Blood Flow, Oxygenation, and Bioenergetic Status of Tumors after Erythropoietin Treatment in Normal and Anemic Rats

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ABSTRACT

Growth, blood flow, oxygenation, and bioenergetic status of experimental tumors were investigated in normal (control) and anemic animals after administration of recombinant human erythropoietin (rhEPO). DS sarcomas were implanted s.c. onto the hind foot dorsum of Sprague-Dawley rats. Tumor-associated anemia was induced by the development of an i.p. hemorrhagic ascites. rhEPO (1000 IU/kg) was administered s.c. three times per week over 14 days, after which it was found to have significantly increased hematocrit values in both normal and anemic animals. Tumor growth in anemic animals was slower than in normal animals, and rhEPO administration did not influence tumor growth in either group. Tumor blood flow in anemic animals was lower than in control animals and was only increased in larger tumors in animals in which anemia was prevented by prophylactic rhEPO application. Tumor oxygenation, determined using polarographic needle electrodes and oxygen partial pressure histography, was poorer in anemic animals than in normal animals. This reduction could be reversed partially, but not compensated fully by rhEPO treatment in smaller tumors (≤1.4 ml). These changes suggest that rhEPO, by improving tumor oxygenation, may increase the efficacy of standard radiotherapy in anemic animals and may be of use in anemic tumor patients in whom the success of radiotherapy or O2-dependent chemotherapy might be limited by tumor hypoxia.

INTRODUCTION

The aberrant microenvironment found in most experimental rodent and human malignancies can have a profound effect on tumor growth and response to various nonsurgical modalities (see, e.g., Refs. 1–3). For example, the presence of low O2 levels can confer cell protection from sparsely ionizing radiation and some chemotherapeutic agents, and much evidence exists suggesting that the presence of hypoxia in human tumors may be a major reason for the failure of conventional radiation therapy in the local control of malignancies (e.g., Refs. 4–6) and/or may be responsible for the development of an aggressive phenotype (7, 8). This problem may be exacerbated in situations in which the oxygen-carrying capacity of the blood is decreased. One such situation occurring commonly in tumor patients is progressive anemia (e.g., in approximately 50% of stage IV lung and prostate cancers). Its etiology is multifactorial and not always associated with acute or chronic bleeding. Many patients present with anemia before they receive cytotoxic therapy and even if their bone marrow is not invaded by tumor cells (9). In these patients, factors such as deficiency of erythropoietic factors or suppression of erythropoietin, immune or nonimmune hemolysis, hemodilution, hypersplenism with shorter RBC survival, bone marrow infiltration, nutritional causes (iron, folate, vitamin B12 deficiencies), concurrent infections (hemophagocytosis), and paraneoplastic syndromes may play a role.

Numerous studies have investigated the relationship between anemia and response to radiotherapy (for a recent review, see Ref. 10) and suggest strongly that low hemoglobin levels are an indicator of poor prognosis in patients undergoing radiotherapy. Consequently, homologous blood or RBC transfusion is given frequently to severely anemic cancer patients prior to radiotherapy. Although several investigations have been undertaken to assess the effects of transfusion on therapy outcome, the results of these clinical studies have not shown convincingly a significant improvement in response to radiotherapy (10). This failure may, in some cases, be explained by the recruitment of patients with already very advanced disease, but immunosuppression associated with homologous blood transfusion must also be considered as playing a detrimental role (11–13). In addition, recent focus on transfusion-related infection risks and on the development of hemosiderosis or alloantibodies has brought about a change in general attitudes toward homologous blood transfusion practice. These last two aspects could be overcome by an autologous blood transfusion. Indeed, the first results of a study in which tumor recurrence after surgical treatment of colorectal cancer in patients receiving either autologous or homologous blood transfusion was compared would tend to support this notion (14). However, the use of autologous blood transfusions cannot be considered as a generally feasible approach in already anemic tumor patients. An alternative to transfusion is the use of erythropoietin, a glycoprotein hormone regulating the differentiation and maturation of RBCs and preventing apoptosis of progenitor cells. This approach to the correction of malignancy-associated anemia has received even more attention since rhEPO became available for clinical use. Recent publications have shown that rhEPO can be used effectively and safely in patients to correct malignancy-associated anemia (15–22). To date, however, no studies are available in which the effects of rhEPO on a range of tumor pathophysiological factors have been investigated. In this study, an animal model of tumor anemia was established, and the effects of rhEPO application on RBC-related parameters and on tumor oxygenation, blood flow, and bioenergetic status were determined, to ascertain whether this hormonal approach causes modifications in these parameters that might have consequences for anemic tumor patients in terms of therapy outcome.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River Wiga, Sulzfeld, Germany; body weight, 160–210 g) housed in our animal care facility were used in this study. They received a standard diet and water ad libitum. Experiments were conducted after authorization by the competent ethics committee according to German federal law.

Drugs

rhEPO (Recormon, purity >98%; Boehringer Mannheim, Mannheim, Germany) was dissolved in buffered saline and administered (1000 IU/kg) three times per week, over 14 days, to animals by s.c. injection. Normal (control) animals received an equivalent volume of isotonic PBS. Studies in
rats have shown that there is no significant production of antibodies against rhEPO over this treatment period.3

Tumors

**Control Group.** Solid tumors were induced on day 7 after commencement of rhEPO or isotonic PBS treatment by s.c. injection of DS sarcoma ascites cells (0.4 ml; approximately 10⁶ cells/μl) into the hind foot dorsum (for additional details, see Ref. 23). The tumors investigated appeared as flat, spherical segments. They replaced the subcutis and corium completely and invariably reached the avascular epidermis. Tumor volumes (V) were calculated by an ellipsoid approximation using three orthogonal diameters (d): V = π/6 × d₁ × d₂ × d₃.

**Anemic Group.** Solid tumors were induced on the hind foot dorsum as described above for control animals. In addition, approximately 10⁶ DS sarcoma cells were injected i.p. on day 9. This induced an anemia that resulted primarily from the development of a hemorrhagic ascites. In the rhEPO treatment group, the development of this anemia could be prevented prophylactically.

Measurements

**Surgical Procedure and Measurement of Blood Parameters.** Final investigations took place in all animals on day 14 after commencement of rhEPO or isotonic PBS treatment. Rats were anesthetized with sodium pentobarbital (40 mg/kg i.p., Nembutal; Sanofi Ceva, Paris, France). Polyethylene catheters were surgically placed into the thoracic aorta via the left common carotid artery and connected to a Statham pressure transducer (Type P 23 ID; Gould, Oxnard, CA) for MABP measurement and into the left external jugular vein (for subsequent anesthetic administration). Throughout all experiments, animals lay supine on a heated operating pad, and the rectal temperature was maintained at 37.5°C. Animals breathed room air spontaneously. At the end of a 30-min stabilization period, arterial microblood samples were taken, and the hematocrit (standard microcapillary method), Hb (cyanmethemoglobin method, kit 124729; Boehringer Mannheim), and RBC count (Neubauer hemocytometer) were investigated. The RBC indices mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular Hb could then be calculated. The arterial blood gas status was assessed using a pH/blood gas analyzer (type 178; Ciba Corning, Fernwald, Germany). The SO₂ of the arterial blood was obtained nomographically (24). Tumors then underwent one of the following measurements:

**TBF.** TBF was estimated using the ¹³⁵Xe clearance technique. The indicator (0.1 ml of a solution of ¹³⁵Xe in saline, 185 MBq/ml; Medigenix Diagnostic, Ratingen, Germany) was applied as a bolus injection through the arterial catheter into the thoracic aorta. A Geiger-counting tube (covered with a 6 μm-thick Hostaphan membrane) was placed over the tumor such that no compression of the tumor surface occurred. Shielding of the surrounding tissue was carried out using flexible lead sheeting such that only the tumor was exposed to the counting tube. The registration of the washout process was performed using a linear rate meter (FIT 1100; FAG Kugelfischer, Erlangen, Germany) connected to a chart recorder (LS 64; Linseis, Selb, Germany). With regard to the full-scale counting rate (f = 3000 cpm), the mean statistical error of the rate meter was up to ±5%. Registration of the washout process was carried out until the registered activity returned to the background level. For evaluation of TBF, the clearance curves were transferred to single-logarithmic paper, and the course of the curves was approximated by exponential functions. An index was then used that takes into account both the initial activity for each exponential function as well as the corresponding half-time (for more details, see Ref. 25). Additionally, a tumor tissue/blood partition coefficient (A) was used, in which the actual hematocrit of blood in the animal undergoing measurement was taken into account (26). TBF was then expressed in ml · g⁻¹ · min⁻¹.

**Tumor Oxygen Tension Distribution.** PO₂ values were determined using polarographic needle electrodes (recessed gold in glass electrode; shaft diameter, 300 μm; diameter of the O₂-sensitive cathode, 12 μm) with stainless steel shafts (of the hypodermic needle type) and PO₂ histography (model KIMO C-6650; Eppendorf, Hamburg, Germany; for more details, see Ref. 27). A midline incision was made in the skin covering the lower abdomen, and the Ag/AgCl reference electrode was placed between the skin and the underlying musculature. Calibration was performed in saline solutions equilibrated with either air or pure N₂ immediately before and after tumor PO₂ measurements. A small incision was made into the skin overlying the tumor, and the O₂-sensitive electrode advanced to a depth of approximately 1 mm. The electrode was then advanced automatically through the tissue in preset net steps of 0.7 mm. These steps consisted of a rapid forward step of 1.0 mm, immediately followed by a retraction of 0.3 mm to minimize tissue compression artifacts. The O₂ probe was removed automatically from the tissue at the end of the PO₂ measurement. Four or five parallel electrode tracks were evaluated in each tumor, and a minimum of 70 PO₂ readings was obtained for each tumor. Using an online computing system, data were pooled for individual tumors and for each treatment group, and PO₂ frequency distributions were plotted with class widths of 2.5 mm Hg (27).

**Metabolite Concentrations.** Tumors and thigh adductor muscle were rapidly frozen in liquid N₂, ground to a fine powder using a pestle and mortar, and subsequently freeze-dried. Thereafter, glucose and lactate concentrations were assayed enzymatically using standard test kits (kits 245158 and 256773; Boehringer Mannheim). For determination of ATP, ADP, and AMP, 2-3-mg aliquots of freeze-dried tissue were extracted with 0.3 M perchloric acid and centrifuged, and the supernatant was neutralized with 2 M KOH. The concentrations of the adenylate phosphates were then determined using reversed-phase high-pressure liquid chromatography at 254 nm. The isotropic separation was performed by a Supersphere Rp 18 end-capped column (250 × 4 mm; Knauer, Berlin, Germany) and a guard cartridge system (5 × 4 mm; Knauer). The mobile phase consisted of 0.05 M NH₄H₂PO₄, 0.01 M tetrabutylammonium hydroxide, and 11.5% acetonitrile (v/v), adjusted to pH 6.4. The flow rate was 0.9 ml/min, and the sample size was 50 μl. Concentrations of all metabolites are expressed as μmol/g tissue wet weight (for a general description, see Ref. 28).

**Statistical Analysis**

Results are expressed as means ± SE with the numbers of experiments indicated in brackets. The significance of the differences between the various groups was assessed using the Wilcoxon test for unpaired samples. Results were considered as being significant if P values were less than 5% (P < 0.05).

**RESULTS**

Hb of the blood in anemic animals was found to be reduced significantly when compared with control animals (from 141 ± 2 to 95 ± 6 g/liter; P < 0.001), thus confirming the induction of a tumor-associated, normochromic, normocytic anemia in the experimental system used (Table 1). Application of rhEPO caused significant increases in Hb in animals of the control (184 ± 2 g/l; P < 0.001) and anemia (143 ± 7 g/l; P < 0.001) groups, thus

<table>
<thead>
<tr>
<th>Hb (g/liter)</th>
<th>Control</th>
<th>Control + rhEPO</th>
<th>Anemic</th>
<th>Anemic + rhEPO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>141 ± 2</td>
<td>184 ± 2</td>
<td>95 ± 6</td>
<td>143 ± 7</td>
</tr>
<tr>
<td>Control + rhEPO</td>
<td>184 ± 2</td>
<td>228 ± 1.2</td>
<td>112 ± 7</td>
<td>283 ± 11</td>
</tr>
<tr>
<td>Anemic</td>
<td>95 ± 6</td>
<td>112 ± 7</td>
<td>79 ± 6</td>
<td>121 ± 8</td>
</tr>
<tr>
<td>Anemic + rhEPO</td>
<td>112 ± 7</td>
<td>112 ± 7</td>
<td>79 ± 6</td>
<td>121 ± 8</td>
</tr>
</tbody>
</table>

**Table 1** RBC-related parameters in rhEPO- and isotonic PBS-treated control and anemic animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control + rhEPO</th>
<th>Anemic</th>
<th>Anemic + rhEPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count (10⁶/μl)</td>
<td>4.0 ± 0.2</td>
<td>4.2 ± 0.3</td>
<td>4.5 ± 0.4</td>
<td>4.7 ± 0.5</td>
</tr>
<tr>
<td>Mean corpuscular volume (fl)</td>
<td>72 ± 2.3</td>
<td>70 ± 2.2</td>
<td>68 ± 2.1</td>
<td>66 ± 2.0</td>
</tr>
<tr>
<td>Mean corpuscular Hb (pg)</td>
<td>218 ± 0.3</td>
<td>228 ± 1.2</td>
<td>228 ± 1.2</td>
<td>228 ± 1.2</td>
</tr>
<tr>
<td>Mean corpuscular cHb (g/liter)</td>
<td>335 ± 2</td>
<td>335 ± 2</td>
<td>335 ± 2</td>
<td>335 ± 2</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.001.  
<sup>b</sup> P < 0.05.
fulfilling the recommended treatment criteria for patients (increase in \( cHb >20 \) g/liter, \( cHb >100 \) g/liter). The tumor-induced anemia could therefore be alleviated completely by the application of \( \text{rhEPO} \).

The MABP and the arterial blood gas and pH status, together with the \( sO_2 \) and \( O_2 \) content of animals in the four experimental groups, is shown in Table 2. Although no significant changes were seen in arterial \( pO_2 \), the MABP in anemic animals was significantly lower than in control animals (\( P < 0.001 \)). This difference was reversed partially after \( \text{rhEPO} \) administration to the anemia group. Additionally, \( \text{rhEPO} \) administration to animals of the anemia group resulted in a slight increase in arterial blood carbon dioxide partial pressure and a small decrease in arterial blood pH. Anemia caused a significant decrease in arterial blood \( O_2 \) content (\( P < 0.001 \)). \( \text{rhEPO} \) administration to either control or anemic animals led to a significant increase in \( O_2 \) content (\( P < 0.001 \)), which tended to mirror those changes seen in \( cHb \). In the case of the \( \text{rhEPO} \)-treated anemia group, the arterial \( O_2 \) content was then no longer significantly different from that of control animals.

Anemia in animals with hemorrhagic ascites was found to have a significant effect on tumor growth. Seven days after implantation, the mean volume of tumors in nonanemic animals was \( 1.25 \pm 0.05 \) ml (\( n = 163 \)), whereas in animals with tumor ascites (treated with \( \text{rhEPO} \) or untreated), the mean volume of tumors was \( 0.75 \pm 0.04 \) ml (\( n = 90; \ P < 0.001 \)). For comparison, tumor volume growth in the different treatment groups is shown in Fig. 1. The application of \( \text{rhEPO} \) had no effect on tumor growth in either the control or anemia group. Because in this tumor model a significant correlation has been found between both tumor volume and tissue oxygenation, and tumor volume and blood flow (29), further investigations in this study were carried out on tumors of comparable size ranges. Thus, in the control group, tissue oxygenation, blood flow, and bioenergetic status measurements were carried out on day 7 after s.c. tumor implantation, when tumors had a mean volume of \( 1.25 \pm 0.05 \) ml (\( n = 163 \)), and in the anemia group on day 10, when tumors had a mean volume of \( 1.39 \pm 0.08 \) ml (\( n = 94 \)).

\( ^{133} \text{Xe} \) clearance was used to measure TBF, and results from these investigations are shown in Table 3. As mentioned above, experiments were carried out at times when the mean tumor volumes for the four experimental groups were comparable. Subsequently, for more detailed analysis, tumors were divided into groups of two volume ranges (\( \leq 1.4 \) ml and \( >1.4 \) ml), such that volume-dependent phenomena could be identified, in addition to changes occurring due to anemia and/or application of \( \text{rhEPO} \). As reported previously for this experimental tumor (29), a correlation between tumor volume and TBF was again found in control animals (\( r = -0.45 \)), whereby TBF decreased with increasing tumor volume. This tumor volume dependency was also evident after \( \text{rhEPO} \) administration to control animals. Application of \( \text{rhEPO} \) to control animals led to a significant reduction of TBF in the group of smaller tumors (\( P < 0.05 \)). A similar trend, although not significant, was also seen in the larger tumors. When the control and anemia groups were compared, TBF in the smaller tumors of anemic animals was found to be significantly lower (\( P < 0.01 \)) than in control animals, although TBF still tended to decrease with increasing tumor volume. Upon \( \text{rhEPO} \) administration to the anemia group, this size dependency was no longer evident.

Oxygen availability to the tumor tissue (calculated as the product of arterial blood \( O_2 \) content and TBF) was higher in tumors of control animals than in untreated anemic animals (Fig. 2; \( P < 0.01 \) for both groups of tumors; lines connecting corresponding data points in Figs. 2, 4, 5, and 6 do not imply a linear relationship in between the respective parameters). \( \text{rhEPO} \) treatment of control animals resulted in no significant changes in oxygen availability. After \( \text{rhEPO} \) treatment of the anemia group, the oxygen availability in the larger tumors was no longer significantly different from that of control animals, whereas, in the smaller tumors, no significant improvement could be achieved.

After pooling of tumor \( pO_2 \) data for each treatment group, cumu-

### Table 2. MABP, arterial blood gases, \( pH \), \( sO_2 \), and oxygen content

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>MABP (mm Hg)</th>
<th>( pO_2 ) (mm Hg)</th>
<th>( pCO_2 ) (mm Hg)</th>
<th>( pH )</th>
<th>( sO_2 ) (saturation %)</th>
<th>( O_2 ) content (ml O2/ml blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43</td>
<td>141 ± 3</td>
<td>76 ± 1</td>
<td>42 ± 1</td>
<td>7.43 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95 ± 1</td>
<td>0.18 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control + ( \text{rhEPO} )</td>
<td>44</td>
<td>143 ± 4</td>
<td>75 ± 2</td>
<td>44 ± 1</td>
<td>7.41 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94 ± 1</td>
<td>0.23 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anemic</td>
<td>28</td>
<td>119 ± 3</td>
<td>73 ± 2</td>
<td>38 ± 1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.40 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>93 ± 1</td>
<td>0.12 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anemic + ( \text{rhEPO} )</td>
<td>26</td>
<td>129 ± 3</td>
<td>75 ± 1</td>
<td>43 ± 1</td>
<td>7.37 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>94 ± 1</td>
<td>0.19 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> \( pCO_2 \) carbon dioxide partial pressure.

<sup>b</sup> \( P < 0.01 \).

<sup>c</sup> \( P < 0.001 \).

<sup>d</sup> \( P < 0.05 \).

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By June 30, 1994, 29 experiments were carried out, with a minimum of 68 tumors.

### Table 3. TBF as measured by the \(^{133} \text{Xe} \) clearance technique in \( \text{rhEPO} \) and isotonic PBS-treated control and anemic animals

<table>
<thead>
<tr>
<th>Group</th>
<th>TBF (ml ( \text{g}^{-1} ) ( \text{min}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor volume ( \leq 1.4 ) ml</td>
<td>Tumor volume &gt; 1.4 ml</td>
</tr>
<tr>
<td>Control</td>
<td>0.85 ± 0.06 (16)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control + ( \text{rhEPO} )</td>
<td>0.73 ± 0.06 (23)</td>
</tr>
<tr>
<td>Anemic</td>
<td>0.58 ± 0.06 (16)</td>
</tr>
<tr>
<td>Anemic + ( \text{rhEPO} )</td>
<td>0.54 ± 0.07 (10)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of tumors is indicated in parentheses.
ERYTHROPOIETIN AND TUMOR OXYGENATION

In the case of the median \( pO_2 \) and the low \( pO_2 \) fraction, application of rhEPO to control animals caused no significant changes (Fig. 5, triangles).

No clear tumor volume-dependent relationship was seen for the tissue concentrations of either glucose (1.66 ± 0.06 and 1.43 ± 0.07 \( \mu \text{mol/g} \), for small and large tumor volume groups, respectively) or ATP (0.73 ± 0.06 and 0.81 ± 0.03 \( \mu \text{mol/g} \), for small and large tumor volume groups, respectively), and no significant changes were observed after the induction of anemia or the administration of rhEPO.

In the case of tumor lactate levels, however, a distinct increase was found with increasing tumor volume in all treatment groups (Fig. 6), with much higher levels of lactate being found in the larger tumors of the untreated anemia group (\( P < 0.05 \)).

**DISCUSSION**

For cancer patients, progressive anemia is a common clinical problem and may be at least partially responsible for poor response to radiotherapy in some patients, because several studies have suggested that low hemoglobin levels are an indicator of poor prognosis in patients treated with radiotherapy (for a recent review, see Ref. 10). Although rhEPO has been shown to be effective and safe for the treatment of malignancy-associated anemia in patients (15—22), no clinical studies are available that show whether rhEPO therapy can improve outcomes in patients with cancer.

In the case of tumor lactate levels, however, a distinct increase was found with increasing tumor volume in all treatment groups (Fig. 6), with much higher levels of lactate being found in the larger tumors of the untreated anemia group (\( P < 0.05 \)).

Comparable effects on tumor tissue oxygenation were again seen when median \( pO_2 \) values were considered (Fig. 4). In control animals, a tumor volume dependence of this parameter was seen, with the median \( pO_2 \) decreasing with increasing volume (\( r = -0.377 \); Fig. 4, circles). The median \( pO_2 \) was decreased additionally in animals with untreated anemia (Fig. 4, diamonds). This reduction could be alleviated partially by rhEPO treatment in the group of small tumors (Fig. 4, squares; \( P < 0.001 \) in both cases). However, the median \( pO_2 \) values were still significantly lower than those found in control animals (\( P < 0.001 \)), indicating that a return to preanemia levels was not possible with the rhEPO treatment protocol used. Likewise, when the fraction of \( pO_2 \) measurements between 0 and 2.5 mm Hg was considered (indicating less than half-maximum radiosensitivity), a similar trend was seen (Fig. 5). In control animals, the size of this fraction increased with increasing tumor volume (Fig. 5, circles). In anemic animals, this size dependency is no longer evident (Fig. 5, diamonds), although with both tumor volume ranges, a significantly larger low \( pO_2 \) fraction is seen than that measured in control tumors (\( P < 0.01 \) in both cases). The administration of rhEPO to the anemia group resulted in a decrease in this fraction for tumors of the smallest volume range (\( P < 0.001 \)), although again a recovery to values found in control, nonanemic animals could not be achieved (Fig. 5, squares).

In the case of tumor lactate levels, however, a distinct increase was found with increasing tumor volume in all treatment groups (Fig. 6), with much higher levels of lactate being found in the larger tumors of the untreated anemia group (\( P < 0.05 \)).

For cancer patients, progressive anemia is a common clinical problem and may be at least partially responsible for poor response to radiotherapy in some patients, because several studies have suggested that low hemoglobin levels are an indicator of poor prognosis in patients treated with radiotherapy (for a recent review, see Ref. 10). Although rhEPO has been shown to be effective and safe for the correction of malignancy-associated anemia in patients (15—22), no clinical studies are available that show whether rhEPO therapy can improve outcomes in patients with cancer.
likelihood of distant metastases (32). Reports of experimental studies are also scarce. In one study by Joiner et al. (33), the effect of rhEPO on radiosensitivity in a mouse tumor model was investigated. Here, rhEPO was found to have no influence on tumor radiosensitivity, although it should be noted that at the time of radiotherapy, only a mild anemic state (hematocrit: ~0.39) had been induced.

In the present study, the animal model used allowed the induction of a moderate tumor-associated anemia (hematocrit: ~0.29, equivalent to a clinically relevant anemia found in many tumor patients) at the time of tumor oxygenation and blood flow measurements. rhEPO at a dose of 1000 IU/kg was shown to cause significant increases in the hematocrit values and in hemoglobin content both in control and anemia group animals bearing DS sarcomas, with a correction to preanemia levels in the latter group.

When tumor growth was investigated, tumor-associated anemia caused a significant slowing of tumor growth, a phenomenon that has also been reported for C3H tumors in mice (34). This correlates with the findings of Tannock and Steel (35), who found a slowing down of tumor growth in animals exposed to respiratory hypoxia. In the present study, however, the tumor-induced anemia cannot be responsible for the inhibition of solid tumor growth, because this inhibition also occurred in animals in which anemia was prevented by prophylactic rhEPO administration. In an animal model, Hartveit and Halle raker (36) have shown that the growth of a solid tumor can be inhibited in the presence of an ascites tumor of the same cell line. In the present study, the malignant ascites may therefore be exerting a similar inhibitory effect. That rhEPO treatment was found to have no significant effect on tumor growth either in control or anemic animals is a significant finding with respect to the use of rhEPO in the clinical setting. Similar findings have been made in vitro, where rhEPO was found to have no stimulating effects on the growth of human tumor cell lines (37–39), although erythropoietin receptors are expressed in improve tumor oxygenation, a parameter that is known to modulate the effects of sparsely ionizing radiation and that has been shown to be a significant and independent oncological parameter for prediction of patient survival and local recurrence (7, 30, 31) and for the
some of the cell lines investigated.\(^6\) In an \textit{in vivo} study, rhEPO was found to cause a small slowing of growth rate in mouse tumors at higher rhEPO doses, although growth of the same tumor line \textit{in vitro} was not affected by the presence of rhEPO (33).

Anemia resulted in a decrease in the \(O_2\) content of arterial blood that could be restored to control values by rhEPO application. When tumor oxygen availability was calculated, a decreased \(O_2\) availability that could be restored to control values by rhEPO administration. When restored partially to control values after rhEPO administration. Anemia resulted additionally in a decrease in the MABP, which was seen in tumors in anemic animals as compared to control animals. In a study by Rojas \textit{et al.} (42), however, anemia (induced by bilateral kidney irradiation given several months before tumor implantation) produced an increase in radiosensitivity that could be enhanced further by RBC replacement, whereas Joiner \textit{et al.} (34) showed that oxygenation and decreased tumor radiosensitivity were nevertheless seen. Reasons for these conflicting findings are at present not apparent.

When anemia was corrected by application of rhEPO in the present study, the deterioration of tissue oxygenation could only partially be reversed in terms of a right shift in the cumulative frequency distribution curves, an increase in median \(pO_2\) values, and a decrease in the hypoxic fraction. Because no increase in TBF was seen in animals of the anemia group in the present study after rhEPO administration, the increased tumor oxygenation can presumably be attributed to an increase in the \(O_2\) content of the arterial blood. Alterations in TBF after rhEPO were only seen in control animals, whereas in the group with tumor volume \(\leq 1.4\) ml, a significant reduction in TBF was seen. This is most likely due to increases in viscous resistance to flow in the tortuous, chaotic tumor vasculature resulting from increased blood viscosity as a consequence of the higher hematocrit, which develops after rhEPO application. Sevick and Jain (43), in a study on the effect of hematocrit on intratumor blood viscosity, showed that blood viscosity increases exponentially as a function of hematocrit, with the "threshold" section leading into the steeper part of the curve occurring at a hematocrit of \(\approx 0.45\). For even small increases in hematocrit over \(0.45\), pronounced increases in blood viscosity occur, and therefore, after rhEPO administration to control animals in this study, in which a hematocrit of 0.59 was obtained, a large increase in viscosity would be expected. Even so, the increased arterial blood \(O_2\) content, which occurs as a result of the increased hemoglobin content of the blood in control animals receiving rhEPO, is sufficient to compensate for the reduced oxygen delivery occurring as a result of the increased resistance to flow (decreased flow), such that the oxygen availability to the tumor tissue is not compromised. This is also seen when tumor oxygenation is measured, and no changes in the form of the cumulative frequency curve or median \(pO_2\) occur for tumors in control animals receiving rhEPO.

When the metabolic parameters measured are considered, the increased lactate levels found with increasing tumor volume, in particular in the larger tumors in untreated anemic animals, suggest a shifting of energy metabolism to glycolysis. In addition, the lack of decreases in tumor ATP levels in this experimental model of anemia suggests that oxygen is not a limiting factor in energy metabolism and that \(O_2\) per se cannot be the principal parameter for the genesis of tumor necrosis, as long as glucose is adequately available, a finding that is in agreement with that of Gerweck \textit{et al.} (44).

In conclusion, the changes in tumor oxygenation seen in animals of the anemia group with smaller tumors after rhEPO treatment suggest that rhEPO administration might be of use in "sensitizing" some tumors to sparsely ionizing radiation and oxygen-dependent chemotherapy in anemic animals, and that this form of anemia correction may be effective in increasing tumor radiosensitivity in some anemic tumor patients in whom the success of radiotherapy might be limited by tumor hypoxia. Additional studies of both an experimental and a clinical nature may be warranted to further elucidate the limitations of the effectiveness of rhEPO in increasing tumor radiosensitivity, especially in terms of tumor mass:body weight ratio.

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ERYTHROPOIETIN AND TUMOR OXYGENATION


Blood Flow, Oxygenation, and Bioenergetic Status of Tumors after Erythropoietin Treatment in Normal and Anemic Rats

Debra K. Kelleher, Ulrike Matthiensen, Oliver Thews, et al.


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