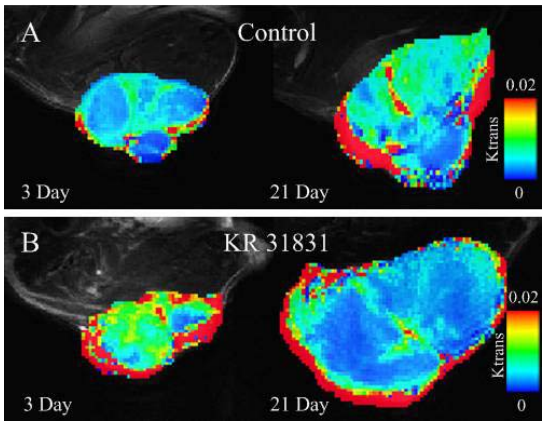

Figure 1. DCE-MR mapping taken on day 3 and 21 after treatment.

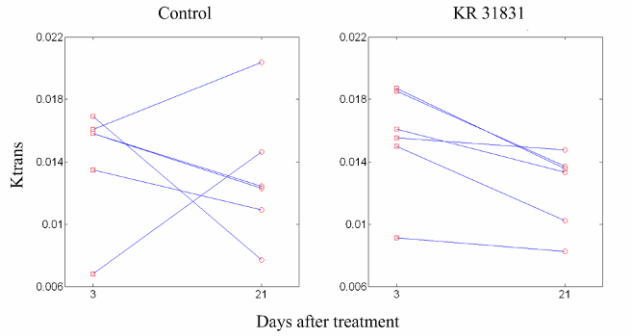

Figure 2. Change of DCE-MRI parameter K^{trans} analyzed with Wilcoxon signed-rank test.

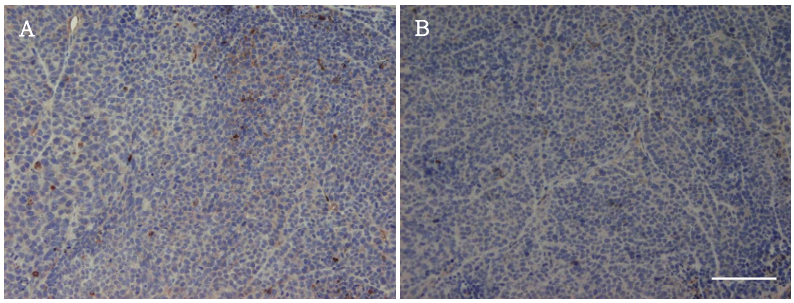

Figure 3. Immunohistochemical analysis of microvascular density on day 21 after treatment. Scale bar, 100 ㎛.

Discussion

The importance of the DCE-MRI technique to evaluate the effect of anti-angiogenic agents has been widely emphasized because of its major advantage to provide pharmacokinetic parameters such as volume transfer constant (K^{trans}) and extravascular extracellular volume fraction (ve). In this study, we demonstrated that the anti-angiogenic effect of the newly developed candidate drug KR-31831 on xenografted human ovarian tumor model could be evaluated by DCE-MRI. Our results also showed a direct relationship of K^{trans} with microvasculature in xenografted animal model. In conclusion, our works suggest DCE-MRI may be a useful tool to evaluate the effect of newly developed candidate drugs on tumor angiogenesis.

References

1. Wilmes et al, *MRI* 25:319-27 (2007). 2. Park et al, *Int J Oncol* 32:1311-5 (2008). 3. Toft et al, *J Magn Reson Imaging* 10:223-32 (1999).