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PENTOXIFYLLINE INCREASES RIF-1 TUMOUR pO_2 IN A MANNER COMPATIBLE WITH ITS ABILITY TO INCREASE RELATIVE TUMOUR PERFUSION

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The time course and dose response of 600 mm^3 subcutaneous RIF-1 tumours to pentoxifylline was measured in terms of relative tumour perfusion, assayed by ^{86}Rb extraction, and pO_2 distribution measured by the Eppendorf histograph. Both perfusion and pO_2 distribution were maximally increased by 20 mg/kg pentoxifylline 15 min after administration, perfusion to 141 ± 15% (2 SE) of control, and median pO_2 from 3 to 15 mmHg, with the percentage of values < 2.5 mmHg falling from 44 ± 8% to 22 ± 7%. Fifteen minutes after administration the pO_2 increases were linearly dose-dependent up to 20 mg/kg. Correlation coefficients for perfusion data with the percentages of low values were, for time course and dose response data respectively, 0.76 and 0.84 for median pO_2, 0.84 and 0.97 for percentage < 2.5 mmHg and 0.81 and 0.87 for percentage < 10 mmHg. The data show good correlation between changes in perfusion and pO_2 distribution parameters, with better correlation for percentage of low values than for median pO_2.

Pentoxifylline is a rheologically active drug known to decrease blood viscosity by a variety of mechanisms. These include increasing the deformability of both white and red blood cells, particularly when they have been subjected to stiffening conditions, and the inhibition of platelet aggregation (1). It has been used to treat peripheral vascular insufficiency (1) and is under investigation as a potential radiosensitiser for clinical application. Radiosensitisation of tumours by pentoxifylline has now been demonstrated in several murine systems, including RIF-1 (2), SCK (3) and FSaII (4) together with increases in tumour oxygenation (3–5), blood flow (5) and relative perfusion (2), providing strong evidence that the radiosensitisation is due to improvement of the oxygenation status. We have previously shown good correlation between the effects of doses of 5 to 100 mg/kg pentoxifylline on RIF-1 relative tumour perfusion and its response to 25 Gy X-rays (2). The aim of the present study was to measure the time-course and dose-response of the effect of the drug on RIF-1 pO_2 distribution, and to assess the degree of correlation of the pO_2 distribution changes with changes in relative perfusion.

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Material and Methods

Tumour system. The RIF-1 tumour was maintained in tissue culture according to the protocol of Twentyman et al. (6). Tumours were initiated in female 10–16-week-old C3H mice of 24–30 g by inoculation of $2–4 \times 10^5$ tumour cells subcutaneously in the lower back, at the base of the tail. Tumour volume in mm^3 was approximated from $\pi abc/6$, where a, b and c are three mutually perpendicular diameters of the shaved tumour in mm. Tumours were treated at a mean volume of 600 mm^3 (SD 100 mm^3). All experiments were carried out in compliance with the UKCCCR guidelines on the welfare of animals in research (7) under Home Office project license number PPL 80/00845.

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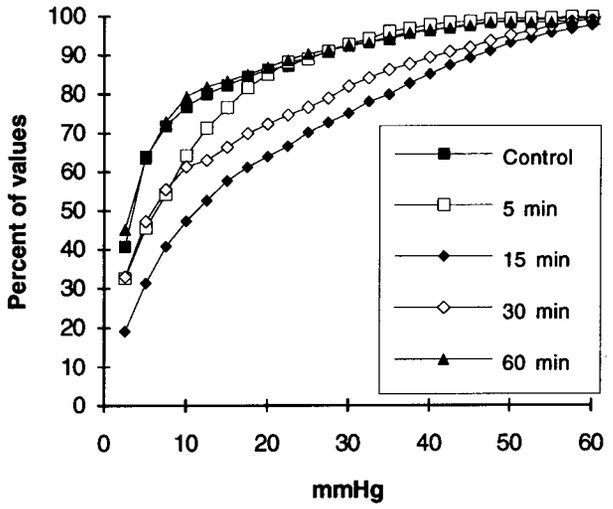

Fig. 1. Cumulative histograms of pO_2 distributions in RIF-1 tumours treated with 20 mg/kg pentoxifylline measured at times from 5 to 60 min after drug administration. Data from two independent experiments were pooled for each treatment; n = 11–27 animals per group.

Pentoxifylline treatment. Pentoxifylline [1-(5′-oxohexyl)-3,7-dimethylxanthine] was kindly provided by D.C. Vaughan, Hoechst U.K. Ltd. It was prepared daily in phosphate buffered saline (PBS) and injected intraperitoneally in 10 ml/kg. Control animals received 10 ml/kg PBS.

Measurement of relative tumour perfusion (RTP)

Relative perfusion of tumour and muscle, as a proportion of cardiac output, were measured by the ^{86}Rb extraction technique (8), as previously described (2), injecting approximately 8 μCi $^{86}RbCl$ intravenously and allowing 60 s for distribution. The percentage uptake of circulating isotope per gram of tissue was calculated for each sample, means for each group were calculated and expressed as a percentage of values for control tumours. Groups were compared using an unpaired, two-tailed t-test.

Oxygen measurement. Tumour oxygen partial pressure (pO_2) was measured using the Eppendorf polarographic oxygen electrode system, KIMOC 6650, as previously described in detail (9). The reference electrode (anode) was attached to the shaved ventral surface of the mouse by means of a trimmed down paediatric adhesive sensor. The cathode, contained in the recessed tip of the bevelled 300 μm probe, was inserted into the tumour, making a small hole in the skin overlying the tumour with a 25 gauge needle if necessary. The tip was manually advanced 1–2 mm into the tumour tissue and was then automatically advanced with 1.0 mm forward steps, each with a 0.3 mm retraction, giving a net forward movement of 0.7 mm before each reading. Path lengths were typically 10 mm but were modified according to the geometry of individual tumours if necessary. Four tracks were used for each tumour, accumulating 50 to 60 readings per tumour, with all tracks starting from almost the same point but taking different directions. Central tumour temperatures

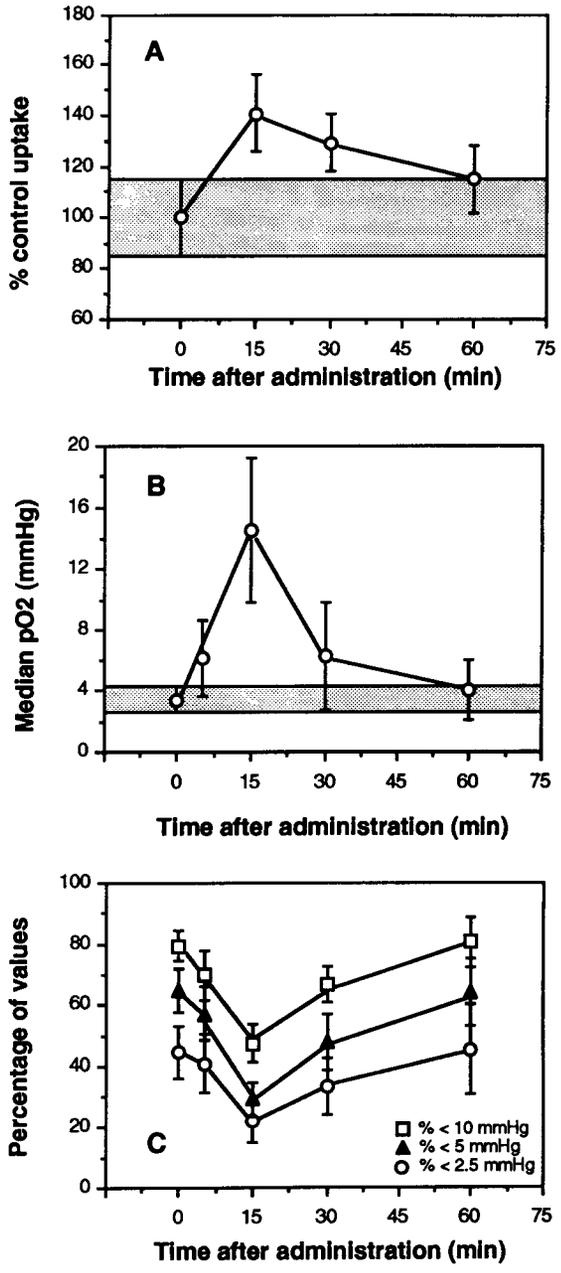

Fig. 2. The time course of the effect of 20 mg/kg pentoxifylline on perfusion (A measured by ^{86}Rb extraction) and oxygen distribution (B and C, measured by polarographic electrode) in RIF-1 tumours. Data points are means of groups, bars indicate ± 2 SE. A RTP; n = 9–10 animals per point from a representative experiment. B Median pO_2; n = 11–27 animals per point from two independent experiments. C Percentages of pO_2 readings less than 2.5, 5 and 10 mmHg; n = 11–27 animals per point, from the same data sets as are presented in B. Stippled areas in panels A and B show the range of ± 2 SE on control values.

were individually measured before pO_2 measurement using a BAT thermometer with a needle thermocouple. Data collection was completed within 2 to 3 min per tumour. Data were transferred from the Eppendorf histograph to a Macintosh SE/30, and subsequently analysed using Statview software (Abacus Concepts Inc). Median values and percentages of measurements at or below 2.5 mmHg, 5 mmHg and 10 mmHg for individual tumours were calculated. Tests for differences between parameters describing oxygen distributions of different groups were carried out using an unpaired, two-tailed t-test. Data for all tumours of each treatment group were also pooled and cumulative pO_2 histograms for each group were plotted. Pooled pO_2 distribution data were compared using the non-parametric Mann-Whitney test. All experiments were carried out twice or three times.

Results

Cumulative pO_2 histograms for pooled groups of tumours treated with 20 mg/kg pentoxifylline for increasing exposure times are shown in Fig. 1, indicating a relatively small shift in the distribution 5 min after administration, with a decrease in the proportion of values below 20 mmHg, but no change at higher pO_2 values. The greatest effect was observed at 15 min after administration, with a substantial reduction in the proportion of low values, e.g., from 64% to 31% of values less than 5 mmHg, commensurate with the shift in the distribution towards higher pO_2 values over the range 0 to 60 mmHg. The data show that at 30 min the shift is considerably reduced, and by 60 min the distribution is indistinguishable from that of controls.

Data for the time course of the effect of 20 mg/kg of pentoxifylline on RTP are shown in Fig. 2A, where the maximum increase also occurs at 15 min, with a rise (± 2 SE) of $41 \pm 15\%$ ($p = 0.002$). The effect is still evident at 30 min with a rise of $29 \pm 11\%$ ($p = 0.007$), but is no longer significant by 60 min ($p = 0.15$). Relative muscle perfusion remained constant during the measured time course at $82 \pm 10\%$, $84 \pm 14\%$ and $83 \pm 7\%$ at 15, 30 and 60 min respectively, indicating that the rise and fall of RTP were specific to the tumour and were not attributable to a transient change in cardiac output. The pO_2 distribution data presented in Fig. 1 are also presented in Fig. 2 as the mean of the median pO_2 (Fig. 2B) and mean of the percentage of values less than 2.5, 5 and 10 mmHg (Fig. 2C). There is good correlation between the changes in RTP and changes in pO_2 distribution parameters, as shown by the correlation coefficients presented in Table 1. The error bars on the pO_2 distribution data presented in Fig. 2B and C indicate the degree of variation found between identically treated animals; it is clear from Fig. 2B that the variation in median pO_2 between control tumours is less than that for treated tumours, unlike the variation in RTP (Fig. 2A) and the variation in percentage of values less than 2.5, 5 and 10 mmHg (Fig. 2B), which are fairly similar for all groups. This indicates that the differences in pO_2 distribution between treated animals in the same group which contribute to the differences in median value are primarily at pO_2 values higher than 10 mmHg, and therefore may not be very important in terms of radiosensitivity which is more likely to be determined by the proportion of low values.

Fig. 3 shows data for the dose response to pentoxifylline, given 15 min before assay, in terms of pO_2 distribution parameters. There is a linear response for doses up to 20 mg/kg, with no further increase at 50 mg/kg. The changes in shape of the cumulative histogram (not shown) were the same as those found for varying the time of assay (Fig. 1), with the right shift apparent over all pO_2 values from 0 to 60 mmHg at all doses tested. Data for the dose response to pentoxifylline of RTP in the same system have previously been published (2), and show a very similar pattern. Control values (± 2 SE, $n = 11$ or 12) were $100 \pm 11.6\%$, 5 mg/kg resulted in an increase to $130 \pm 32\%$, 10 mg/kg an increase to $130 \pm 27\%$, 20 mg/kg an increase to $157 \pm 28\%$ and 50 mg/kg an increase to $169 \pm 22\%$. Correlation coefficients for the comparison of the dose response perfusion data with pO_2 distribution data are shown in Table 1, and show good correlation. The correlation coefficients are higher for the percentages of low values than for the median pO_2 values in both data sets.

The effect of pentoxifylline on tumour temperature is presented in Table 2. An increase in temperature of approximately 0.5°C was observed 15 min after 20 mg/kg pentoxifylline which was significant at the 2% level in both data sets.

Table 1

Correlation coefficients for relative tumour perfusion data (RTP) compared with pO_2 distribution parameters

Experiment	Data sets compared	Correlation coefficient (R^2)[1]
Time course data (0, 15, 30, 60 min)	RTP v median pO_2	0.76
	RTP v % < 2.5 mmHg	0.84
	RTP v % < 5 mmHg	0.87
	RTP v % < 10 mmHg	0.81
Dose response data (0, 5, 10, 20, 50 mg/kg)	RTP v median pO_2	0.84
	RTP v % < 2.5 mmHg	0.97
	RTP v % < 5 mmHg	0.87
	RTP v % < 10 mmHg	0.87

[1]Pearson correlation

Discussion

The time course of the effect of pentoxifylline in increasing both RTP and pO_2 distribution, with a maximum

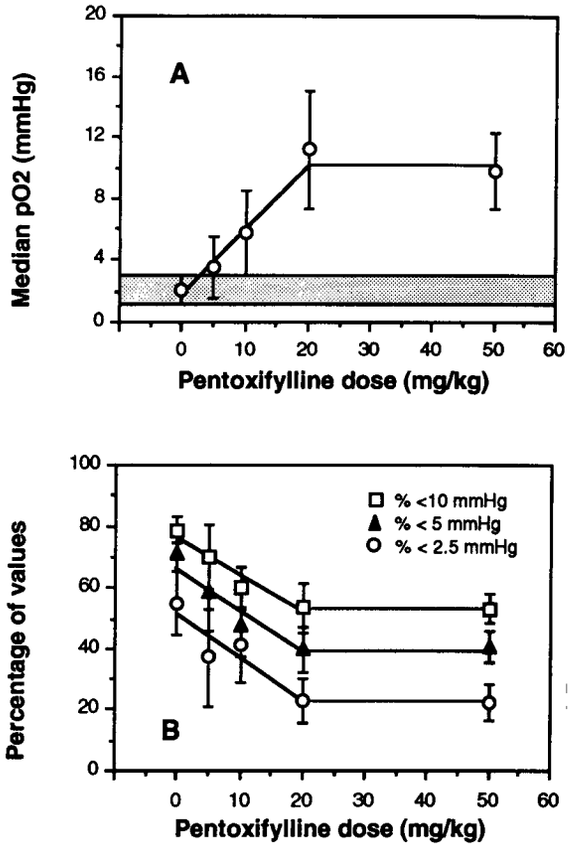

Fig. 3. The effect of varying doses of pentoxifylline on the pO_2 distribution of RIF-1 tumours, measured 15 min after drug administration. Data points are means of groups, bars indicate ± 2 SE. A Median pO_2; n = 9–27 animals per point, from three independent experiments. B Percentages of pO_2 readings less than 2.5, 5 and 10 mmHg; n = 9–27 animals per point, from the same experiments as are presented in A. Stippled area in A shows the range of ± 2 SE on control values.

effect at 15 min after drug administration, is in keeping with the pharmacokinetic profile of the drug in mice, and suggests that the effect is closely linked to the concentrations of the drug and its active hydroxy metabolite. Peak plasma concentrations of both occur 5 min after intraperitoneal injection, and both are eliminated with a half-life of 5 to 6 min (2), hence the maximum effect on pO_2 occurs when plasma concentrations are reduced to around 12% of the peak. Tumour concentrations are, however, likely to lag behind plasma concentrations and so the effect may be dependent either on tumour drug concentrations, or on the critical cells in the blood having been exposed to the active drug for sufficient time for rheological changes to be expressed.

Table 2

Mean central tumour temperatures following pentoxifylline treatment

Pentoxifylline treatment	Mean tumour temperature (°C) ± 2 SE		p-value (for t-test against control)
Time course data at 20 mg/kg	Control	35.3 ± 0.28	~
	5 min	34.9 ± 0.42	0.10
	15 min	35.8 ± 0.28	0.02
	30 min	35.7 ± 0.28	0.10
	60 min	35.1 ± 0.44	0.49
Dose reponse data at 15 min	Control	35.9 ± 0.28	~
	5 mg/kg	36.2 ± 0.26	0.17
	10 mg/kg	36.3 ± 0.25	0.12
	20 mg/kg	36.3 ± 0.18	0.02
	50 mg/kg	35.6 ± 0.37	0.23

The increase in tumour temperature found 15 min after 20 mg/kg pentoxifylline is consistent with the improvement in perfusion and associated efficiency of vascular warming. Although 50 and 20 mg/kg of drug resulted in essentially identical increases in perfusion and pO_2 distribution, 50 mg/kg did not cause an absolute increase in tumour temperature (p for 20 mg/kg v 50 mg/kg = 10^{-3}). This is probably because 50 mg/kg causes a small drop in core temperature of around 0.5°C, which offsets any perfusion-driven increase in tumour temperature, while 20 mg/kg does not affect core temperature (2).

The present data show a close correlation between the observed increases in RTP and pO_2 distribution. Tumour pO_2 is measured as the distribution of multiple measurements of volumes of 5×10^{-4} mm^3 throughout each tumour and requires an adequate number and spatial distribution of the measurements for the sampling to be representative of the entire tumour. The relative proportion of low pO_2 values is generally thought to be a more likely determinant of radiosensitivity than other parameters describing the pO_2 distribution, e.g. the median. The perfusion estimate however is a true volume average of the isotope uptake by the tumour. It is interesting that better correlation was consistently found between perfusion and the proportion of low pO_2 values than between perfusion and the median pO_2 value (Table 1), adding weight to the hypothesis that perfusion limitation is the reason for the low pO_2 values.

A correlation between response to a single dose of 25 Gy and increases in tumour perfusion induced by pentoxifylline over the dose range 0 to 50 mg/kg has already been demonstrated for this tumour system (2). The present data showing the close correlation between tumour perfusion and proportion of low pO_2 values supports the conclusion that the reduction of the proportion of low pO_2 values increases radiosensitivity where it is compromised by hypoxia and points to a potential role for pentoxifylline as a clinically effective radiosensitiser.

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