EPR study of the tumor reoxygenation following inhibition of the MAPKinase pathway: underlying mechanisms and radiosensitizing effects

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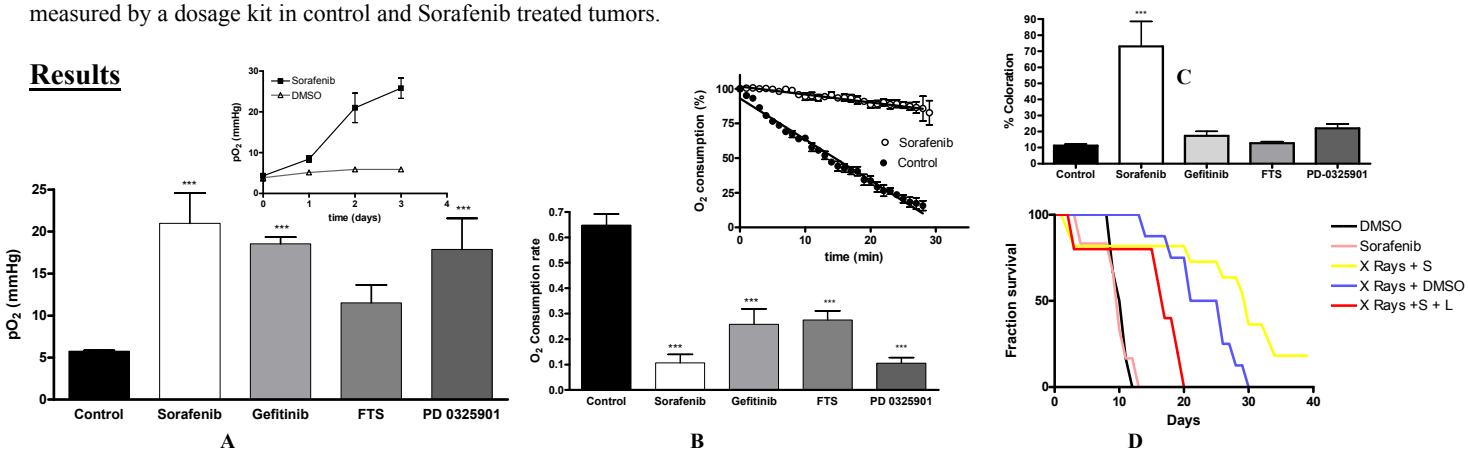
Purpose

The Mitogen Activated Protein Kinase (MAPK) pathway plays a central role in promoting cell proliferation and survival. It has been shown that this pathway directly affects the formation, progression, and metastasis of human tumors (1). It is also well recognized that tumor hypoxia is a critical determinant for response to radiotherapy or chemotherapy (2). A link between the MAPK pathway and tumor hypoxia has not been established yet. In the current study, the chronic effects of different MAPK inhibitors were monitored in vivo using EPR (Electron Paramagnetic Resonance) oximetry in experimental tumors and the window of reoxygenation was exploited in order to sensitize tumors to radiation therapy.

Material and Methods

FSa II (Fibrosarcoma murine) tumor bearing C3H mice were injected daily (IP) with either vehicles (DMSO) or different MAPK inhibitors (Sorafenib and Gefitinib at 45 mg/kg/Day in DMSO and PD-0325901 and Farnesyl Thiosalycilic Acid (FTS) at 20 mg/kg/Day) . Mice were anesthetized using isoflurane. Local pO_2 was estimated using in vivo EPR oximetry (L-band, 1.2 GHz). The therapeutic relevance was assessed for Sorafenib by measuring the regrowth delay following a 20 Gy irradiation of X-rays using a RT-250 device (Philips). For all MAPK inhibitors, changes in blood flow were probed by patent blue staining and oxygen consumption was measured ex vivo using X-Band EPR (9.1 GHz). Mitochondrial activity was assessed in vitro using a fluorescent probe (MitoTracker) after treatment with Sorafenib (10µM). Glutathion levels were measured by a dosage kit in control and Sorafenib treated tumors.

Results



Sorafenib, gefitinib and PD 0325901 as well as 3 on 4 FTS treated mice; dramatically modified FSaII tumor pO_2 upon one day post-injection and the effect was prolonged until day 4, with a peak at days 2-3 (A). This window of reoxygenation (day 2) was used for the rest of the experiments. Oxygen consumption decreased in treated tumors for all MAPK inhibitors tested (B). A rough estimate of tumor blood flow, assessed by patent blue staining at day 2, showed a high increase for Sorafenib, and no blood flow modification for Gefitinib, FTS and PD 0325901 compared to control tumors (C). The tumor radiosensitivity was enhanced by a factor of 1.5 in the treated and irradiated group (XRays+S) compared to X-rays alone (XRays+DMSO). The Kaplan Meier plot shows that the survival rate in the irradiated and treated group is higher than the irradiated group. Moreover, a group including clamped tumors (deprivation of oxygen at the time of irradiation) and Sorafenib (XRays+S+L) did not show any enhancement of response, which definitely proves the involvement of an oxygen effect. The Mitotracker assay showed a decrease of mitochondrial activity for Sorafenib treated tumors as well as a decrease of the glutathion level.

Discussion

In vivo EPR oximetry showed an increase in pO_2 for all MAPK inhibitors in FSaII experimental tumors and allowed the identification of a window of reoxygenation, which was further successfully exploited with sorafenib for improving radiation response. The increase in tumor oxygenation was shown to be the result of two major factors: (i) an increase in blood flow for sorafenib only which could be explained by one of the numerous target of Sorafenib, such as CRAF, VEGFR-2, VEGFR-3 and PDGFR-ß, that are all implicated in tumor vascularisation; and (ii) the decrease of oxygen consumption for all MAPK inhibitors in FSaII tumors, likely to be due to an alteration of the mitochondrial activity observed using the Mitotracker probe in tumors treated with Sorafenib. This effect could be due to the observed GSH depletion. Indeed, literature shows that Sorafenib is implicated in mitochondria-dependent oxidative stress mechanism in Hep G2 celles (hepatocellular carcinoma) provoked by a rapid production of ROS followed by a nearly 90% of GSH depletion (3).

(1)Clin Cancer Res 3651 2008; 14(12) June 15, 2008 (2) Semin Radiat Oncol 2004;14:233 (3) Cancer Biology and Therapy, 2009. 8:1904-1913.