Recombinant human erythropoietin increases the radiosensitivity of xenografted human tumours in anaemic nude mice

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Abstract Purpose: The effect of recombinant human erythropoietin (Epo) on the radiosensitivity of human tumour xenografts growing in anaemic nude mice was studied. Methods and materials: Anaemia was induced by total body irradiation (TBI) of mice prior to tumour transplantation. The development of anaemia was prevented by Epo (1000 U/kg s.c.) given 3 times weekly starting 2 weeks prior to TBI (5 Gy). Epo treatment did not influence the growth rate of the tumours, which were transplanted into the subcutis of the hind leg of mice. Thirteen days after TBI (tumour volume of approx. 40 mm³), a single-dose irradiation (12 Gy) of the tumour was performed resulting in a growth delay with subsequent regrowth of the tumours. Results: In Epo-treated animals the tumour growth delay was significantly longer compared to anaemic mice. However, the radiosensitivity of tumours in non-anaemic animals’ (non-Epo-treated) tumours could not fully be restored. Conclusion: These data give evidence for restored radiosensitivity after correction of anaemia with Epo.

Key words Radiosensitivity · Anaemia · Hypoxia · Erythropoietin · Human tumour xenografts

Introduction

A major factor contributing to radioresistance is tumour hypoxia. Over the last few years it has become clear that experimental tumours contain significant amounts of hypoxic cells (Moulder and Rockwell 1984; Rockwell and Moulder 1990; Stüben et al. 1998) and more and more evidence is accumulating which suggests that hypoxia is relevant in the clinical situation (Overgaard 1989; Nordsmark et al. 1996; Höckel et al. 1993, 1996; Kaanders et al. 1998). For several pathophysiological reasons, anaemia is common in clinical oncology and its incidence also increases with the use of modern neoadjuvant protocols. In this context, chemotherapy-induced anaemia might reduce the efficacy of radiation treatment.

Historical observations have already implicated reduced efficacy of radiation treatments in anaemic patients. This is thought to result from a reduced oxygen-carrying capacity of the blood leading to a decreased arterial O₂ supply to the tumour. If pronounced anaemia is present, one way of reducing tumour hypoxia might be the application of recombinant human erythropoietin (Epo) in order to overcome anaemia-associated radioresistance. A previous experimental study showed that correcting anaemia by Epo treatment can improve tumour oxygenation substantially (Kelleher et al. 1996). Despite the fact that there are several ongoing clinical trials, experimental data on the effects of Epo on radiosensitivity in anaemic tumour models are still scarce. In contrast to previous studies (Thews et al. 1998), we evaluated Epo treatments on the radiation response of human tumour xenografts in mice with radiation-induced anaemia.

Materials and methods

Animals

Nude mice (nu/nu of NMRI inbred background) were used in this study. The mice were obtained from the central animal care facility of Essen University, where breeding was performed under pathogen-free conditions. Animals were housed in the Dept. of Radiation Oncology in laminar air-flow units and had unlimited access to water [supplemented with chlorotetracycline (1.35 g/l) and potassium sorbate (10 g/l) acidified to a pH = 3.0] and a high calorie laboratory diet. The animals entered the experiment at an age of
6–9 weeks. All experimentation had previously been approved by the regional animal ethics committee.

Tumours and transplantation

The rapidly growing glioblastoma HTZ11 cell line established from a biopsy of the primary tumour with a volume doubling time of 1.7 ± 0.3 days, was used for the investigation. Tumour pieces of 2–3 mm were transplanted into the subcutis of the right hind leg of the mice. Tumours were repeatedly characterised by means of DNA content, volume doubling time, and isoenzyme pattern of LDH and GPD (Badach et al. 1989). During the experimental period, no changes in these parameters were observed, confirming the human origin of the tumour.

Tumour growth

Animals were assigned to treatment groups when tumours reached a volume of approximately 40 mm³. Tumour size was measured using two perpendicular diameters 2–3 times a week and tumour volume was calculated as

\[ V = \frac{a \times b^2}{2} \]

where \( a \) and \( b \) are the long and the short axes, respectively.

Anaesthesia

Details of the experimental setting used for the irradiation treatments have been described previously (Stüben et al. 1994). Briefly, mice were positioned concentrically to the midpoint of the experimental set-up, spontaneously breathing an anaesthetic gas mixture through openings in the distributor. Enflurane (Etherean) was circulated by a membrane pump and was mixed with air. For further details of the anaesthetic procedure see Ang et al. (1982). A decrease in body temperature during anaesthesia was avoided by surrounding the animal gently with a Perspex tube. In addition, two thermostatically controlled fan heaters were positioned at a distance of 40 cm to the experimental setting during irradiation.

Induction of anaemia

Animals received total body irradiation (TBI) at a dose of 5 Gy (5 MeV photons generated by a linear accelerator at a dose rate of 2.5 Gy/min), 6 h prior to tumour transplantation. The focus isocenter distance was 100 cm with field sizes of 20 × 20 cm² at the isocenter.

Tumour irradiation

The tumour-bearing mouse legs were irradiated with 5 MeV photons generated by a linear accelerator at a dose rate of 2.5 Gy/min. The focus isocenter distance was 100 cm with field sizes of 3 × 2 cm² at the isocenter. The remainder of the animal body was shielded from the direct beam such that the animals were mainly exposed to scattered radiation. The whole body dose of mice was 8% of the total tumour-absorbed dose.

Based on previous experiments which showed the nadir of the haemoglobin content 12–14 days after TBI, the single-dose irradiation of the tumour was applied 13 days after transplantation. At that time, the tumours reached a volume of approx. 40 mm³ (40.7 ± 3.4) mm³. The single dose of radiation delivered was 12 Gy.

Blood cell count

Erythrocyte and leucocyte parameters were assessed using a multiparameter, automated haematology analyser (Coulter MD II, Coulter, Fla., USA). All measurements were performed using a sample of venous blood (100 µl) from the retrobulbar plexus.

Erythropoietin treatment

The development of anaemia was prevented by Epo (1000 U/kg s.c.) given 3 times weekly starting 2 weeks prior to the TBI. Epo treatment was continued for 4 weeks (13 injections).

Experimental design

The experiments consisted of six groups, as detailed in Table 1.

Statistical analysis

Results are expressed as means ± standard error of the mean (SEM). Differences between the groups were assessed by two-tailed Wilcoxon test for unpaired samples. The significance level was set at \( z = 5\% \) for all comparisons.

Results

Total body irradiation resulted in a substantial anaemia in the nude mice. With a TBI dose of 5 Gy the initial haemoglobin level dropped, typically reaching a nadir at days 12–14 after TBI. All animals recovered from the radiation-induced anaemia. Haemoglobin concentrations (Hb) and haematocrit values measured at the time of tumour irradiation are given in Table 2.

Figure 1 illustrates the volume growth curves of non-irradiated tumours in control, anaemic, and Epo-treated animals, respectively. Regardless of the haemoglobin concentration of animals all tumours had comparable growth characteristics.

The single-dose irradiation of the tumour with a dose of 12 Gy resulted in a significant tumour growth delay in all experimental groups (see Fig. 2). Tumours growing in anaemic animals were significantly less sensitive to irradiation compared to control animals (24 ± 3 days to reach 4 times the initial tumour volume in anaemic mice compared to 42 ± 2 days in control animals). The prevention of anaemia by Epo-treatment resulted in a significantly improved radiosensitivity compared to tumours of anaemic mice (36 ± 3 days to reach 4 times the initial tumour volume in Epo-treated mice compared to 23 ± 3 days in anaemic animals). However, the radiosensitivity of tumours growing in animals in which

<table>
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