Acta Oncologica Vol. 34, No. 3, pp. 405–408, 1995

THE ROLE OF TUMOR VOLUME IN 'REOXYGENATION' UPON CYCLOPHOSPHAMIDE TREATMENT

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The effect of cyclophosphamide (CP) injection (60 mg/kg i.p., single dose) on volume growth and tissue oxygenation (pO_2 distribution) was investigated in rat DS-sarcomas. CP was administered 4 days after subcutaneous (s.c.) tumor implantation (volume $\sim$ 0.35 ml). Polarographic pO_2 measurements were performed in the subcutis at the hind foot dorsum and in tumors 72 h after CP administration. The oxygenation status of these tissues was compared with that of saline-treated controls. CP-injection caused a mean growth delay of 11 days in DS-sarcomas and had no impact on the oxygenation status of the subcutis. In contrast, in s.c. growing DS-sarcomas the pO_2 distribution improved significantly when treated tumors (0.59 ml volume) were compared with their untreated counterparts (1.15 ml volume). Comparison of the oxygenation data of CP-treated tumors with size-matched controls revealed an identical oxygenation status in the experimental tumors used. Thus, when 'reoxygenation' is discussed, one should consider whether it is solely the result of tumor shrinkage or a volume-independent phenomenon.

Reoxygenation is the process whereby cells that are radiobiologically hypoxic at the time of irradiation become oxygenated thereafter (1). In analogy to this phenomenon, the increase in tissue pO_2 values following chemotherapy was also termed reoxygenation by several investigators (e.g., 2–4) although this term historically has been used to refer to a radiobiological phenomenon. Like standard irradiation, cyclophosphamide (CP) seems to be more toxic to well-oxygenated cells than to their chronically hypoxic counterparts (5) and has been extensively studied in order to clarify whether or not CP is able to induce tumor 'reoxygenation' upon administration of a single dose of this drug.

Summarizing data available in the literature, there is some confusion concerning changes in tumor oxygenation upon CP due to the use of differing doses, routes of application, tumor cell lines investigated, pO_2 measurement protocols following CP, and use of bulky tumors (3, 4, 6). Therefore, in the present study the impact of a single dose administration of CP on tumor tissue oxygenation of a well-established rat tumor line (DS-sarcoma) has been investigated with special emphasis focussing on the comparison of pO_2 data before and after CP treatment with and without size-matching.

Received 6 October 1994.
Accepted 7 December 1994.
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Paper presented in part at the Tumour Microenvironment Workshop in Granada, Spain, September 23–25, 1994.

Material and Methods

Animals and tumors. Sprague-Dawley rats of both sexes (Charles River Wiga, Sulzfeld, Germany; body weight 304 ± 9 g) were used and were allowed access to food and acidified water ad libitum prior to experiments. Experimental tumors were grown subcutaneously (s.c.) after injection of ascites cells of DS-sarcoma (0.4 ml; approx. 10^4 cells/μl) into the dorsum of the hind foot (7). Animals were kept under SPF conditions. All experimentation had been approved by the regional animal ethics committee. Tumor size was estimated by measuring three orthogonal diameters with calipers and calculating tumor volume (V) by the formula $V = \pi/6(d_1 \cdot d_2 \cdot d_3)$.

 ISSN 0284-186X

Drug. Cyclophosphamide (Endoxan 100, Asta Medica, Frankfurt/M., Germany) was dissolved in sterile water and injected intraperitoneally (i.p.) at a single dose of 60 mg/kg 4 days after tumor implantation when the mean tumor volume was approximately 0.35 ml. Controls received comparable volumes of saline (3 ml/kg i.p.).

Tissue oxygenation measurements. Tissue oxygen tension (pO_2) measurements in the normal subcutis and in tumors were performed 72 h after CP administration in anesthetized (sodium pentobarbital, 40 mg/kg i.p., Nembutal, Ceva, Paris, France), spontaneously breathing animals with surgically placed polyethylene catheters in the thoracic aorta and in the right jugular vein. Throughout pO_2 measurements, animals were laid supine on a heated operating pad and the rectal temperature was maintained at 37.5–38.5°C. Mean arterial blood pressure (MABP) was continually monitored through the connection of the arterial catheter to a Statham pressure transducer (type P 23 ID, Gould, Oxnard, Ca., USA). Micro blood samples (50 μl) were used to measure O_2 and CO_2 partial pressures, pH, hematocrit values and hemoglobin concentrations in the arterial blood.

Tumor oxygen 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 (8)). 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 saline solution immediately before and after tumor pO_2 measurements. A small incision was made into the skin overlying the tumor and the O_2-sensitive electrode advanced to a depth of approximately 2 mm. The electrode was then automatically advanced through the tissue in pre-set steps of 0.7 mm. Each rapid forward movement was immediately followed by a backward step of 0.3 mm in order to minimize compression artifacts caused by the forward motion of the O_2-sensitive electrode. This motion pattern led to an effective forward step length of 0.4 mm. Three to five radial electrode tracks were evaluated in each tumor and at the end of each measurement the O_2 probe was automatically removed from the tissue. pO_2 studies of individual tumors were generally carried out in less than 10 min. In each tumor a minimum of 50 pO_2 readings was obtained. Using an on-line computing system, data were pooled for control or CP-treated tumors and pO_2 frequency distributions plotted with class widths of 2.5 mmHg.

pO_2 studies of the subcutis of the contralateral foot in controls and in CP-treated animals served as an 'internal control' for the assessment of intra-laboratory measurement quality.

Statistical analysis. The Wilcoxon-Mann-Whitney U-test was used to test for differences between groups. The significance level was set at $p < 0.05$. Results are presented as mean values ± standard error.

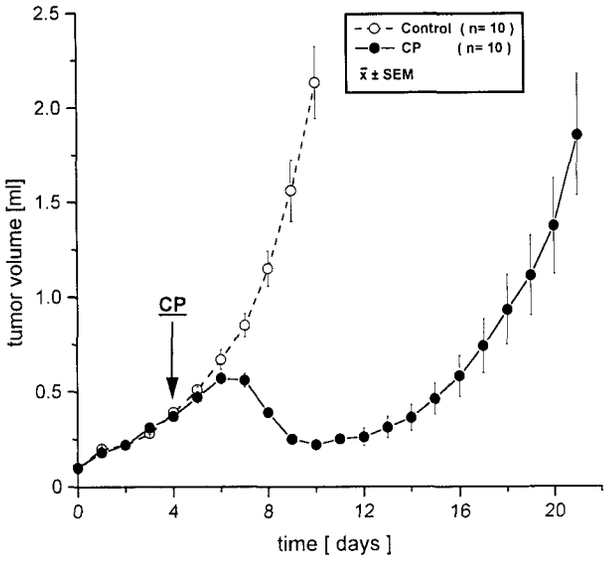

Fig. 1. Volume changes of untreated DS-sarcomas in rats (○) and of tumors treated with cyclophosphamide (CP; 60 mg/kg i.p.) 4 days after tumor implantation (●). n = number of tumors investigated.

Table

Relevant systemic parameters in control and cyclophosphamide (CP)-treated animals at the time of tissue pO_2 measurements. Values are means ± SEM. n = number of animals investigated

	Controls (n = 15)	CP-treated rats (n = 15)
Body weight (g)	311 ± 10	297 ± 9
Arterial pO_2 (mmHg)	77 ± 1	77 ± 1
Arterial pCO_2 (mmHg)	42 ± 1	43 ± 1
Arterial pH	7.44 ± 0.01	7.44 ± 0.01
Arterial sO_2 (%)	95 ± 1	95 ± 1
[Hb] (g/dl)	14.4 ± 0.1	13.6 ± 0.3
Hct (v/v)	0.43 ± 0.04	0.39 ± 0.04
MABP (mmHg)	142 ± 6	138 ± 3

Results and Discussion

Cyclophosphamide (CP) at a single dose of 60 mg/kg i.p. resulted in a mean growth delay of 11 days as shown in Fig. 1 ($p < 0.005$). Volume growth in the CP-treated tumors reached its nadir of 0.2 ml, one week after drug administration. Thereafter, growth resumed and tumors were monitored both in control and in CP-treated animals up until tumor volumes of ~2 ml were reached to avoid too large tumor burdens. Arterial blood gas data (pO_2, pCO_2, pH, oxyhemoglobin saturation sO_2) were in the physiological range in both controls and CP-treated animals (Table). Body weight, hematocrit values (Hct), hemoglobin concentrations ([Hb]) and mean arterial blood pres-

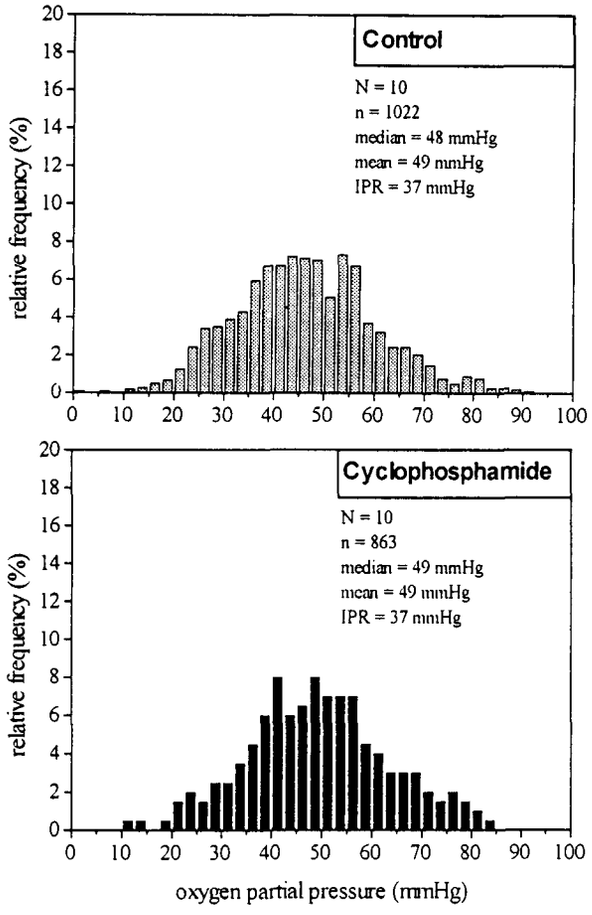

Fig. 2. Frequency distribution of measured pO_2 values (pO_2 histogram) in the subcutis of the hind foot dorsum in the rat. Upper panel: controls, lower panel: measurement performed 72 h following cyclophosphamide treatment (60 mg/kg i.p.). N = number of animals investigated, n = number of pO_2 measurements performed, IPR = 10–90 interpercentile range.

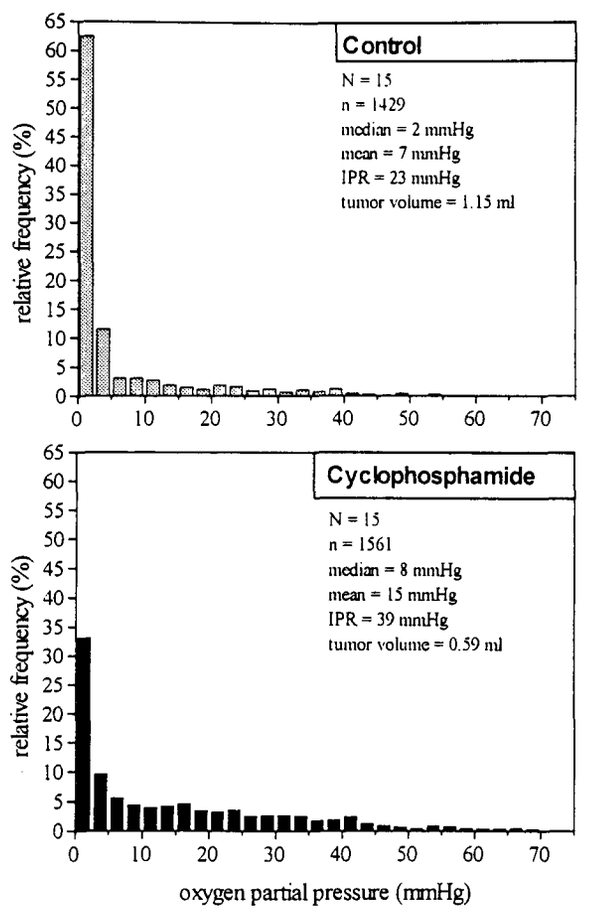

Fig. 3. pO_2 histogram of measured O_2 tensions in control tumors (upper panel) and in tumors 72 h following cyclophosphamide treatment (60 mg/kg i.p., lower panel). N = number of tumors investigated, n = total number of pO_2 measurements performed, IPR = interpercentile range (comparison of tumors without size-matching).

sure (MABP) were slightly (n.s.) lower in the CP-treated group. Effects of a possible CP-induced anemia on tissue oxygenation thus can be excluded in the treatment group.

Cyclophosphamide treatment had no influence on the oxygenation status of the normal rat subcutis at the hind foot dorsum as shown in Fig. 2. Both pO_2 distribution curves were Gaussian with a mean and median pO_2 of 49 mmHg. The 10–90 interpercentile range (IPR) was identical in both groups with practically no pO_2 values below 10 mmHg. Hypoxia was not detectable in the subcutis of control and CP-treated animals.

In contrast, in subcutaneously growing DS-sarcomas, the median and mean tissue pO_2 values were significantly higher ($p < 10^{-4}$) in the treatment group (see Fig. 3). The 10–90 interpercentile range (IPR) was 23 mmHg in the control and 39 mmHg in the treatment group. In control tumors, the fraction of pO_2 values between 0 and 2.5 mmHg (i.e., in the pO_2 range indicating less than half-maximum radiosensitivity) was 63 vs. 33% in the cyclophosphamide group. The data in Fig. 3, however, indicate tumor oxygenation without any size-matching of the malignancies investigated. Control tumors had a mean volume of 1.15 ml whereas in the cyclophosphamide group the mean tumor volume was 0.59 ml. It is well known that the tissue oxygenation status in many experimental tumor systems deteriorates with enlarging tumor mass (9, 10). This is shown in Fig. 4 for the experimental tumor used in the present study (unpublished results). For this reason, the improvement in oxygenation seen may just be the result of tumor shrinkage following CP treatment. This notion is supported by the data presented in Fig. 4, because the mean pO_2 values (shown by the filled dots; mean volume = 1.15 ml) and of the CP-treated tumors (mean volume = 0.59 ml) fit well into the general trend of decreasing tissue pO_2 values with increasing tumor volumes. Comparison of the oxygenation data of CP-treated tumors with size-matched controls have thus been performed in this study. When these data are compared, results clearly show that CP-treatment has no significant impact on tissue oxygenation in the experimental tumor

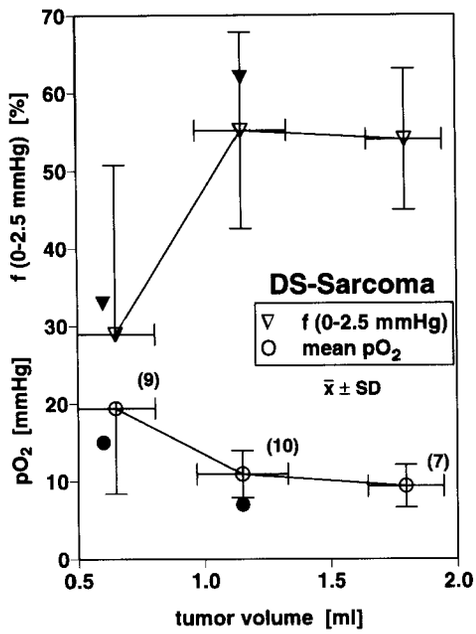

Fig. 4. Mean pO_2 values ($\pm$ SD, circles) and fraction of pO_2 values between 0 and 2.5 mmHg (triangles) in untreated DS-sarcomas of the rat as a function of tumor volume (open symbols, number of tumors investigated in an earlier study are given in brackets). Data obtained in this study are added (filled symbols, mean tumor volume in control tumors = 1.15 ml, mean tumor volume in the cyclophosphamide treated group = 0.59 ml).

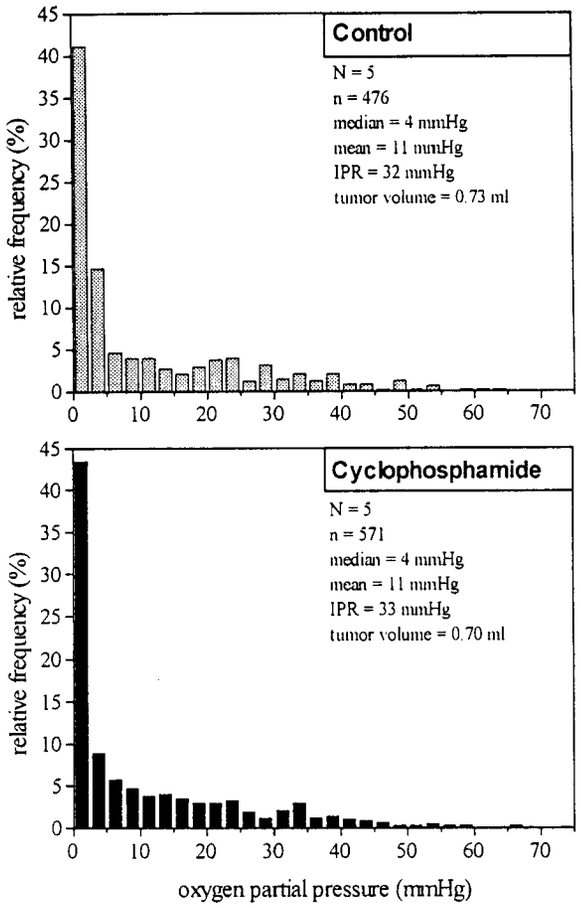

Fig. 5. pO_2 histogram of measured O_2 tensions in control tumors (upper panel) and in tumors 72 h following cyclophosphamide treatment (60 mg/kg i.p., lower panel). N = number of tumors investigated, n = total number of pO_2 measurements performed, IPR = interpercentile range (comparison of tumors with size-matching).

system used (Fig. 5). In both groups with mean tumor volumes of ~ 0.7 ml, the median and mean pO_2 values are identical with practically no differences in the interpercentile range and in the fraction of pO_2 values between 0 and 2.5 mmHg. This behavior may indicate that tumor shrinkage upon treatment is the major factor for the 'improvement' ('reoxygenation') of tissue oxygenation observed upon CP-treatment.

Whenever an improvement in tumor tissue oxygenation upon drug treatment is discussed, one should thus consider whether this is solely the result of treatment-related tumor shrinkage or a volume-independent phenomenon.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Krebshilfe (grant No. M 40/91 Va 1).

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