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Combretastatin-induced hypertension and the consequences for its combination with other therapies

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ABSTRACT

Purpose: Combretastatin A-4 phosphate (CA4P) is a promising vascular disrupting agent in cancer treatment, but elicits hypertension in patients. The aim of this study was to use a mouse model to investigate whether hypertension or its modification influenced the treatment efficacy of CA4P in combination with other therapies.

Material and methods: C3H mammary carcinoma bearing or non-bearing CDF1 mice were used. The effects of CA4P alone or in combination with the antihypertensive drug hydralazine (HDZ) on mean arterial blood pressure (MABP), hematocrit (Hct) and hemoglobin concentration ([Hb]) were characterized in non-tumor-bearing animals. Tumor-bearing mice were also treated locally with radiation and/or hyperthermia (41.5 °C; 60 min) in combination with CA4P alone or CA4P plus HDZ, and TCD50 values (radiation dose that controls 50% of tumors) determined.

Results: Hct, [Hb] and MABP respectively increased from 49.3 ± 0.3%, 9.1 ± 0.1 mM and 110 ± 7 mm Hg, to 54.7 ± 0.2%, 10.3 ± 0.1 mM and 127 ± 5 mm Hg, within 1 h after injecting 100 mg/kg CA4P. For each parameter the magnitude of the peak increase was largely dose independent within the CA4P dose range tested (10–250 mg/kg). However, high CA4P doses delayed the return to baseline and Hct and [Hb] recovered more slowly than MABP. Co-administration of HDZ (0.2 mg/kg) was able to suppress the CA4P-induced increase in MABP for several hours but did not noticeably affect the changes in Hct and [Hb]. The TCD50 value (± 95% confidence interval) for radiation alone was 53 (51–55) Gy. Tumor irradiation followed by injection of either CA4P (100 mg/kg) or CA4P + HDZ 30 min later reduced the TCD50 values to 50 (46–54) Gy and 48 (45–52) Gy, respectively. Heating tumors after irradiating further decreased the TCD50 value to 46 (43–48) Gy. When all treatments were combined the TCD50 was 35 (32–38) Gy, regardless of whether the drugs were CA4P or CA4P + HDZ.

Conclusions: CA4P significantly increased Hct, [Hb] and MABP. Hypertension, but not increases in Hct and [Hb], could be reversed with the antihypertensive drug HDZ. CA4P significantly improved tumor response to radiation or thermoradiation, neither of which was influenced by the addition of HDZ.

1. Introduction

When tumors reach a size of approximately ~1 mm^3 further expansion depends critically on the development of new vessels to maintain oxygen and nutrient delivery (Carmeliet and Jain, 2000; Kerbel and Folkman, 2002). Dividing endothelial cells are thus more prevalent in tumors than in normal tissues, making the developing vasculature a relevant target to deprive the tumor of oxygen and nutrients (Horsman and Siemann, 2006; Siemann et al. 2004). Endothelial cells are easily accessible to drugs and represent a particularly attractive target because they, unlike tumor cells, are genomically stable, which prevents them from acquiring treatment resistance (Ahmed et al., 2008). Two classes of drugs are currently receiving considerable attention (for recent reviews see: Siemann et al. 2005; Siemann and Horsman, 2009). Angiogenic inhibitors prevent the development of new vessels, while vascular disrupting agents target the existing tumor vasculature. Although treatment with VDAs typically has rapid and dramatic effects on tumor vasculature and causes a substantial decrease in blood flow as well as excessive killing of cells in the tumor core, monotherapy with these agents cannot control tumor growth (Chaplin et al., 1999). This may be because peripheral tumor cells can derive sufficient quantities of oxygen and nutrients from nearby normal and largely treatment-insensitive vessels in non-malignant tissue (Chaplin et al., 1999). The efficacy of VDAs may therefore be improved significantly when combined by conventional treatment regimes that also target the well-oxygenated tumor rim. In accordance, a combination of VDA

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therapy with radiation, hyperthermia, mild temperature thermoradiation or chemotherapy improves the efficacy substantially (for a thorough review see: Horsman and Siemann, 2006). Nevertheless, treatment sequence and timing are critical since VDAs may compromise drug accessibility or exacerbate hypoxia (Murata et al., 2001b,c; Siemann and Rojiani, 2002).

Combretastatin A-4 phosphate (CA4P) belongs to the tubulin depolymerizing agents and is a lead candidate VDA currently undergoing clinical testing in combination with conventional cancer therapies (Siemann et al., 2009). Clinical studies show that CA4P may cause hypertension and since blood pressure and frequency of cardiac disorders increase with age, cancer patients may be particularly vulnerable to a further medication-induced elevation of blood pressure which may ultimately limit the tolerable dose (Rustin et al., 2003). However, the mechanism by which CA4P cause hypertension is poorly understood and it is not clear whether pharmacological correction of hypertension may affect (positively or negatively) the anti-tumor efficacy of CA4P. Specifically, we hypothesize that elevated perfusion pressure may partly offset the reduced tumor blood flow by forcing blood through constricted vessels or by preventing injured vessels from collapse. The aims of this study were to use a mouse model to investigate the CA4P-induced hypertension in mice, whether that hypertension can be pharmacologically controlled, and how it affects the efficacy of CA4P in combination with other therapies.

2. Materials and methods

2.1. Animal and tumor model

CDF1 mice were used for all experiments. Hematological and blood pressure measurements were done in non-tumor-bearing mice, whereas the radiation and heat experiments were performed in mice bearing 200 mm^3 C3H mammary carcinomas in the right rear foot. Tumors were grown by subcutaneous injection of freshly minced tumor material obtained from a previously established flank tumor as detailed in an earlier study (Overgaard, 1980).

2.2. Mean arterial blood pressure (MABP) measurements

To monitor drug induced changes in MABP the carotid artery was cannulated as previously described from our laboratory (Christensen et al., 1990) using PE10 catheter (gently pulled to reduce the tip diameter) containing heparinised saline. In short, mice were anaesthetized with a mixture of hypnorm and diazepam and the carotid artery was carefully exposed through a midline incision over the trachea and throat. Three sutures were placed around the artery for immobilization, to prevent bleeding during insertion of the catheter, and to secure the catheter following insertion. The catheter was externalised in the neck and secured to the skin by a single suture. Body temperature was monitored and maintained at ~37 °C during surgery by means of a heating bulb. Following surgery mice were administered 0.5 ml of physiological saline subcutaneously and left overnight at 30 °C to buffer fluid and electrolyte imbalances and prevent hypothermia during the awakening period. When fully recovered following surgery, mice were immobilized in special designed jigs and their catheters were lengthened with PE50 tubing and connected to pressure transducers (Baxter Edward, model PX600, Irvine, CA), which were calibrated before use against a water column. Blood pressure was recorded at a sampling rate of 500 min^{-1} using a BIOPAC MP100 unit (Goleta, CA) and displayed, stored and analyzed using the Acknowledge software. Blood pressure was monitored continuously and allowed to stabilize for 1.5 to 2 h, before mice were administered intraperitoneally (i.p.) with saline alone or saline containing CA4P (OXiGENE Inc., Watertown, USA) using an injection volume of 0.02 ml/g body weight. In some experiments mice were also injected i.p. with the vasodilator hydralazine (0.2 mg/kg in ~0.1 ml saline). To ensure appropriate water and electrolyte balance and prevent blockage of the catheter mice were slowly administered with 0.1 ml weakly heparinised saline through their catheters every 0.5 h. Mice were monitored until blood pressure was back to baseline level or until irreparable catheter blockage occurred. To minimize visual disturbance mice were shielded from the observer during measurements.

2.3. Hematological parameters

In a separate experimental series, hematocrit (Hct) and hemoglobin concentrations ([Hb]) were determined from blood samples taken from the sub-orbital sinus in animals treated for various time periods with the same drug concentrations and combinations as above. To avoid repetitive blood sampling from animals with small blood volumes only one sample was obtained from each mouse. Hct was determined using a Hematocrit centrifuge (Andreas Hettich GmbH & Co KG, Germany) and an Adams A2970 Micro-Hematocrit reader (Clay Adams, Parsippany, NJ) and [Hb] was measured using an OSM3 Hemoximeter (Radiometer, Copenhagen, Denmark).

2.4. Radiation and heat experiments

Tumor-bearing mice were transferred to restraining jigs and their tumor-bearing legs exposed and loosely attached to the jig with tape and the tumor was treated with single graded radiation doses (240 kV X-rays) as monotherapy or in combination with additional treatment as follows:

(1) CA4P (100 mg/kg) 30 min post-irradiation; (2) CA4P (100 mg/kg) and HDZ (0.2 mg/kg) 30 min post-irradiation; (3) local tumor heating (41.5 °C, 60 min) by immersing the tumor-bearing foot in a water bath 60 min post-irradiation; (4) CA4P (100 mg/kg) 30 min post-irradiation followed by heat 60 min post-irradiation; (5) CA4P (100 mg/kg) and HDZ (0.2 mg/kg) 30 min post-irradiation followed by heat 60 min post-irradiation. After treatment, mice were returned to their cages and observed on a weekly basis. The percentage of animals in each treatment group showing local tumor control at 90 days was recorded and from logit analysis of the radiation dose response curves, TCD50 values (radiation dose that controlled 50% of tumors) were calculated.

2.5. Statistics

TCD50 values are reported as mean values with 95% confidence intervals. Other parameters are reported as means ± SEM. For each dose the effect of CA4P on cardiovascular parameters over time was tested using one-way ANOVA with repeated measures (MABP) or without repeated measures (Hct and [Hb]) followed by appropriate post-hoc tests. The magnitude of peak values for MABP, Hct and [Hb] was analyzed using one-way ANOVA. Recovery time following treatment was calculated as the time for each parameter to return to within 0.5 standard deviation of the pretreatment levels. TCD50 values were analyzed using a chi-square test. Significance level was set at $p<0.05$.

3. Results

3.1. Cardiovascular physiology and haematology

Baseline MABP of the carotid-cannulated non-anaesthetized restrained mice was approximately 110 mm Hg and did not change upon infusion of saline (Fig. 1). Injection of CA4P, however, caused a rapid and significant increase in MAPB at all concentrations tested that reached a near-plateau phase within 0.5–1 h at approximately 15% above baseline level (Fig. 1). The magnitude of the peak change in