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Effect of Angiotensin II on Intermittent Tumour Blood Flow and Acute Hypoxia in the Murine SCCVII Carcinoma

Martin J. Trotter, David J. Chaplin and Peggy L. Olive

Effects of the vasoconstrictor angiotensin II on tumour microvascular perfusion and oxygenation were examined in the murine SCCVII carcinoma grown subcutaneously in C3H/He mice. Angiotensin II infusion (2 μg/kg/min) caused an increase in mouse arterial blood pressure from 85 (2) mm Hg (mean, S.D.) to 112 (7) mm Hg. During drug infusion, tumour red blood cell (RBC) flow (measured by laser doppler flowmetry) increased 206 (50%) ($P < 0.001$) in unanaesthetised animals and 305 (90%) ($P < 0.001$) in mice immobilised with ketamine and diazepam. As assessed using a fluorescent double-staining technique, angiotensin II reduced staining mismatch (indicative of intermittent blood flow) in SCCVII microvasculature from 8.1 (2.5%) of total vessels to 2.0 (1.3%) ($P < 0.001$). However, a large proportion of this reduction could be attributed to volume loading. Angiotensin II reduced but did not completely eliminate the radiobiological acute hypoxia which results from intermittent tumour vessel non-perfusion. We propose that angiotensin II improves tumour microcirculatory flow distribution via its systemic actions, by elevating perfusion pressure, thereby preventing collapse and/or temporary flow stasis in tumour microvessels.
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INTRODUCTION

SELECTIVE MANIPULATION of tumour blood flow using vasoactive drugs has been extensively investigated as a means to improve conventional methods of cancer diagnosis and treatment [1]. Modulation of tumour blood flow is undertaken with the goal of reducing or eliminating perfusion heterogeneity, a factor considered detrimental to successful radiation therapy and delivery of chemotherapeutic agents. Increases in tumour perfusion, especially if they produce a homogeneous flow distribution, could result in improved tumour oxygenation and drug delivery.

Tumour blood flow is very sensitive to changes in systemic blood pressure [2, 3]. Flow autoregulation is apparently absent; at perfusion pressures > 40 mm Hg, tumour blood flow is linearly related to mean arterial blood pressure [4, 5]. This passive pressure–flow relationship, together with evidence that vascular smooth muscle and adrenergic innervation are often

poorly developed or absent in tumour neovasculature [6], suggests that elevation of systemic blood pressure by vasoactive drugs might selectively increase tumour blood flow.

The vasoconstrictor angiotensin II, a potent pressor agent, has been shown, with one exception [7], to increase absolute and relative blood flow in solid experimental tumours [8–11]. Administration of angiotensin II can enhance tumour visualisation during angiography [12], improve the efficacy of systemic chemotherapy [8, 13–16], and selectively increase tumour blood flow under conditions of applied local hyperthermia [11].

The mechanism of angiotensin-induced increases in tumour blood flow is assumed to be indirect, secondary to an elevation of systemic blood pressure [8]. Since angiotensin II causes vasoconstriction via specific receptors on vascular smooth muscle, the contribution of indirect effects and direct effects on tumour vasculature will obviously depend on the number of tumour vessels (likely incorporated host arterioles) which possess such musculature. Angiotensin II has also been shown to increase tumour vascular area [17] and to reduce the macroscopic heterogeneity in tumour blood flow distribution [9]. Methods have not been widely available to measure drug-induced flow changes at the microregional or single vessel level in solid experimental tumours and therefore, an anticipated improvement in blood flow distribution at the microvascular level has not, to date, been investigated. Angiotensin-induced increases in tumour blood flow, if they reduce heterogeneity in microvascular perfusion, especially intermittent tumour blood flow [18], might potentially enhance tumour oxygenation (by eliminating acute hypoxia) and thereby improve the tumour response to radiation therapy.

In this study, we have examined the effect of angiotensin II on microvascular function in the murine SCCVII carcinoma. Measurements of tumour microregional blood flow have been made using a variety of techniques: (1) use of fluorescent markers of functional vasculature to detect vessels subject to intermittent periods of nonperfusion, (2) application of fluorescence-activated cell sorting methods to isolate acutely hypoxic tumour cells resulting from such flow fluctuations and (3) measurement of tumour blood flow using laser doppler flowmetry. Despite methodological restrictions preventing direct intercomparison of the results obtained, we have used these approaches, each an independent assessment of tumour perfusion at the microregional level, to examine the hypothesis that systemic hypertension induced by angiotensin II infusion will increase tumour perfusion, reduce intermittent tumour blood flow, and result in a reduction in the number of radiation-resistant acutely hypoxic tumour cells.

MATERIALS AND METHODS

Drugs

Angiotensin II was obtained from Sigma. The drug was dissolved in phosphate-buffered saline (PBS) (NaCl 120 mmol/l, KCl 2.7 mmol/l, phosphate buffer 10 mmol/l, pH 7.4) at 10 μg/ml and infused intravenously using a microlitre infusion pump (Harvard Microliter Syringe Pump, Harvard Apparatus, South Natick) via a 25-gauge winged infusion needle placed in the lateral tail-vein. Angiotensin II was administered at 6.4 μl/min corresponding to a dose of 2 μg/kg/min. Hoechst 33342, also obtained from Sigma, is a DNA-binding fluorescent stain which emits blue fluorescence when excited by UV light. The stain is a useful *in vivo* marker of perfused vasculature in experimental tumours [19–21]. Hoechst 33342 was dissolved in phosphate-buffered saline (PBS) at 8 mg/ml. For visualisation of tumour vasculature, or for fluorescence-activated cell sorting of disaggregated tumour cell populations, the stain was injected intravenously via the lateral tail-vein at 10–15 mg/kg in 50 μl. The fluorescent carbocyanine derivative 3,3'-diheptyloxacarbocyanine, $DiOC_7(3)$, was purchased from Molecular Probes (Eugene, Oregon). Like Hoechst 33342, $DiOC_7(3)$ can be employed as a perfusion probe in experimental tumours [22]. The stain exhibits green fluorescence when excited by blue light and can be easily discriminated from Hoechst 33342 when both dyes are present in tissue frozen sections examined by fluorescence microscopy. $DiOC_7(3)$ was dissolved in dimethylsulphoxide (DMSO) and then diluted to 75% (v/v) with PBS before use (final concentration 0.6 mg/ml). The stain was administered intravenously in 50 μl via the lateral tail-vein at 1 mg/kg. This dose provided optimal visualisation of tumour blood vessels.

Table 1. Summary of experimental methods

Experimental method	Mice	Tumour site	Anaesthetic	Infusion duration (min)	Volume infused (μl)
Blood pressure	6	None	Halothane	15	96
Laser doppler	5	Back	Ket/diaz	15	96
	5	Foot	None	15	96
Double staining	18	Back	None	0	0
	9(AngII)	Back	None	30	192
	10(PBS)	Back	None	30	192
Irradiation and sorting	6	Back	None	8	50

Ket/diaz = ketamine and diazepam, AngII = angiotensin II, PBS = phosphate-buffered saline.

Mice and tumours

For all experiments, the transplantable murine SCCVII squamous cell carcinoma was used. The SCCVII is a rapidly growing, poorly differentiated malignant tumour composed of a mixture of spindle and epithelioid cells. Details of tumour maintenance and implantation techniques have been reported [23]. Tumours were grown subcutaneously over the sacral region of inbred male or female C3H/He mice (20–30 g weight) and were used for experimentation at a size of 450–700 mg. For laser doppler tumour blood flow measurements in unanaesthetised mice, tumours were grown subcutaneously in the hindfoot dorsum and used at a size of 200–300 mg.

Blood pressure

The effect of angiotensin II infusion on mean arterial blood pressure and heart rate was measured in non-tumour-bearing male C3H/He mice ($n=6$) anaesthetised with halothane, administered continuously, via vaporiser, at 0.5–2.5% mixed with 100% oxygen. Mice were allowed to breathe spontaneously. Rectal temperature was monitored and maintained at 35–37°C using a heating pad. The left femoral artery was dissected free

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and the superficial circumflex iliac branch was ligated. The femoral artery was then catheterised using saline filled PE10 tubing (outside diameter 0.61 mm). Pressure measurements were recorded using a Statham P23D pressure transducer (Gould, Oxnard, California) connected to an amplifier and recorder (General Electric, Liverpool, New York). Heart rate could be read directly from the arterial pressure waveform. Prior to intravenous infusion of angiotensin II, mean arterial blood pressure was titrated to a baseline level of 80–90 mm Hg by adjusting the concentration of inhaled halothane.

Laser doppler flowmetry

Relative changes in tumour or skin blood flow in response to angiotensin II infusion were measured using a laser doppler flowmeter (Laserflo Blood Perfusion Monitor, Model BPM 403, TSI, St Paul, Minnesota). This instrument is capable of monitoring tissue microvascular flow continuously and non-invasively with a spatial resolution of approximately 1 mm^3 [24]. Details of our technique for flow measurement in murine tumours have been reported [22, 25]. Mice bearing tumours subcutaneously implanted over the sacral region require immobilisation to obtain a non-artifactual signal. This was achieved using ketamine (50 mg/kg intraperitoneally) and diazepam (10 mg/kg intraperitoneally) anaesthesia. Measurement of tumour blood flow was also performed using unanaesthetised, restrained mice bearing subcutaneous tumours implanted in the hindfoot dorsum. In both tumour sites, measurements were made from superficial tumour regions using a laser doppler needle probe with a 0.7 mm tip diameter.

Transient tumour perfusion

Spontaneous fluctuations in tumour microvascular perfusion were measured using a double fluorescent staining technique [26]. Simultaneous intravenous administration of Hoechst 34342 and $DiOC_7(3)$ results in labelling, with both stains, of all tumour vessels perfused for the several minutes following injection of the stains, both of which have short distribution half-lives [19, 22]. If, however, $DiOC_7(3)$ is injected 20 minutes after Hoechst 33342, some tumour vessels, in frozen sections examined by fluorescence microscopy, exhibit staining mismatch (marked with one stain but not the other) indicative of intermittent vessel perfusion. This double staining protocol was performed in unrestrained mice (n = 18) and in restrained mice in which angiotensin II (n = 9) or a PBS control (n = 10) were continuously infused prior to, between and after stain injections. The total length of intravenous infusion was 30 minutes (total volume of angiotensin II or saline infused was 190 μl). A 30 minute infusion was required in order to allow the necessary 20 minute interval between injection of fluorescent vascular markers: Hoechst 33342 was given 5 minutes after the start of infusion and $DiOC_7(3)$ was administered 20 minutes later. Animals were killed and tumours removed for histological examination 5 minutes after $DiOC_7(3)$ injection.

Acute hypoxia

Acutely hypoxic tumour cells resulting from transient non-perfusion of tumour blood vessels can be isolated from experimental tumours following X-irradiation using the vascular marker Hoechst 33342 in conjunction with fluorescence-activated cell sorting [18, 23]. We have employed this method to determine the impact of angiotensin II infusion on tumour oxygenation as assessed by the response of radiation tumour cell subpopulations defined with Hoechst 33342.

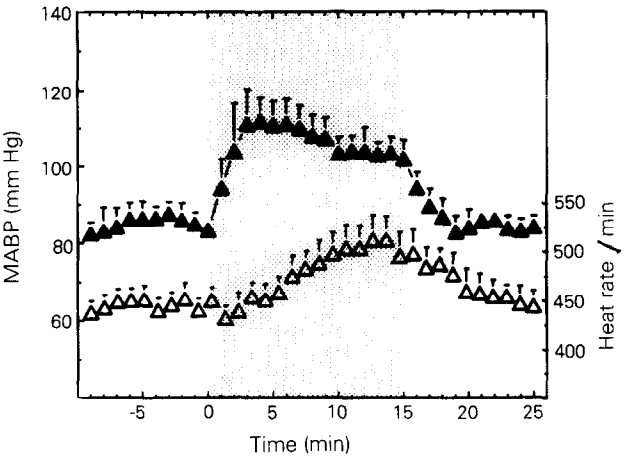

Fig. 1. Effect of angiotensin II infusion (2 μg/kg/min) on mean arterial blood pressure (MABP) (▲) and heart rate (△) in 8-week-old male C3H/He mice anaesthetised with halothane via vaporiser [mean (S.D.), n = 6]. During angiotensin II infusion the blood pressure was significantly increased over pretreatment levels ($P < 0.001$). Angiotensin II also significantly increased heart rate ($P < 0.005$).

Subcutaneous SCCVII tumours were locally irradiated using 270 KVp X-rays at a dose rate of 1.5 Gy/min. Mice were restrained but unanaesthetised during the 7 minutes required to deliver 10 Gy at this rate. Hoechst 33342 was injected 20 minutes prior to irradiation. Immediately after irradiation, mice were killed and tumours were excised, enzymatically dissociated into a single cell suspension and analysed for Hoechst 33342 fluorescence intensity using a fluorescence-activated cell sorter (FACS 440, Becton-Dickinson) [18, 23]. In the present study, cells were separated on the basis of Hoechst 33342 fluorescence intensity into two sorted fractions representing the brightest and dimmest 10% of cells. An "all sort" fraction was also collected to measure the average response of tumour cells to treatment. Following sorting, tumour cell viability was assessed using a soft agar clonogenic assay [23]. Cells brightly stained with Hoechst 33342 are those located in close proximity to tumour vessels perfused during the short period that the stain is present in the circulation. Such cells should in most instances display an oxic response to radiation, that is, the cells should be radiosensitive relative to dimly stained cells located more distant from the blood supply. If transient vessel non-perfusion occurs such that tumour vessels perfused at the time of Hoechst 33342 injection are subsequently non-perfused during the period of irradiation, then brightly stained cells may exhibit a hypoxic radiation response. Such cells are thus considered to be subject to acute, perfusion-limited hypoxia.

To examine the effect of angiotensin II on acute hypoxia in the SCCVII tumour, the drug was infused intravenously beginning 1 minute prior to and continuing throughout irradiation (n=6; total infusion time 8 minutes; infusion volume 50 μl).

RESULTS

Intravenous infusion of angiotensin II in anaesthetised mice caused an increase in arterial blood pressure from a pretreatment value of 85 (2) mm Hg to a maximum pressure of 112 (7) mm Hg [mean (S.D.), $P < 0.001$]. A significantly elevated blood pressure was sustained for the duration of drug infusion (Fig. 1). Heart rate also increased, although with a delayed time course, from 446 (5) beats per minute (bpm) to 518 (60) bpm ($P < 0.005$).

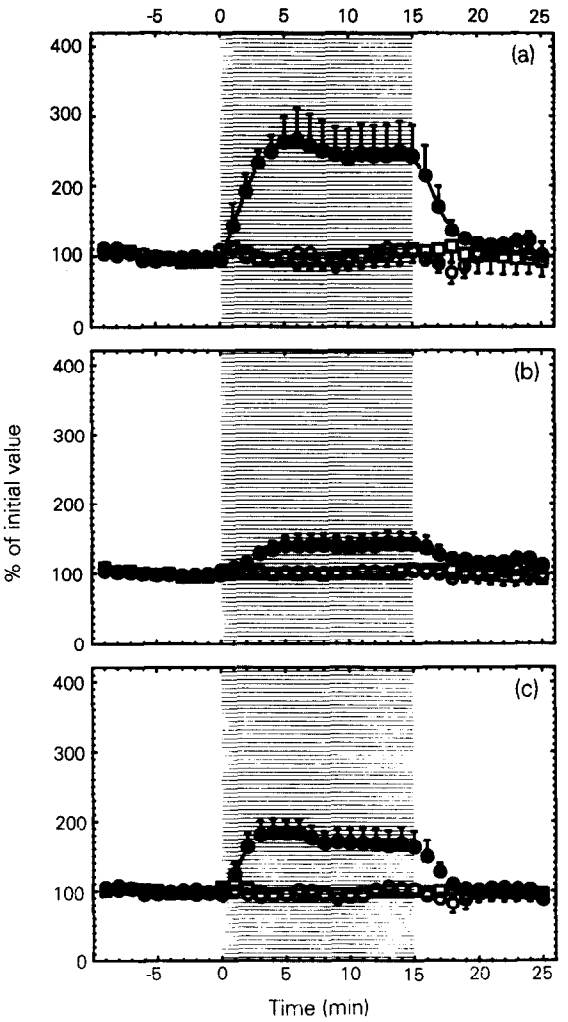

Fig. 2. Effect of angiotensin II infusion (shaded area) on RBC flow (a), number of moving RBCs (indicative of functional microvascular volume) (b), and mean RBC velocity as assessed by laser doppler flowmetry (c). Results are shown for tumour (●) and for normal skin adjacent to the tumour site (□). The effect of PBS infusion on tumour RBC flow is also indicated (○). Error bars represent S.E. for 5 mice per treatment.

Angiotensin II infusion resulted in a 2–3 fold increase in SCCVII tumour (RBC) flow as measured by laser doppler flowmetry (Fig. 2). The maximum tumour flow increase occurred 2–7 minutes after starting angiotensin II infusion and, in anaesthetised mice bearing subcutaneous back tumours, this maximum flow was 305 (90)% (range 169–386%) of pretreatment values ($P < 0.001$). In unanaesthetised, restrained mice bearing foot tumours, RBC flow increased to a maximum value of 206 (50)% (range 150–270%). This difference between back tumour (with anaesthesia) and foot tumours (no anaesthesia) was significant ($P < 0.01$). The elevation in RBC flow observed was due both to an increase in the number of moving RBCs [157 (35)% of normal] and an increase in mean RBC velocity [199 (44)%]. Interestingly, the number of moving RBCs, indicative of functional microvascular volume, remained slightly elevated relative to pretreatment values even 10 minutes after cessation of angiotensin II infusion [117 (4)%, $P < 0.001$]. Infusion of saline had no effect on tumour RBC flow and angiotensin II had no significant effect on blood flow in normal skin.

An estimation of the pressure-flow relationship for tumour vasculature during angiotensin II infusion was obtained by

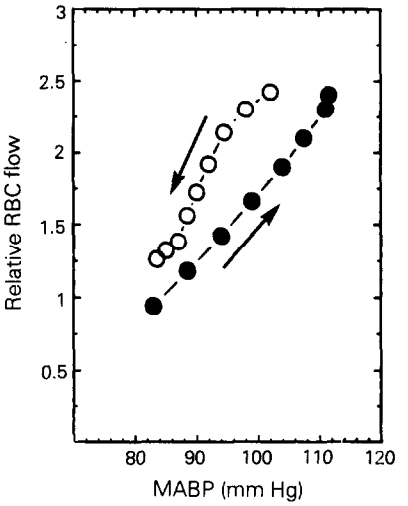

Fig. 3. Relationship of tumour RBC flow to mean arterial blood pressure changes induced by angiotensin II infusion. (●) = increasing blood pressure and (○) = decreasing blood pressure (following cessation of drug infusion). Linearity of pressure–flow relationship suggests lack of flow autoregulation in SCCVII tumour tissue.

plotting relative tumour RBC flow changes measured in anaesthetised mice using laser doppler flowmetry against mean arterial blood pressure measured in a separate experiment. Pressure-flow curves for conditions of increasing blood pressure at the start of infusion and decreasing pressure after infusion ceases are shown in Fig. 3. The linearity of this relationship suggests absence of autoregulation. Some hysteresis was noted; that is, for a given blood pressure, flow was higher *after* angiotensin II infusion than when pressure was increasing at the start of infusion.

In unrestrained C3H/He mice bearing subcutaneous SCCVII carcinoma, staining mismatch (indicative of intermittent tumour perfusion) is found in approximately 8% of tumour vessels. Angiotensin II caused a reduction in intermittent tumour blood flow (Table 2); during angiotensin II infusion, vessels exhibiting mismatch comprised only 2.0 (1.3)% of total tumour vessels, a

*Table 2. Effect of angiotensin II or saline infusion on intermittent tumour blood flow**

	Staining mismatch (%)		
	Peripheral	Central	Overall
Simultaneous injection ($n = 11$)	1.1 (0.5)	1.6 (0.5)	1.3 (0.5)
No restraint ($n = 18$)	6.1 (2.1)§	11.7 (4.1)§	8.1 (2.5)§
Saline infusion ($n = 10$)	2.4 (1.5)†	5.5 (3.8)‡	3.6 (1.8)§
Angiotensin II infusion ($n = 9$)	1.6 (0.9)	2.7 (2.2)	2.0 (1.3)

*Percent (S.D.) of SCCVII tumour vessels with staining mismatch (indicative of transient non-perfusion) measured after sequential intravenous injection of the vascular markers Hoechst 33342 (15 mg/kg) and $DiOC_7(3)$ (1 mg/kg). Values for the entire tumour (overall) and for peripheral (< 500 μm from surface) and central regions are shown. Mismatch levels significantly different from background levels (measured after simultaneous stain injection) are indicated.
† $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$.

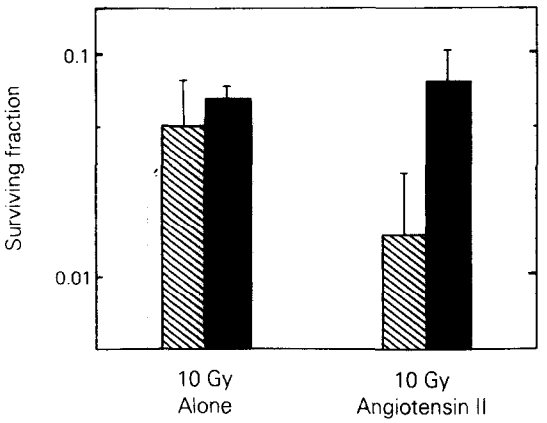

Fig. 4. Effect of angiotensin II infusion on survival of SCCVII tumour cells irradiated *in vivo* with 10 Gy X-rays. The radiation response of the brightest (▨) and dimmest (■) 10% of cells is shown [mean (S.E.), $n = 6$]. Angiotensin II infusion significantly reduced the relatively radioresistant response of acutely hypoxic cells located in the bright sort fraction ($P < 0.05$).

value not significantly different than the background levels obtained when both vascular markers are injected simultaneously [1.3 (0.5)]. The reduction in vessel nonperfusion induced by angiotensin II occurred in both peripheral and central tumour regions. Restraint of the mice and infusion of saline also resulted in a similar significant reduction in staining mismatch compared to unrestrained, non-infused animals. This decrease to 3.6 (1.8)% remained however, significantly different than background levels ($P < 0.001$).

A reduction in intermittent tumour blood flow brought about by angiotensin II infusion might be expected to decrease acute tumour hypoxia caused by these flow fluctuations. In the absence of angiotensin II, perivascular tumour cells stained brightly with Hoechst 33342 (injected 20 minutes before X-rays) exhibited a partially hypoxic response to radiation (Fig. 4) as a result of transient vessel non-perfusion during the period of irradiation. This effect was reduced if angiotensin II was infused throughout the irradiation period. Surviving fraction of bright cells was decreased from 0.049 (0.032) to 0.016 (0.014) [mean (S.D.)] by angiotensin II ($P < 0.05$). No effect on the radiation response of dim cells was seen.

DISCUSSION

This study provides evidence that acute radiobiological hypoxia resulting from intermittent tumour blood flow can be reduced by angiotensin II induced systemic hypertension. Our results support the hypothesis that angiotensin II does not act locally on SCCVII tumour vasculature (since intratumour vascular smooth muscle is very rare [27]) but rather that the drug improves tumour microcirculatory flow distribution via its systemic actions, by elevating perfusion pressure, thereby preventing collapse and/or temporary flow stasis in microvessels. Intermittent non-perfusion can also be significantly reduced by saline infusion alone; thus large increases in blood pressure and in overall tumour blood flow are not absolute prerequisites for the reduction of flow heterogeneity in tumour microvasculature.

The methods used in this study to quantify intermittent tumour microvascular flow and acute hypoxia in solid three-dimensional tumours are based on *in vivo* staining of perivascular tumour cells with fluorescent vascular markers. Effects of these probes on mouse cardiovascular function and tumour perfusion are short-lived and do not significantly influence tumour microvascular flow when employed in the manner described [21, 22]. The techniques provide a unique method to investigate both spontaneous and drug-induced changes in the microregional perfusion of experimental tumours.

Our observation that angiotensin II increases functional microvascular volume by a factor of 1.6 (as measured by laser doppler flowmetry) supports the results obtained by Hori *et al.* [17] using tumours grown in transparent chambers in which perfused vascular area during angiotensin II infusion was 2.1 × pretreatment levels. The slight increase in microvascular volume noted even 10 minutes after cessation of drug infusion is consistent with the hysteresis-like response of the pressure-flow relationship, an effect described previously by Suzuki *et al.* [8]. Angiotensin II caused a greater increase in RBC flow in back tumours than in foot tumours. Our data do not allow a direct comparison of these flow values since measurements in back tumours were performed using anaesthetised animals while those in foot tumours used awake but restrained mice.

Tumour selectivity of the angiotensin II-induced blood flow increase is likely a result of abnormal tumour vascular morphology and function, specifically, an almost complete absence of intratumour vascular smooth muscle [27] and an inability to autoregulate tumour perfusion in response to changing perfusion pressure [4, 5]. Angiotensin II, because it acts primarily at the level of precapillary sphincters, is unlikely to have any large direct effect on tumour neovasculature, which, at least in the SCCVII tumour model, has few contractile elements.

The mechanism underlying intermittent non-perfusion of tumour blood vessels has not been clearly defined. Vasomotion in incorporated host arterioles affecting flow in downstream capillaries [28, 29], vessel plugging by leucocytes, RBC rouleaux, or circulating tumour cells [30], or collapse of vessels in regions of high tumour interstitial pressure [5] are all plausible explanations for the phenomenon. Angiotensin II or volume infusion would be expected to influence the latter two of these proposed mechanisms. An increased non-zero pressure intercept of the pressure flow relationship in a solid tumour perfused *ex vivo* [5] supports the concept of an elevated "critical closing pressure" [31, 32] in tumour microvasculature. The tumour microvascular pressure required to maintain perfusion is elevated, not due to excessive vasomotor tone as might occur in normal tissues, but rather as a result of elevated tumour interstitial pressure [30]. However, in tumour vessels, intravascular pressure is actually lower than normal [33–35], increasing the likelihood of vessel collapse. Angiotensin II infusion, by increasing systemic blood pressure (and presumably tumour perfusion pressure) could suppress or reverse such vessel non-perfusion. In addition, increased tumour microvascular pressure could prevent vessel plugging by relatively non-deformable circulating cells [36]. Since angiotensin II acts to increase vascular tone, intermittent perfusion resulting from arteriolar vasomotion would be reduced only if the increased systemic pressure was sufficient to overcome vasoconstriction of a small number of tumour vessels possessing smooth muscle.

Infusion of angiotensin II during irradiation caused a reduction in radiobiological acute hypoxia. In separate experiments, the drug decreased the amount of staining mismatch caused by transient tumour vessel non-perfusion. Note, however, that the histological double-staining technique required a 30 minute infusion of angiotension II and that most of the reduction in staining mismatch can be explained by volume loading effects. Cell sorting experiments employed only an 8 minute drug infusion (volume = 50 μl) therefore volume loading

does not account for the decrease in acute hypoxia observed. Angiotensin does not completely eliminate hypoxia in brightly stained perivascular cells, since the relative survival level of a fully oxic population of SCCVII tumour cells irradiated with 10 Gy *in vitro* is expected to be 0.005 [18]. The observed survival level of 0.016 is consistent with a residual hypoxic fraction of 2% of brightly stained cells (0.2% of total tumour cells). Angiotensin II induced vasoconstriction of the rare tumour vessel containing vascular smooth muscle might explain this small, residual population of acutely hypoxic cells. Flow of RBC-free plasma through tumour vessels which remain partly collapsed or occluded despite elevated systemic pressure could also account for a lack of staining mismatch but retention of an acutely hypoxic radiation response. Since laser doppler studies suggest that the maximum increase in tumour RBC flow occurs 2–7 minutes after the start of angiotensin II infusion, the irradiation experiments could likely have been further optimised by beginning drug infusion, for example, 7 minutes before irradiation, rather than the 1 minute employed. All of the residual acute hypoxia could perhaps be eliminated under optimal experimental conditions.

Restraint of the mice and infusion of saline also resulted in a significant reduction in staining mismatch although tumour RBC flow was not increased. The effect of large volume saline infusion on acute hypoxia was not examined. Restraint-induced hypertension might account for the reduction in mismatch; this hypothesis predicts an increase in tumour flow, an effect that was not observed during saline infusion. However, the use of anaesthesia in the laser doppler studies would clearly prevent blood pressure elevation induced by stress/restraint.

Alternatively, haemodilution caused by saline infusion (the total infusion volume of 190 μl represents approximately 10% of mouse blood volume given over 30 minutes) could, by lowering intratumour haematocrit, improve tumour perfusion at the microvascular level [36]. Haemodilution following volume infusion would, like elevation of perfusion pressure, reduce vessel occlusion by, for example, RBC aggregates. Agents which improve RBC deformability, such as the calcium channel blocker flunarizine (a drug which does not cause hypertension or require intravenous infusion), have been shown to reduce tumour intermittent blood flow [1]. Similar effects have been observed using nicotinamide [37]. Finally, restraint of small rodents reduces body temperature, an effect likely to inhibit spontaneous vasomotion [38]. Thus, homogeneity of tumour blood flow distribution can be improved by a variety of mechanisms, angiotensin II induced hypertension being only one method to achieve this objective. The possible effect of angiotensin II on chronic tumour cell hypoxia was not examined directly in this study. Since the radiosensitivity of dimly stained tumour cells (distant from the blood supply) was not influenced by angiotensin II infusion (Fig. 4), we hypothesise that chronic hypoxia is not reduced by this drug. Further experiments using standard assays to measure tumour hypoxic fraction would be required to confirm this hypothesis.

If acutely hypoxic cells influence the response of human tumours to radiation, a reduction in intermittent tumour blood flow by angiotensin II infusion (or by other methods designed to increase tumour perfusion homogeneity) would have important implications for radiotherapy. Angiotensin II induced changes in tumour microregional perfusion are certainly consistent with improvements in tumour visualisation [12] and in chemotherapeutic drug delivery [8, 13–16]. However, the effects of vasoactive drugs on tumour perfusion are notoriously variable, being highly dependent on tumour type and experimental technique. A goal in our laboratory is to investigate, using well-characterised experimental tumours and several complimentary methods for measuring blood flow, the effect of both vasoactive and non-vasoactive agents on tumour microvascular perfusion. Measurements in human tumours of vascular morphology, host-arteriole incorporation, and perhaps even the amount of functional as opposed to collapsed vasculature, would then allow a prediction of how such tumours might respond to pharmacological manipulation of blood flow.

The use of angiotensin II to improve diagnostic tumour visualisation or chemotherapeutic drug delivery has been previously described. This study extends these observations to the microvascular level in solid tumours and has important implications for the development of radiotherapeutic strategies designed to overcome tumour hypoxia, especially if human tumour oxygenation is influenced by temporal heterogeneity in blood flow.

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Densitometric Evaluation of DNA Content in Colorectal Cancer

Luigi Matturri, Bruna Biondo, Franco Uggeri and Anna Maria Lavezzi

The aim of the present study was to provide information on the DNA content in colorectal tumours using densitometric techniques on histological samples and correlating the findings with age, sex, histological grade, stage, presence or absence of lymph-node metastasis and survival time. The distribution of DNA values was significantly related with the histological grade, Dukes' stage and infiltration of the peritumoral lymph-nodes. The distribution of DNA values was not significantly correlated with age and sex. From the data obtained in this study it can be concluded that evaluation of DNA content in colorectal adenocarcinoma can be used as a prognostic test that is complementary to histological investigation. The ploidy can provide information for classifying the degree of malignancy and can also be used to determine which tumours are biologically most aggressive.
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INTRODUCTION

IN THE diagnostic and prognostic evaluation of tumours, in addition to the well-known parameters such as histological type, degree of differentiation and stage, biological factors such as cell kinetics, cytogenetics, DNA content and morphometry have recently become increasingly important.

These indicators are not only of diagnostic value but can also be correlated with clinical development of the disease and survival. In the last few years many studies, using a variety of methods, have been carried out on nuclear DNA content in different types of cancer such as carcinomas of the bladder, breast, liver, stomach, ovary and lung [1–7].

These studies have demonstrated the existence of a correlation between the degree of ploidy of tumour cells and the histological characteristics and clinical development of the disease.

Various researchers have subdivided the tumours into three or four subtypes according to the percentage of cells with different DNA content. In all classifications type I, with a DNA content superimposable on that of normal cells, corresponds to diploidy whereas the last type (type III or type IV), with a wide