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Abstract #4010

DCE-MRI as a tool to detect tumor vascular normalization by E7820, a novel angiogenesis inhibitor

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Abstract

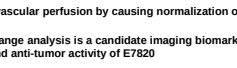
E7820 is a novel angiogenesis inhibitor, which modulates integrin α2 expression on endothelial cells and shows anti-tumor activity in a variety of xenograft models in nude mice. In this study, we tested the effect of E7820 on tumor vasculature using DCE-MRI with Gd-DTPA as contrast agent in human colon HCT-116 and renal Caki-1 tumor xenograft models in nude rats. The activity (IC_{50}) of E7820 in in vitro proliferation assay was 0.011 ± 0.010 μM for HCT-116 and 12 ± 2.8 μM for Caki-1 cells, respectively. In nude rats, E7820 was administered orally at 6.25 mg/kg bid. for 14 days (D0-D13) and showed significant anti-tumor activity in both tumor models (optimal T/C% of 9% and 47% for HCT 116 and Caki-1 model, respectively). In the HCT-116 model, the mean K^{trans} value in the tumor rim was increased by 76% at D13. Since imaging data suggested an alteration of K^{trans} distribution within tumors, K^{trans} histograms were computed to determine the ratio of voxels having low (0.0 - 0.4, 1/min), medium (0.4 - 0.8, 1/min) and high (0.8 - 1.2, 1/min) K^{trans} values. With this K^{trans} range analysis, the proportion at D13 of high K^{trans} was increased by 89%, while the proportion of low K^{trans} was decreased by 71% within HCT-116 tumor in nude rats treated with E7820. In the Caki-1 model, the proportion of low K^{trans} at D2 was decreased by 46%, and then the proportion of high K^{trans} at D7 was increased by 293% and both effects were sustained until D13 with E7820 treatments. ADC parameter measured by DW-MRI was not changed during the course of E7820 treatment. IHC analysis of tumors showed that the number of large vessels decreased after E7820 treatment. The percentage of pericyte-covered vessels (SMA/CD31 staining) and Hoechst33342 extravasation in tumors increased after E7820 treatment. These results confirmed the data obtained by DCE-MRI and suggested that E7820 induced vascular normalization at a pharmacological dose showing a significant anti-tumor activity in both models. In summary, E7820 affected tumor vasculature and might cause an improvement of vascular perfusion. DCE-MRI with K^{trans} range analysis is a good imaging biomarker to evaluate the normalization effect of E7820 on tumor vascular and to design further combination studies of E7820.

Key findings

- **E7820 might improve vascular perfusion by causing normalization of tumor vasculature**
- **DCE-MRI with Ktrans range analysis is a candidate imaging biomarker for anti-tumor vasculature and anti-tumor activity of E7820**

Background

E7820



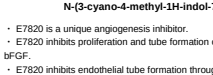
N-(3-cyano-4-methyl-1H-indol-7-yl)-3-cyanobenzenesulfonamide

- E7820 is a unique angiogenesis inhibitor.
- E7820 inhibits proliferation and tube formation of endothelial cells induced by both VEGF and bFGF.
- E7820 inhibits endothelial tube formation through the suppression of integrin α2.
- E7280 inhibits tumor-induced angiogenesis in mice
- E7820 shows anti-tumor activity to some types of tumor cells

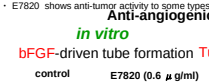
Anti-angiogenic activity of E7820

in vitro

bFGF-driven tube formation

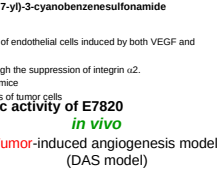


VEGF-driven tube formation



in vivo

Tumor-induced angiogenesis model (DAS model)



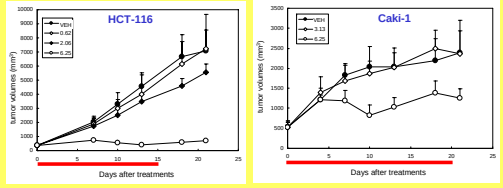
Cancer Res., vol 62, 6116-6123, 2002
Clin. Cancer Res., vol10, 1430-1438, 2004

Summary

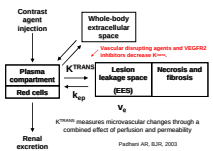
- **E7820 has a significant antitumor activity in HCT 116 and Caki-1 nude rat models.**
- **K^{trans} range analysis is useful to evaluate the effect on tumor vasculature by DCE-MRI analysis**
- **E7820 altered distribution of K^{trans} values within tumor.**
- **E7820 increased the ratio of high K^{trans} probability (K^{trans} range: 0.8-1.2), while decreasing the ratio of low K^{trans} probability (K^{trans} range: 0.0- 0.4) by K^{trans} range analysis.**
- **E7820 increased pericyte coverage of vessels and improve vascular perfusion within tumor.**

Results

Effect of E7820 on s.c. tumor growth in nude rats



Bi-compartmental, bi-directional pharmacokinetic model

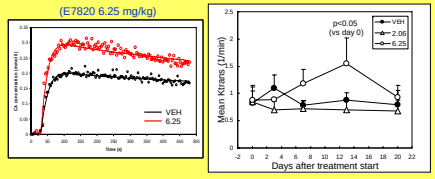


K^{trans} measures microvascular changes through a combined effect of perfusion and permeability

Effect of E7820 on K^{trans} at the tumor rim (HCT-116 model)

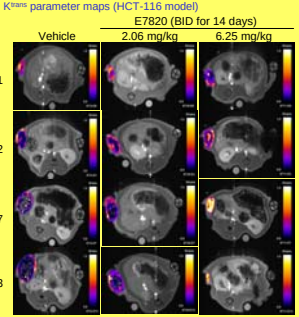
(a) Gd-DTPA uptake curve (E7820 6.25 mg/kg)

(b) Mean Ktrans (1/min)

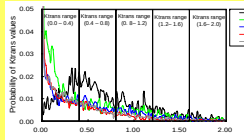


K^{trans} range analysis (HCT-116 model)

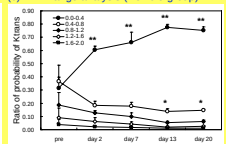
(a) K^{trans} parameter maps (HCT-116 model)



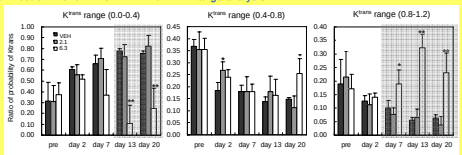
(b) K^{trans} histograms (Vehicle group)



(c) K^{trans} range analysis (Vehicle group)

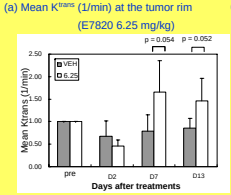


(d) Effect of E7820 in DCE-MRI with K^{trans} range analysis

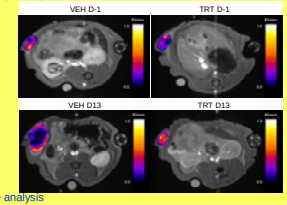


K^{trans} range analysis (Caki-1 model)

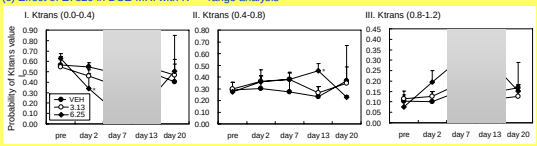
(a) Mean K^{trans} (1/min) at the tumor rim (E7820 6.25 mg/kg)



(b) K^{trans} parameter maps

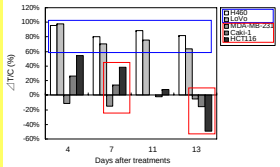


(c) Effect of E7820 in DCE-MRI with K^{trans} range analysis

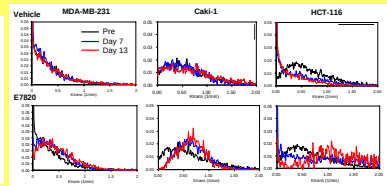


Activity of E7820 (6.25 mg/kg, BID) in DCE-MRI with K^{trans} range analysis reflects anti-tumor activity among five human tumor xenograft models in nude rats

(a) Anti-tumor activity of E7820 in five human tumor xenograft models in nude rats



(b) Evolution of K^{trans} histogram with treatment of E7820



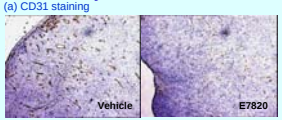
(c) Summary of the effect of E7820 in DCE-MRI with K^{trans} range analysis in rat xenograft models

Cell		Ktrans range 0.0-0.4 (Low perfusion) day 7	0.0-0.4 (Low perfusion) day 13	0.4-0.8 (Medium perfusion) day 7	0.4-0.8 (Medium perfusion) day 13	0.8-1.2 (High perfusion) day 7	0.8-1.2 (High perfusion) day 13
LoVo	Vehicle	1.20 ± 0.30	1.21 ± 0.19	0.61 ± 0.27	0.61 ± 0.23	1.31 ± 0.98	0.91 ± 0.35
	E7820	1.59 ± 0.63	1.52 ± 0.68	0.44 ± 0.18	0.54 ± 0.29	0.49 ± 0.31	0.86 ± 0.58
	T/C	133%	125%	73%	89%	37%	95%
MDA-MB-231	Vehicle	1.04 ± 0.15	1.01 ± 0.13	0.96 ± 0.34	0.98 ± 0.23	1.12 ± 0.47	1.03 ± 0.21
	E7820	0.81 ± 0.22	0.70 ± 0.31	1.55 ± 0.54	1.66 ± 0.43	1.73 ± 1.40	2.52 ± 2.55
	T/C	78%	69%	162%	170% *	155%	244%
H460	Vehicle	1.21 ± 0.12	1.25 ± 0.11	0.53 ± 0.14	0.50 ± 0.17	0.55 ± 0.37	0.40 ± 0.22
	E7820	1.12 ± 0.15	1.23 ± 0.13	0.69 ± 0.19	0.47 ± 0.18	0.93 ± 0.55	0.38 ± 0.31
	T/C	92%	98%	130%	94%	169%	94%
Caki-1	Vehicle	1.81 ± 0.79	1.17 ± 0.18	1.02 ± 0.30	0.67 ± 0.07	0.47 ± 0.33	0.52 ± 0.47
	E7820	0.30 ± 0.17	0.36 ± 0.15	1.29 ± 0.45	1.43 ± 0.07	3.70 ± 1.91	3.21 ± 2.28
	T/C	17% *	31% *	121%	213%	790% *	613% *
HCT-116	Vehicle	2.56 ± 1.23	3.08 ± 1.74	0.49 ± 0.09	0.37 ± 0.03	0.74 ± 0.64	0.38 ± 0.26
	E7820	1.13 ± 0.79	0.32 ± 0.48	0.52 ± 0.19	0.47 ± 0.20	1.27 ± 0.84	2.12 ± 1.14
	T/C	44%	10% **	106%	125%	172%	565% **

Effect of E7820 on tumor vasculature

IHC analysis of tumor vessels

(a) CD31 staining

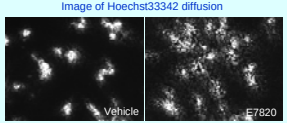


(b) Effect of E7820 on micro vessel density

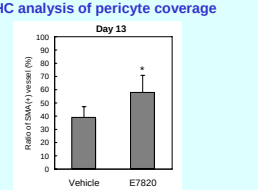
Treatment	Dose (mg/kg)	CD31 staining (score) Median	Area
Vehicle	-	3	(2.5, 3, 3, 3, 3, 3, 3.5, 3.5, 3.5)
E7820	6.25	2.5 *	(2, 2.5, 2.5, 2.5, 2.5, 3, 3, 3, 3)

Effect on diffusion of Hoechst33342

Image of Hoechst33342 diffusion



IHC analysis of pericyte coverage



Materials & Methods

Test substance : E7820 prepared in Glucose 5% DMSO:Tween (0.35:0.65)

Tumor cell lines : HCT 116 human colon carcinoma ; Caki-1 human renal carcinoma; NCI-H460 human lung ; MDA-MB-231 human mammary adenocarcinoma ; LoVo human colon adenocarcinoma

Animals : *Nude* rats (Charles River, France)

Drug administration : oral route (PO) via a cannula

Tumor induction and treatment schedule: SC inoculation of $2x10^7$ HCT 116, Caki-1, NCI-H460 and LoVo cancer cells and tumor fragment xenografting for MDA-MB-231 to irradiated Nude rats (7 Gy, Co60, INRA, Dijon, France),.Randomization at D0 in 3 to 4 groups of 6 to 8 rats when the range of tumor volume reached about 400 to 800 mm^3.At D0, the treatment starts in the different groups of rats with PO administrations of vehicle or E7820 at 5 ml/kg twice a day for indicated consecutive days

Anti-tumor activity measured as T/C% or △T/C% parameter is defined as followed:

T/C (%) = (Median TVn of treated group) x 100 / (Median TVn of vehicle group) at day n

△T/C (%) = (TVn-TV1)/(TVm-TV1) x 100, where TVn is the tumor volume of treated mice at day n.

The effective criteria for T/C% is <42% (IV. Tumor Volume). The animal care unit is authorized by the French ministries of Agriculture and Research (Agreement No. A21231011). Animal experiments were performed according to the European ethical guidelines of animal experimentation and the English guidelines for welfare of animals in experimental neoplasia. All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon).

DCE-MRI protocol: Three to five rats/group dedicated to MRI exams were selected on tumor volumes at D0 (before start of treatment) (in case of death, alternative rats were chosen in the same group). MRI was performed at D0, D3, D7, D13 and D20. All imaging was performed at 4.7T with an horizontal bore magnet (Pharmascan, Bruker, Germany). During the imaging protocol, the animals were maintained under anesthesia via a constant flow of isoflurane at 2-3% delivered by a nose cone. A T2-weighted RARE sequence (TE/TR=38/2500 ms) with a FOV=70x50 mm and a slice thickness of 1.5mm was used for morphological description and tumor volume measurement. DCE-MRI data was acquired during 8 minutes using a T1-weighted FLASH2D sequence (TE/TR/flip angle= 3ms/50ms/60° ; Slice thickness=2mm) with FOV=60x50 mm and matrix size= 108x80 at a temporal resolution of 4s per image. An intravenous bolus injection of Gd-DTPA (Magnevist®, Bayer Healthcare Pharmaceuticals, Germany) at the dose of 0.1 mmol/kg was performed 30s after acquisition start. Tracer uptake curves derived from signal enhancement in selected regions of interest (ROI) (i.e in tumor rim and core) were fitted using a two-compartment kinetic model (Tofts et al, JMRI, 1999) for the determination of the volume transfer constant (K^{trans}) using an in-house developed plug in of ImageJ. K^{trans} distributions were computed from ROI data and normalized to voxel count to assess vascular heterogeneity and to allow for visualization of changes not shown by the mean value over a ROI.

Hoechst perfusion protocol: At D13, three rats/group from Caki-1 model received IV Hoechst injection 1 min. prior to sacrifice. Their tumor was collected, frozen and used for immunofluorescence microscopy and digitalization under a Cell Observer Zeiss apparatus (2 sections per tumor, 6 images per section).

CD31/α-SMA immunostaining: At D13, three rats/group from Caki-1 model were sacrificed. Their tumor was collected, frozen and stained sequentially with the following two pairs of antibodies: mouse anti-rat CD31 antibody / anti-mouse coupled to Alexa Fluor® 488 and mouse anti-rat α-SMA antibody / anti-mouse coupled to Alexa Fluor® 568. 2 sections per tumor and 6 images per section were digitized for CD31 and α-SMA co-localization quantification. For immunochemical characterization of microvascularization through CD31 staining in HCT 116 model, the tumor was stained with mouse anti-rat CD31 antibody revealed with the complex anti-mouse antibody coupled to biotin / avidin biotin-peroxidase conjugate which converts the DAB chromogen to visualize the reaction.