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CHANGES IN PERFUSION PATTERN OF EXPERIMENTAL TUMORS DUE TO REDUCTION IN ARTERIAL OXYGEN PARTIAL PRESSURE

Oliver Thews[*], Debra K. Kelleher[**], and Peter Vaupel[**]

1. INTRODUCTION

In many solid tumors, oxygen deficiency (hypoxia) can be found which results from a discrepancy between tissue O_2 supply and O_2 consumption by the cells. The presence of low O_2 partial pressures (pO_2) in tumors can influence the efficacy of O_2-dependent treatment modalities and also the biological properties of tumor cells, e.g., by modulating expression of hypoxia-dependent proteins (for a review see Höckel & Vaupel, 2001)[1]. Major parameters determining the O_2-supply are the convective transport with the blood as well as the O_2-diffusion from blood to the cells which is mainly a function of the diffusion distance. Convective O_2-transport in tumors is fundamentally different from that found in normal tissues, since tumor vessels show many structural abnormalities such as blind vessel endings, irregular branching patterns or loss of vascular hierarchy [2] but also functional differences compared to normal blood vessels, e.g., intermittent flow stop or pronounced arterio-venous shunt perfusion.

Since hypoxia can limit the efficacy of non-surgical treatment modalities (e.g., irradiation or O_2-dependent chemotherapy) [3, 4] new treatment alternatives have been developed which preferentially target hypoxic tumor cells [5, 6]. In order to improve the efficacy of these treatments, it might be beneficial to further increase tumor hypoxia temporarily, e.g., by breathing a gas mixture with a reduced O_2 fraction. However, since it is known that hypoxia affects tumor biology and thus may have an impact on the malignant progression of the tumor [1], the question arises of whether a reduction of the arterial pO_2 during ventilation of a hypoxic gas mixture may change the metabolic microenvironment of the tumor. Since hypoxia at least partially affects the tumor by influencing gene expression, both acute and long-term effects of a lower tumor pO_2 have to be considered. This study focuses on changes in tumor perfusion during acute (20 min) and chronic (several days) inspiratory hypoxia.

[*] Institute of Physiology, University of Würzburg, 97070 Würzburg, Germany
[**] Institute of Physiology and Pathophysiology, University of Mainz, 55099 Mainz, Germany

2. MATERIAL AND METHODS

2.1. Animals and Tumors

All studies were performed using the DS-sarcoma of the rat. Following subcutaneous injection of DS-ascites cells (0.4 ml; approx. 10^4 cells/µl) into the dorsum of the hind foot of male Sprague Dawley rats (Charles River Deutschland, Sulzfeld, Germany; body weight 190-240 g) experimental tumors grew as flat, spherical segments and replaced the subcutis and corium completely. The volume was determined by measurement of the three orthogonal diameters of the tumor and using an ellipsoid approximation with the formula: $V = d_1 \times d_2 \times d_3 \times \pi/6$. Perfusion distribution experiments were performed when tumors reached a target volume of 0.5 - 3.0 ml approx. 6 to 14 days after inoculation of tumor cells. Studies had previously been approved by the regional ethics committee and were conducted according to UKCCCR guidelines [7] and the German Law for Animal Protection.

2.2. Inspiratory Hypoxia

Animals were housed either under normoxic ambient conditions (room air; 21% O_2) or in a hypoxic atmosphere containing 8% O_2 (chronic hypoxia) for the whole period of tumor growth. For experiments analyzing the impact of acute hypoxia, animals were housed under normoxic conditions during tumor growth but breathed the hypoxic gas mixture for 20 min prior to and during measurements (e.g., oxygenation measurements).

2.3. pO_2-Measurement

The distribution of tumor O_2 partial pressures (pO_2) was measured polarographically using steel-shafted microelectrodes (outer diameter: 300 µm) and the pO_2 histography system (Eppendorf, Hamburg, Germany)[8]. A small midline incision was made in the skin covering the lower abdomen and the Ag/AgCl reference electrode placed between the skin and underlying musculature. For tumor pO_2 measurement, a small incision was made into the skin covering the tumor and the electrode was then moved automatically through the tissue in pre-set steps with an effective step length of 0.7 mm. Approximately 100 pO_2 values were obtained in less than 20 min from each tumor in up to 8 parallel electrode tracks. The O_2 status of each tumor was described by the median pO_2 and the fraction of pO_2 values $\leq$ 5 mmHg. Additionally, arterial blood gas analysis was performed immediately before and after tumor tissue pO_2 measurements using a pH/blood gas analyzer (type ABL 5, Radiometer, Copenhagen, Denmark) to ensure that values for arterial blood gases were within the physiological range during the measurement period.

2.4. Analysis of Perfusion Distribution

In order to assess parameters related to the perfusion distribution within a tumor, the fluorescent dye Hoechst 33342 (Sigma, Deisenhofen, Germany) was injected i.v. (25 mg/kg body weight). For this, the dye was dissolved in isotonic saline (10 mg/ml) and approx. 0.5 ml of this stock solution rapidly injected into a tail vein. Sixty seconds after injection, animals were sacrificed by an overdose of anesthetic and the tumors rapidly frozen in liquid nitrogen. Following subsequent tumor removal, cryosections were prepared. Using fluorescence microscopy (filter set #02, Zeiss, Jena, Germany, excitation wavelength: 365 nm, emission filter: low-pass 420 nm) at low resolution (range of vision