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MODULATION OF SPATIAL O_2 TENSION DISTRIBUTION IN EXPERIMENTAL TUMORS BY INCREASING ARTERIAL O_2 SUPPLY

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Tumor oxygenation has been measured polarographically in s.c. implanted DS-sarcomas on the dorsum of the hind foot of male Sprague-Dawley rats. pO_2 was determined in all 3 spatial dimensions and 3-dimensional pO_2 distributions as well as the mean extent of confluent areas with $pO_2 < 5$ mmHg were calculated. Finally, the effect of elevating arterial pO_2 (by carbogen breathing) as well as of increasing tumor blood flow (by angiotensin infusion) on the spatial pO_2 distribution was analyzed. Depending on the tumor volume, the spatial pO_2 distribution is more or less anisotropic. In smaller tumors, areas with physiological pO_2 values are found adjacent to large hypoxic areas whereas larger tumors are almost completely hypoxic/anoxic. With carbogen breathing, the mean tissue pO_2 is elevated although hypoxia is not eradicated in larger tumors. In small tumors, angiotensin leads to a vasoconstriction of tumor vessels followed by a worsening of tumor oxygenation whereas in large tumors the increased systemic perfusion pressure resulted in an improvement of oxygenation. Thus, carbogen predominately affects pO_2 diffusion by increasing the arterial pO_2 whereas angiotensin influences tumor perfusion and leads to an increased oxygen supply to the tumor tissue.

Tumor tissue pO_2 has been shown to be an important modulator of the response of malignancies to radio- or chemotherapy (1, 2). It has also been demonstrated that oxygenation in fast-growing tumors shows no uniform pattern (3, 4). Most of these studies describe the heterogeneity of oxygenation by the dispersion (expressed by the 10–90% interpercentile range) of the pooled distribution of all measured pO_2 values. With pO_2 histography, a technique is available allowing determination of pO_2 in different regions of the tumor and the measurement of spatial distribution of oxygenation (3).

Several approaches have been studied to reduce chronic and acute tumor tissue hypoxia (5–9) which can be caused by diffusion and perfusion limits within the tumor. Thus, modulation of either of these limiting factors should reduce hypoxia. O_2 diffusion can be enhanced by increases in arterial pO_2 achieved by breathing air with higher O_2 concentrations (e.g., carbogen with 95% O_2 and 5% CO_2) (8). Tumor perfusion can be modulated either by increasing systemic perfusion pressure (e.g., by angiotensin infusion) (9) or by lowering local vascular resistance (e.g., by increasing local pCO_2 which in turn leads to a peripheral vasodilation (10) but this effect may only be observed under conditions of reduced sympathetic innervation which may be found in fast-growing tumors). All of these mechanisms would be expected to influence the oxygenation pattern of a tumor.

The aim of the present study was to analyse 3-dimensional pO_2 distributions in fast-growing experimental tumors and their modulation through modification of the arterial O_2 supply to the tumor.

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Material and Methods

Animals and tumors. Male Sprague-Dawley rats (Charles River Wiga, Sulzfeld, Germany; body weight 253 ± 25 g) were used with experimental tumors growing subcutaneously after injection of ascites cells of DS-sarcoma

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(0.4 ml; approx. 10^4 cells/μl) into the dorsum of the hind foot. Tumors were used in experiments when they reached a volume of between 0.44 and 2.12 ml, 6–10 days following tumor implantation. All experimentation had previously been approved by the regional animal ethics committee.

Surgical procedures. When tumors reached the desired size, rats were anaesthetized with sodium pentobarbital (40 mg/kg i.p., Nembutal, Ceva, Paris, France). Throughout all experiments, animals were laid supine on a heated operating pad and the rectal temperature maintained at 37.5–38.5°C. Animals breathed room air spontaneously. Mean arterial blood pressure (MABP) was continually monitored through connection of the arterial catheter to a Statham pressure transducer (type P 23 ID, Gould, Oxnard, CA, USA).

Carbogen breathing. Carbogen (95% O_2 + 5% CO_2) was flushed around the tracheotomy tube at a flow rate of 2 l/min and animals breathed this gas mixture spontaneously. Gassing started 10 to 15 min before pO_2 measurement or angiotensin infusion and was maintained throughout the measurement period. Arterial blood gas parameters were assessed before commencement of carbogen breathing and immediately before and after tumor oxygenation measurement.

Angiotensin infusion. Angiotensin (Hypertensin, Ciba-Geigy, Wehr, Germany) was dissolved at a concentration of 6 μg/ml in 0.9% NaCl solution and was infused i.v. at an infusion rate of 0.5–2 $\mu g \cdot min^{-1} \cdot kg^{-1}$ body weight using an infusion pump. The infusion rate was chosen to increase the preinfusion mean arterial blood pressure (MABP) by 50 mmHg during the whole observation period. In most cases an infusion rate of 1–1.5 $\mu g \cdot min^{-1} \cdot kg^{-1}$ body weight was sufficient, and pO_2 measurement commenced 5 min after attainment of the target MABP.

Tumor oxygen tension measurements. Tumor O_2 tension values were determined using O_2 sensitive electrodes (probe diameter 250 μm) with stainless steel shafts (of the hypodermic needle type) and pO_2 histography (KIMOC-6650, Eppendorf, Hamburg, Germany; for more details of this method see (3)). A small midline incision was made in the skin covering the lower abdomen and the Ag/AgCl reference electrode was inserted between the skin and the underlying musculature. Calibration was performed in 0.9% saline solution equilibrated with room air or 100% nitrogen gas ($pO_2 = 0$ mmHg) immediately before and after each tumor pO_2 measurement. A small incision was made into the skin overlying the tumor using a 24-gauge needle and the O_2-sensitive electrode advanced to a depth of approximately 1 mm. The electrode was then automatically advanced through the tissue in pre-set steps of 0.4 mm. Most of the tumors were analyzed in two horizontal layers with a vertical distance of between 1.5 and 2.6 mm. Because of the ellipsoid shape of the tumor, the upper layer was smaller than the lower one. In the upper layer, 3 to 6 parallel electrode tracks with a horizontal track-to-track distance of between 1.2 and 2.9 mm, and in the lower layer 5 to 10 tracks with a distance of between 1.0 and 3.1 mm were measured. The coordinates of the penetration point of the pO_2 electrode into the skin in the three spatial dimensions were measured relative to a fixed point using precision calipers. pO_2 studies of individual tumors were generally carried out in less than 20 min. In each tumor a minimum of 80 pO_2 readings was obtained.

Computation and statistical analysis. The oxygenation status was expressed by the median pO_2 of all pooled measurements of a single tumor as well as by the fraction of pO_2 measurements lower than 2.5 mmHg. For visualization, the measured pO_2 values in all tumor layers were interpolated using an inverse-distance weighting algorithm. For quantification of the anisotropism of the spatial distribution of hypoxic measurements, the mean extent of confluent areas with pO_2 values less than 5 mmHg were determined. For this, each measured point in one layer was surrounded by a rectangle. The X-extent of this rectangle was taken as half of the distance to the neighboring electrode tracks and the Y-extent as 0.4 mm, the step size of the electrode movement. The confluent hypoxic area (HA) was defined as the sum of all of these rectangles with pO_2 less than 5 mmHg which have contact with at least one other rectangle with pO_2 values <5 mmHg. Where several such defined areas occur in one tumor separated by better oxygenated regions, the mean extent of all confluent hypoxic areas has been calculated. To compare the extent of the mean hypoxic areas between tumors of different sizes the extent of these regions has been normalized by dividing it by the area of the analyzed tumor layer. Thus, mean relative size of confluent hypoxic regions is expressed as a percentage of the tumor layer size.

All results are presented as mean values $\pm$SEM. Comparisons between the groups were performed with the two-tailed Wilcoxon rank-sum test for unpaired samples.

Results

Since tumor size is a very important factor influencing oxygenation in the experimental system investigated, tumors were divided into two groups of different volume ranges (small tumors with volumes <1.1 ml and large tumors $\geqslant 1.1$ ml). In the untreated tumors, the well-known size dependency of oxygenation was demonstrated. In small tumors ($n = 16$) the mean pO_2 of the pooled data was 21 (± 3) mmHg, the median pO_2 was 14 (± 4) mmHg, the fraction of measured values <2.5 mmHg was 32 (± 6)%, the mean relative extent of confluent hypoxic areas being 15.4 (± 4.8)%. In large tumors, the mean pO_2 was 8 (± 1) mmHg, the median pO_2 2 (± 1) mmHg, the hypoxic fraction 61 (± 4)%, and the mean area of confluent hypoxic regions 24.8 (± 3.3)% (Fig. 1). Especially in

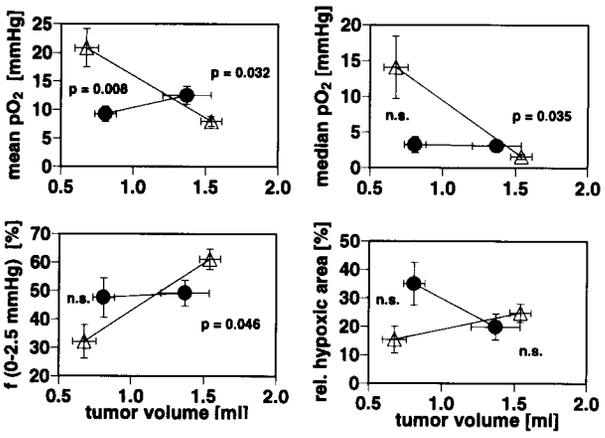

Fig. 1. Size dependency of tumor oxygenation (expressed as the mean and median pO_2, the fraction of O_2 readings <2.5 mmHg, and the mean relative size of confluent hypoxic areas) in the control groups (△) and during angiotensin infusion (●) (values are means ±SEM). Statistical comparisons were performed between the control and angiotensin-treated groups for tumors in a given volume range.

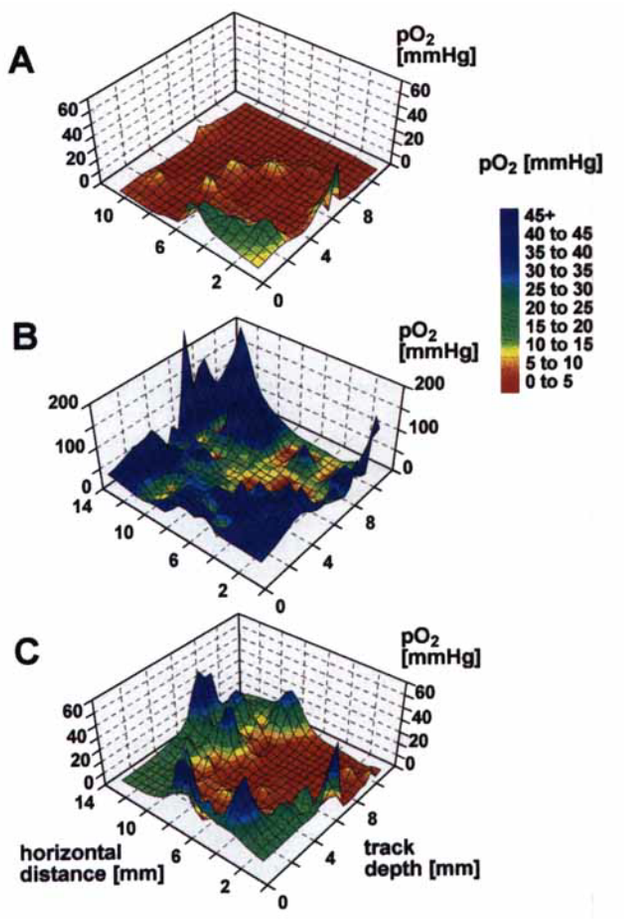

Fig. 2. Example of the reconstructed spatial pO_2 distributions in the lower layers of three large tumors (volume ≈ 2 ml) in (A) control, (B) during carbogen breathing, and (C) with angiotensin infusion. The tumor is displayed in the center graph where a horizontal distance of 0 mm corresponds to the rightmost edge of the tumor and a track depth of 0 mm to the front edge.

the largest tumors (volume ≈ 2.0 ml) the confluent hypoxic area was found to include most of the tumor layer. As an example, Fig. 2A shows the pO_2 distribution in a large untreated tumor where the horizontal distance describes the tumor size from its right to the left edge and the track depth the size from the front to the end of the tumor. This tumor is almost completely hypoxic whereas at the tumor periphery higher pO_2 values were measured. Additionally, some areas with higher pO_2 values which were surrounded by hypoxic areas could also be localized in the more central regions of the tumors.

Carbogen breathing. With carbogen breathing tumor oxygenation was found to improve in both tumor groups, although this improvement was more evident in small tumors. The Table shows the oxygenation parameters for both tumor size groups. In small tumors, hypoxia was almost nondetectable, but in larger ones hypoxic areas were still evident (Fig. 2B).

Angiotensin infusion. With angiotensin infusion, both tumor size groups show approximately the same oxygenation status and the size dependency of oxygenation found in untreated tumors disappears. Fig. 1 shows the oxygenation status of both tumor groups compared to control tumors. In small tumors, oxygenation was slightly worse following angiotensin administration than in control tumors whereas in large tumors a significant improvement in oxygenation was found. As an example, Fig. 2C shows a spatial pO_2 distribution in a large tumor treated with angiotensin. The tumor periphery is better oxygenated than the control whereas the central region of the tumor is nearly completely hypoxic.

Finally, the effect of combining carbogen breathing and angiotensin infusion on oxygenation of small tumors has been analyzed (Fig. 3). In these tumors, angiotensin reduces the beneficial effect of carbogen breathing. Compared to the carbogen group the mean and median pO_2 decrease and the fraction of pO_2 readings <2.5 mmHg as well as the mean confluent hypoxic area increase significantly.

Discussion

The present study has demonstrated that the spatial distribution of the oxygen tension in fast-growing DS-sarcomas, especially in small tumors, is heterogeneous whereas larger tumors are almost completely hypoxic/anoxic (Fig. 2A). However, it must be pointed out that the interpolation algorithm used in the present study could lead to an underestimation of the heterogeneity in these tumors because the distance between neighboring measuring tracks is 1 to 2 mm. If two hypoxic measurement points separated

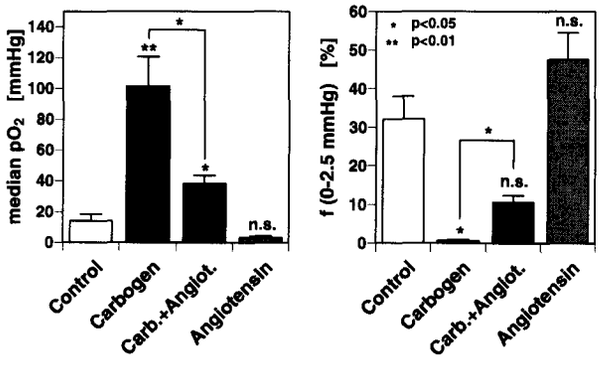

Fig. 3. Tumor oxygenation (expressed as median pO_2 and the fraction of O_2 readings <2.5 mmHg) in small tumors (volume <1.1 ml) during carbogen breathing (n = 3), angiotensin infusion (n = 8), the simultaneous administration of carbogen and angiotensin (n = 7), and in untreated controls (n = 16) (values are means ±SEM). Statistical comparisons are performed between the control level and the different treatment groups (p-values at the top of the column) as well as between the carbogen breathing group and the animals treated with the combination of carbogen and angiotensin.

by a distance of 1.5 mm are considered then theoretically a better-oxygenated area smaller than the track-to-track distance could be situated between these two points. In this case the interpolating algorithm smoothes the pO_2-profile and could lead to a more homogeneous visual impression.

In the present study, tumor oxygenation shows a strong size-dependency with oxygenation becoming worse with increasing tumor volume (Fig. 1). In addition, oxygenation heterogeneity varies with tumor size. With increasing volume the spatial distribution becomes more uniform with a preponderance of low pO_2 values. The improvement of tumor oxygen supply either by increasing arterial pO_2 or by increasing tumor blood flow, reduces hypoxia and therefore would be expected to result in a greater sensitivity of tumor tissue to radiation (11–13).

The predominant effect of carbogen on tumor oxygenation is an increase of arterial pO_2 which reduces diffusion-limited hypoxia (7). In untreated small tumors oxygenation is very heterogeneous. During carbogen breathing these tumors still show anisotropism with large pO_2 differences within the tumor but on a much higher pO_2 level so that hypoxia is no longer detectable. In large tumors, which are almost completely hypoxic under control conditions (Fig. 2A), carbogen leads to a sufficient oxygenation in some regions of the tumor although large confluent hypoxic areas still remain, mainly in the center of the tumor (Fig. 2B). In the tumor model used in the present study, hypoxia can only be completely eradicated by hyperbaric oxygen breathing (5). Additionally, carbogen may reduce perfusion-limited hypoxia by means of CO_2-induced dilation of reactive vessels feeding the tumor. This effect has been seen in laser Doppler flowmetry experiments (preliminary results) where a slight increase in red blood cell flux was measured and is further evidenced by a more pronounced bleeding from the measurement track following removal of the electrode from the tumor.

The effects of angiotensin on tumor blood supply as reported in the literature are somewhat contradictory. In some studies an increase in tumor blood flow (TBF) due to the loss of reactivity to vasoconstrictory agents (14–16) and thus an improvement of tumor oxygenation (9) has been found. Several other studies however showed a reduction of TBF (17) or a variable effect (18, 19) by angiotensin. The present study also reflects this non-uniform effect of angiotensin on oxygenation although here, this effect appears to be strongly correlated with tumor volume. In small tumors a worsening of tumor oxygenation can be observed whereas in larger tumors an improvement can be found. Even so, in large tumors this beneficial effect of angiotensin is less pronounced than during carbogen breathing and large confluent hypoxic areas still remain (Fig. 2C). The completely different behavior of different-sized tumors to angiotensin may result from differences in the vasoreactivity of feeding vessels. In small tumors, a large fraction of reactive host tissue vessels may be present

Table

Effect of carbogen breathing on tumor oxygenation as a function of tumor size (p-values for comparison with untreated tumors)

	Small tumors (<1.1 ml)		Large tumors (≥1.1 ml)	
	Carbogen (n = 3)	Control (n = 16)	Carbogen (n = 6)	Control (n = 15)
Mean pO_2 (mmHg)	118 (±20)	21 (±3)	57 (±7)	8 (±1)
	$p < 0.01$		$p < 0.001$	
Median pO_2 (mmHg)	101 (±19)	14 (±4)	44 (±6)	2 (±0)
	$p < 0.01$		$p < 0.001$	
f (0–2.5 mmHg) (%)	1 (±0)	32 (±6)	5 (±2)	61 (±4)
	n.s.		$p < 0.001$	
Mean rel. hypoxic area (%)	0.3 (±0.3)	15.4 (±4.8)	1.5 (±0.3)	24.8 (±3.3)
	n.s.		$p < 0.001$	

which lead to strong local vasoconstriction and thus to a reduction of TBF. In larger tumors, this fraction of reactive feeding vessels may be lower so that the predominant effect of angiotensin is an increase in systemic perfusion pressure which increases TBF. It may therefore be necessary to take tumor size into account when considering the effect of angiotensin or other vasoactive drugs on TBF or oxygenation.

The detrimental effect of angiotensin on the oxygenation of small tumors restricts its applicability as a radiosensitizing drug, especially since this vasoconstriction cannot be counteracted by the CO_2-induced vasodilation during carbogen breathing. In these small tumors the hypoxia induced by angiotensin infusion due to a reduction of perfusion partly overrides the decrease of diffusion limited hypoxia by carbogen. Further studies are needed to show whether the vasoconstrictive effect of angiotensin can be counteracted by other localized vasodilative therapies (e.g., low-dose local hyperthermia) in order to take advantage of the increased systemic perfusion pressure on TBF and oxygenation.

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