

DCE-MRI informs on proteasome activity on apoptosis of CT26 colon cancer by vascular disrupting agent KML001.

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Introduction

Tumor growth and metastasis require vascular network to provide oxygen and nutrients. So the tumor vasculature is an excellent target of anticancer therapy. Vascular disrupting agent (VDA) is a new class of antivascular agents that are currently undergoing clinical trials. The biologic activity of Arsenic Trioxide(ATO) that has been reported so far explains the anti-cancer effects with a variety of mechanisms including anti-tubulin effect, differentiation induction, apoptosis, anti-proliferative activity and angiogenesis inhibition. Young S. Lew et al. (1999. Cancer Research) found out acute tumor vascular shutdown and massive tumor necrosis similar to those observed in VDAs when ATO was administered to the murine tumor model. Given the fact that KML001 is the derivative of arsenic trioxide, it is highly possible that the anti-cancer effect of the agent might result from tumor vascular disruption and it is needed to validate the therapeutic efficacy on the tumor angiogenesis by the VDA in vivo.

In this study, we quantitatively visualized vascular disrupting effects of KML001 by Dynamic contrast enhanced MRI (DCE-MRI) correlated with cytoskeleton assay. We used xenograft mouse model of CT26 colon cancer cell line. Central tumor necrosis and skin discoloration were found in mice treated with KML001. Quantitative DCE-MRI parameter Kep was evaluated. The signal intensity and Kep of the tumor were significantly decreased at the post-treatment than the pre-treatment. Moreover, we found that KML001 degrades tubulin protein quantitatively in HUVEC cells, which may relate with vascular disrupting properties.

Material and Methods

The MRI examination was performed when the tumor size became 1cm in diameter after subcutaneous inoculation of 2×10^6 CT26 mouse colon cancer cells to Balb/c mice. Pre treatment baseline MRI images were obtained 8 day after tumor cell injection, then each group were treated with 100ul of KML001 at a concentration of 10mg/kg with 5% dextrose or sham injection of 100ul saline. Post treatment MRI was performed 24hr after treatment. Mice were anesthetized by exposure to 2% of isoflurane and a polyethylene catheter was maintained in the tail vein for injection of Gd-DTPA (Magnevist, schering, Germany, 281mg/kg) contrast agent during MRI scan.

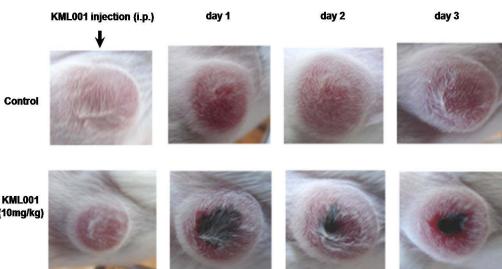
Imaging was performed using a whole-body 3T Signa Philips scanner. T2-weighted spin-echo images were acquired on coronal planes using the following parameters: TE=60ms, TR=3200ms, field of view (FOV) = 50mm, RFOV (%) = 69.94%, Matrix scan = 224, reconstruction = 512, 0.5mm thick slices, 14 slices, acquisition time = 8m 38s. T1-weighted spin-echo images were acquired on coronal planes before and after dynamic scan using the following parameters: TE=7.9ms, TR=427ms, field of view (FOV) = 50mm, RFOV (%) = 70%, Matrix scan = 128, reconstruction = 256, 1mm thick slices, 11 slices, acquisition time = 2m 8s. Gd-DTPA (Magnevist, schering, Germany) was then administrated at a dose of 281mg/kg as a tail-vein injection. DCE-MRI was performed using a perfusion-weighted spin echo sequence with the following acquisition parameters: FOV (mm) = 50 x 35, RFOV (%) = 71, Matrix scan = 112, reconstruction = 224, TR/TE (ms) = 12 / 4.0, slice number = 11, dynamic scans = 60, 1mm slice thickness with a total acquisition time of 2min 6s.

Raw data were transferred to a processing workstation and converted into analyze format. Pride tool DEC-MRI2010 (Philips Medical system, Netherlands) were used for fitting dynamic data. Data was analyzed by BRIX model method (Journal of computer assisted tomography 15(4):621-628).

Results

Figure 1 shows tumor morphology pre and post injection of the KML001. The central part of tumors of the mice injected with KML001 became discolored at 24hours after the injection. We found the tissue in the central part became necrosis on histopathologic examination.

DCE-MRI results show comparison between pre-treatment and 24hr post-treatment Gd-DTPA contrast enhancement T1-weighted MRI image (Figure 2a). Enhancement has been significantly decreased in KML001 treated group than sham injection group (KML001 p= 0.032, saline p= 0.659) (Figure 2b). Cytoskeleton assay performed on HUVECs proved vascular disrupting effect resulted from the cytoskeleton-associated protein degradation of tubulin by KML001's. (Figure 3)



Conclusion

DCE-MRI could quantitatively visualized the therapeutic efficacy of the KML001 acting as tubulin polymerization inhibitor by disrupting the tubulin network of endothelial cytoskeleton of colon cancer. DCE-MRI could be useful to monitor the antiangiogenic effect of vascular disrupting agent in the clinic to monitor the therapeutic efficacy of the antiangiogenic agent.

References

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Figure 1. Tumor necrosis after treatment KML001.

KML001 treatment group shows tissue necrosis in the central portion of the tumor.

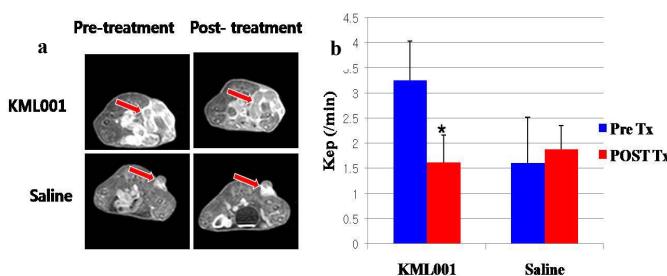


Figure 2. Quantitative analysis of the DCE-MRI parameter in the CT26 colon cancer xenograft mouse model.

a. T1-weighted gadolinium contrast enhanced MRI of pre-treatment and 24h post-treatment. Arrows indicates enhancing tumors at the proximal hind leg of the mice.
b. Changes in the mean values of Kep measured pre and 24h post-treatment. (* indicate p<0.05)

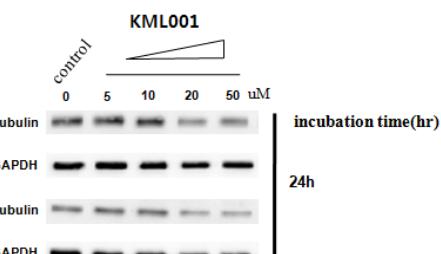


Figure 3. KML001 promotes a degradation of α - and β -tubulin in HUVEC cells. KML001 leads the protein of α -tubulin and β -tubulin to be specifically destroyed and then reduced the amount of protein production.