NEOPLASIA
www.neoplasia.com

Volume 20 Number 7 July 2018 pp. 734–744 734

DCE-MRI of Sunitinib-Induced Changes in Tumor Microvasculature and Hypoxia: A Study of Pancreatic Ductal Adenocarcinoma Xenografts[1,2]

Catherine S. Wegner, Anette Hauge, Trude G. Simonsen, Jon-Vidar Gaustad, Lise Mari K. Andersen and Einar K. Rofstad

Group of Radiation Biology and Tumor Physiology, Department of Radiation Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway

Abstract

The purpose of this study was dual: to investigate (a) whether sunitinib may induce changes in tumor microvasculature and hypoxia in pancreatic ductal adenocarcinoma (PDAC) and (b) whether any changes can be detected by DCE-MRI. Sunitinib-treated and untreated control tumors of two PDAC xenograft models (BxPC-3 and Panc-1) were subjected to DCE-MRI before the imaged tumors were prepared for quantitative analysis of immunohistochemical preparations. Pimonidazole was used as a hypoxia marker, and fraction of hypoxic tissue (HF_{Pim}), density of CD31-positive microvessels (MVD_{CD31}), and density of αSMA-positive microvessels ($MVD_{\alpha SMA}$) were measured. Parametric images of K^{trans} and v_e were derived from the DCE-MRI data by using the Tofts pharmacokinetic model. BxPC-3 tumors showed increased HF_{Pim}, decreased MVD_{CD31}, unchanged $MVD_{\alpha SMA}$, and increased vessel maturation index (VMI = $MVD_{\alpha SMA}/MVD_{CD31}$) after sunitinib treatment. The increase in VMI was seen because sunitinib induced selective pruning rather than maturation of αSMA-negative microvessels. Even though the microvessels in sunitinib-treated tumors were less abnormal than those in untreated tumors, this microvessel normalization did not improve the function of the microvascular network or normalize the tumor microenvironment. In Panc-1 tumors, HF_{Pim}, MVD_{CD31}, $MVD_{\alpha SMA}$, and VMI were unchanged after sunitinib treatment. Median K^{trans} increased with increasing MVD_{CD31} and decreased with increasing HF_{Pim}, and the correlations were similar for treated and untreated BXPC-3 and Panc-1 tumors. These observations suggest that sunitinib may induce significant changes in the microenvironment of PDACs, and furthermore, that K^{trans} may be an adequate measure of tumor vascular density and hypoxia in untreated as well as sunitinib-treated PDACs.

Neoplasia (2018) 20, 734–744

Introduction

In 1971, Judah Folkman proposed that antiangiogenesis would be important in the therapy of cancer [1], and in 1990, he provided significant evidence that tumor growth is angiogenesis dependent [2]. The pioneering work of Folkman created strong interest in tumor angiogenesis and antiangiogenic treatment of cancer, and today, antiangiogenic therapy is considered to be a promising cancer treatment modality [3]. Several molecular pathways promoting tumor angiogenesis have been identified, including the interleukin-8 (IL-8)/nuclear factor-κB (NF-κB) pathway, the angiopoietin (ANGPT)/tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains (TIE) pathway, and the vascular endothelial growth factor (VEGF)/VEGF receptor (VEGF-R) pathway [4,5]. The VEGF/VEGF-R pathway seems to be the most important

Abbreviations: αSMA, α smooth muscle actin; ANGPT/TIE, angiopoietin/tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; FOV, field of view; HE, hematoxylin and eosin; HF, hypoxic fraction; IL-8/NF-κB, interleukin-8/nuclear factor-κB; K^{trans}, volume transfer constant; MVD, microvessel density; PDAC, pancreatic ductal adenocarcinoma; ROI, region of interest; TE, echo time; TR, repetition time; v_e, fractional distribution volume; VEGF/VEGF-R, vascular endothelial growth factor/VEGF-receptor

Address all correspondence to: Einar K. Rofstad, PhD, Department of Radiation Biology, Institute for Cancer Research, Norwegian Radium Hospital, Box 4953 Nydalen, 0424 Oslo, Norway.
E-mail:; einar.k.rofstad@rr-research.no

[1] Funding: This work was supported by the Norwegian Cancer Society and the South-Eastern Norway Regional Health Authority.

[2] Conflict of Interest: The authors have no conflicts of interest.

Received 28 March 2018; Revised 16 May 2018; Accepted 21 May 2018

© 2018 The Authors. Published by Elsevier Inc. on behalf of Neoplasia Press, Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1476-5586
https://doi.org/10.1016/j.neo.2018.05.006

pathway, and many therapeutic drugs targeting this pathway have been developed, including the antibody bevacizumab and the tyrosine kinase inhibitors sorafenib and sunitinib [5,6]. A large number of clinical studies have been conducted, and it has been revealed that antiangiogenic therapy may not have a future as monotherapy, but used in combination with conventional chemotherapy or radiation therapy, antiangiogenic drugs may be important in the treatment of a large number of malignant diseases [5,7].

However, many factors limiting the effect of antiangiogenic drugs have been identified, one of which is the impact on the physicochemical microenvironment of tumors, particularly tumor oxygenation. This is a controversial issue, as some investigations suggest that antiangiogenic therapy may cause excessive vessel pruning leading to increased tumor hypoxia, whereas other investigations provide evidence that antiangiogenic therapy may lead to vessel normalization, increased perfusion, and improved tumor oxygenation [8,9]. Improved tumor oxygenation may decrease tumor aggressiveness and increase the effect of radiation therapy, immunotherapy, and some forms of chemotherapy, whereas elevated tumor hypoxia may impair the effects of most treatment modalities and promote tumor cell dissemination, invasion, and metastatic growth [10,11]. Importantly, it has been demonstrated that inhibition of the VEGF/VEGF-R pathway may increase the invasiveness and augment the metastatic potential of experimental tumors [12,13].

Noninvasive methods are needed to monitor the effect of antiangiogenic therapy on tumor oxygenation, and it has been suggested that physiological magnetic resonance imaging (MRI) may provide relevant parametric images [14–16]. This suggestion is supported by some preclinical studies that have compared the effect of antiangiogenic therapy on MRI-derived tumor parameters with that on tumor microenvironmental parameters assessed by invasive methods. Thus, it has been shown that multiparametric MRI, susceptibility contrast MRI, and MRI methods making use of ultrasmall superparamagnetic iron oxide particles as contrast agent may provide valid information on microvascular changes induced by antiangiogenic drugs [17–20]. However, similar studies examining the potential usefulness of established clinical MRI methods are sparse [21,22].

In our laboratory, studies of orthotopic A-07 melanoma xenografts – tumors that show a microenvironment characterized by highly permeable blood vessels and limited amounts of extracellular matrix components – have suggested that dynamic contrast-enhanced (DCE)-MRI is a potentially useful method for monitoring sunitinib-induced changes in tumor hypoxia [22]. This possibility was challenged in the study reported here by using pancreatic ductal adenocarcinoma (PDAC) xenografts as tumor models. The microenvironment of PDACs is characterized by an abundant desmoplastic stroma that may occupy large fractions of the tumor volume [23], and a dense extracellular matrix and poorly permeable blood vessels are important hallmarks of this stroma [24–26].

The purpose of this study was to investigate whether sunitinib can induce significant changes in the physicochemical microenvironment of PDAC xenografts, and furthermore, whether any changes can be detected by DCE-MRI. Tumors of two PDAC models (BxPC-3 and Panc-1) were included in the study. These models were selected because they differ substantially in tumor growth rate, differentiation, extracellular matrix distribution, angiogenic activity, microvascular density, and fraction of hypoxic tissue [27]. Sunitinib-induced changes in tumor microvasculature and hypoxia were assessed by quantitative analysis of histological preparations, and to investigate whether DCE-MRI is sensitive to sunitinib-induced changes, parametric images derived by pharmacokinetic analysis of DCE-MRI series were compared with the histological data.

Materials and Methods

Tumor Models

BxPC-3 and Panc-1 (American Type Culture Collection, VA, USA) human PDAC xenografts grown in adult (8–12 weeks of age) female BALB/c *nu*/*nu* mice were used as tumor models. Tumors were initiated from cells cultured in RPMI-1640 (25 mmol/l HEPES and L-glutamine) medium supplemented with 13% bovine calf serum, 250 mg/l penicillin, and 50 mg/l streptomycin. Approximately 2.5×10^6 cells in 25 μl of Hanks' balanced salt solution were inoculated intramuscularly in the left hind leg, and tumors were included in MRI experiments when having grown to a volume of 50–1200 mm^3.

Sunitinib Treatment

Sunitinib L-malate (LC Laboratories, Woburn, MA, USA) was dissolved in hydrochloric acid (1.0 molar ratio of sunitinib). Polysorbate 80 (0.5%; Sigma-Aldrich, Schnelldorf, Germany), polyethylene Glycol 300 (10%; Sigma Aldrich), sodium hydroxide to adjust to a pH of 3.5, and sterile water were added to the solution. Tumor-bearing mice were treated orally with sunitinib (40 mg/kg/day) or vehicle for 4 days.

Magnetic Resonance Imaging

MRI was carried out by using a Bruker Biospec 7.05-T bore magnet and a mouse quadrature volume coil (Bruker Biospin, Ettlingen, Germany). The tumors were positioned in the isocenter of the magnet and were imaged with axial slices covering the entire volume. The mice were given gas anesthesia (~4.0% Sevofluran in O_2; Baxter, IL, USA) at a flow rate of 0.5 l/min during imaging. Respiration rate and body core temperature were monitored continuously by using an abdominal pressure sensitive probe and a rectal temperature probe (Small Animal Instruments, New York, NY, USA). The body core temperature was kept at 37 °C by automated hot air flow regulation, and the gas anesthesia was adjusted manually to maintain a stable respiration rate.

DCE-MRI with Gd-DOTA (Dotarem, Guerbet, Paris, France) as contrast agent was performed as described earlier [27]. Anatomical T_2-weighted images were obtained prior to DCE-MRI by using a fast spin echo pulse sequence (RARE) with a repetition time (TR) of 2500 ms, an echo time (TE) of 35 ms, an image matrix of 128 × 128, a field of view (FOV) of 3 × 3 cm^2, a slice thickness of 0.7 mm, a slice gap of 0.3 mm, 2 averages, and fat suppression. A fast spin echo pulse sequence (RARE) with TRs of 200, 400, 800, 1500, 3000, and 5000 ms, TE of 8.5 ms, an image matrix of 128 × 128, a FOV of 3 × 3 cm^2, a slice thickness of 0.7 mm, and a slice gap of 0.3 mm was used to measure precontrast T_1-values (T_{10}-map). Gd-DOTA was diluted to a final concentration of 0.06 M and administered in the tail vein in a bolus dose of 5.0 ml/kg body weight during a period of 5 s by using an automated infusion pump (Harvard Apparatus, Holliston, MA, USA). A 3D SPGR pulse sequence (3D-FLASH) with a TR of 10 ms, a TE of 2.07 ms, a flip angle (α) of 20°, an image matrix of 128 × 128 × 10, and a FOV of 3 × 3 × 1 cm^3 was used to produce postcontrast T_1-weighted images at a temporal resolution of 14.8 s.

Download English Version:

https://daneshyari.com/en/article/8456797

Download Persian Version:

https://daneshyari.com/article/8456797

Daneshyari.com