Evolution of the tumor oxygenation and perfusion at the early stage of the treatment with SU5416, an inhibitor of Flk-1 tyrosine kinase.

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Introduction

The use of anti-angiogenic drugs as therapeutic agents for tumor treatment is well known to produce a significant inhibition of tumor angiogenesis and tumor growth in vivo. Recently, a new approach for anti-angiogenic agents was proposed consisting of a transient normalisation of tumor vasculature during the early stage of an anti-angiogenic treatment (1). This consists of pruning the immature and inefficient blood vessels, leading to a transient improvement of tumor perfusion and oxygenation and therefore to a better radiotherapy and chemotherapy efficacy. Our previous study concerning the non specific anti-angiogenic agent Thalidomide confirmed this process in FSAII and TLT tumors (2). Similarly, we decided to investigate whether another anti-angiogenic agent acting on another target (Flk-1 tyrosine kinase activity) could also normalize the tumor vasculature and enhance treatments. We chose to study SU5416, which is known to inhibit endothelial cell proliferation.

Materials and methods

TLT tumor-bearing mice received daily 100µl i.p. SU5416 injections (25mg/kg) (100µl i.p. DMSO alone for controls). Oxygen pressure (pO_2) was measured daily by EPR oximetry with a 1.2 GHz spectrometer (Magnettech, Germany). This measurement is based on the variation of the EPR linewidth of a paramagnetic oxygen sensor implanted in the tumor. To estimate tumor perfusion and tracer penetration, DCE MRI (4.7 Tesla, Bruker Biospec, T1-weighted GRE sequence) was performed using P792 as a contrast agent. A pharmacokinetic analysis using a bicompartmental model was performed to extract the plasma volume fraction and the transcapillary flux. The consumption rate of oxygen in tumor cells was measured using an X-band EPR spectrometer operating at 9 GHz (Bruker).

Results

Daily SU5416 treatments increased tumor oxygenation with a maximum level attained two days after the beginning of the treatment (fig.1). Surprisingly, this dramatic oxygen increase was not correlated with an increase of tumor perfusion or plasma volume fraction (fig.2). It is therefore likely that the increase in tumor oxygenation was due to a decrease in tumor cell oxygen consumption (fig.3). At the day of the maximum oxygenation increase (day 2), the efficacy of radiotherapy (10 Gy of 250 kV RX) was improved (regrowth delay for doubling the tumor size was increased by 7.1± 2.8 days for pre-treated group versus control group). For chemotherapy (cyclophosphamide 50mg/kg), no improvement was observed for the pre-treated group.

Discussion

The improvement of the radiotherapy treatment but not the chemotherapy treatment implied that only the tumor oxygenation status was changed during the early phase of SU5416 treatment. In contrast to our previous study with Thalidomide, this increase in pO_2 was not likely the result of a normalisation process of the tumor vasculature, but instead due to a decrease in tumor cell oxygen consumption rate. This new mechanism for an anti-angiogenic agent offers a new way to treat tumors by improving the response to radiation. Also, this study demonstrates that combined measurements of pO_2, oxygen consumption and tumor blood flow may serve as a guide for planning treatment combinations.

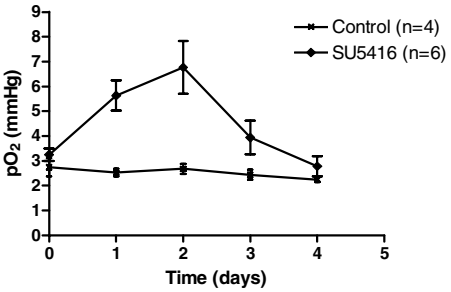

Fig.1 Effect of SU5416 on TLT tumor oxygenation monitored by EPR Oximetry. Note the significant increase ($p<0.01$) in pO_2 24 hours after the beginning of the treatment

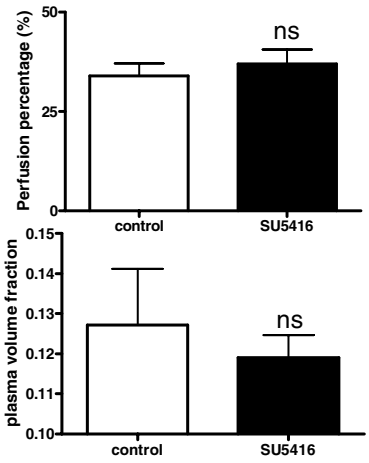

Fig.2 Effect of SU5416 on TLT tumor perfusion or plasma volume fraction monitored by DCE MRI 2 days after the beginning of the treatment. No significant change (ns) was observed 24 hours after the beginning of the treatment

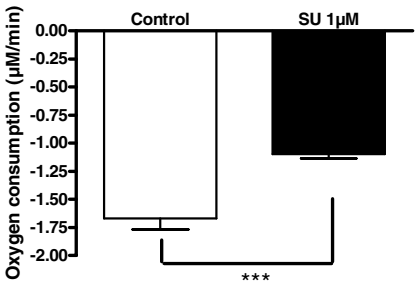

Fig.3 Effect of SU5416 1µM on TLT tumor cell oxygen consumption using EPR X-band. Note the significant decrease (***;$p<0.001$) of oxygen consumption.

(1) Jain R.K Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nat Med. 2001 Sep;7(9):987-9. Review.

(2) Ansiaux R. *et al.* Thalidomide radiosensitizes tumors through early changes in the tumor microenvironment. Clin. Cancer Res. 2005 Jan 15;11:743-50.