The potential for prazosin and calcitonin gene-related peptide (CGRP) in causing hypoxia in tumours

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Summary Using ³¹P NMR spectroscopy, changes in tumour metabolic status were studied in a transplanted rat fibrosarcoma following the administration of vasodilators. Mean Arterial Blood Pressure (MABP) was monitored simultaneously. Two vasodilators were studied, prazosin and CGRP, which altered the NMR parameters Pi/ΣP, δNTP/Pi, PCr/Pi and PME/Pi in a dose dependent manner. There was a good correlation between the various NMR parameters; for analysis, Pi/ΣP was used for convenience. With increasing doses of vasodilator, Pi/ΣP increased and the MABP decreased. Reduction in pH_Na showed a correlation with decreasing MABP following the administration of prazosin but not after CGRP.

Both prazosin and CGRP produced changes in ³¹P NMR spectra consistent with a reduction in tumour blood flow. The results for prazosin and CGRP were comparable and showed a 15–20% increase in Pi/ΣP for a 20% reduction in MABP. These results were compared with those from hydralazine. With hydralazine an acceptable reduction in blood pressure (up to approximately 25%) has little effect and may even alter NMR parameters consistent with an increase in blood flow, a reduction of approximately 40% is required for a significant decrease in flow.

Both prazosin and CGRP are shown to be far more effective than hydralazine in causing tumour hypoxia at a clinically acceptable reduction in blood pressure. CGRP may be the more suitable for clinical use because of its short half life, its capability to achieve controlled hypotension and the relatively few side effects associated with its use.

Selective reduction in tumour blood flow and the consequent increase in hypoxia could have several advantages in cancer treatment, for example in combination with drugs which are selectively toxic to hypoxic cells (Brown, 1987; Chaplin & Acker, 1987; Stratford et al., 1989; BRENNER et al., 1990). It might also be an advantage in treatment by hyperthermia, resulting in higher tumour temperatures (Babbs et al., 1982) and more uniform heating together with increased tumour sensitivity resulting from decreased nutrient delivery and reduced pH (GERWECK et al., 1979; HORSMAN et al., 1989).

The potential for selective manipulation of tumour blood flow depends on differences in vasculature between tumours and normal tissues. Tumours are thought to have long, tortuous, leaky vessels with frequent A-V shunts, lack of smooth muscle and probably no innervation. Interstitial pressure tends to be high due to leaky vessels (Wig et al., 1982) and lack of lymph drainage (see REINHOLD & ENDRICH, 1986 for review). The response of the vasculature of a tumour to a vasodilator is likely to be different from that of a normal tissue for two reasons (Chan et al., 1984). One is that the tumour arterioles may react minimally or not at all to the vasoactive stimuli so that vasodilatation elsewhere results in 'stealing' of blood from the tumour. The second result from the normally high tumour interstitial fluid pressure so that any reduction in capillary hydrostatic pressure may lead to tumour vascular collapse leading to flow stasis or even reversal (Chaplin et al., 1987; TROTTER et al., 1988; TROTTER et al., 1989).

The most successful attempts to reduce tumour blood flow has been by the administration of hydralazine, a directly acting vasodilator (Babbs et al., 1982). This has been shown to decrease tumour blood flow in experimental animals and also to potentiate the action of bioreductive compounds and hyperthermia (Brown, 1987; Chaplin et al., 1987; HORSMAN et al., 1989). However, to achieve these effects with hydralazine, a substantial reduction in blood pressure is required, which is likely to limit the clinical use of hydralazine as a modifier of tumour blood flow (OKUNIEFF et al., 1989; TOZER et al., 1990). For a reasonable, clinically acceptable reduction in blood pressure, hydralazine has even been shown to cause an increase in tumour blood flow (KALMUS et al., 1990; ROWELL et al., 1990). A further disadvantage of hydralazine is its long half-life (GROSS, 1977). Clearly, there is a need to investigate drugs which could be of greater therapeutic benefit.

Information related to tumour oxygenation and perfusion status can be obtained from changes in bioenergetic status using ³¹P Magnetic Resonance Spectroscopy (EVELHOCH et al., 1986; ROFSTAD et al., 1989). This technique has been used as a non-invasive means of monitoring tumour metabolic status following the administration of vasodilators hydralazine and prostaclyn (OKUNIEFF et al., 1989; BHUJWALLA et al., 1990a; TOZER et al., 1990). Recently a direct relationship between tumour metabolic status and blood flow has been demonstrated (BHUJWALLA et al., 1990b). In the present study, we have used ³¹P MRS to obtain information on tumour blood flow by monitoring changes in tumour metabolism following administration of various vasoactive compounds. Blood pressure was monitored simultaneously, since this facilitates following administration of vasodilators. The results from two vasodilators, prazosin and CGRP, are reported. Prazosin is a quinazoline derivative which acts by competitive blockade of post-synaptic α-adrenergic receptors. Calcitonin gene-related peptide (CGRP) is an endogenously occurring peptide (ROSENFIELD et al., 1983; MORRIS et al., 1984), which acts by binding to its receptors in the vessel wall (LUNDBERG et al., 1985). When given intravenously CGRP is one of the most potent vasodilators known (BRAIN et al., 1985; STRUTHER et al., 1986). It has the advantage of a short half-life (BENJAMIN et al., 1987). The mechanism of action of each of these compounds is different from that of hydralazine, which acts by directly relaxing the arteriolar smooth muscle.

Materials and methods

Tumour model

A transplanted rat fibrosarcoma, designated LBDS1, was used for these experiments. The tumour arose spontaneously

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in the flank of a male BD0 rat and was serially transplanted. Further details of these tumours may be found elsewhere (Tozer & Morris, 1990). Only early generation transplants were used. Tumour pieces 1–2 mm$^3$, taken from a previous generation isograft were implanted subcutaneously into the right flanks of 10–12 week old BD0 rats. The rats were then returned to their cages and housed in a temperature controlled and light-cycled room. They were fed on normal rat chow and water ad libitum. It took an average time of 30 ± 4 days for the tumours to reach 15–20 mm in diameter, at which size they were used for experiments.

Drugs used

In order to restrain the animals for NMR spectroscopy, they were anaesthetised with a combination of fentanyl citrate (0.315 mg kg$^{-1}$), fluanisone (10 mg kg$^{-1}$) 'Hypnorm' (Crown Chemical Co.) and midazolam (5 mg kg$^{-1}$) 'Hypnovel' (Roche).

Prazosin (Sigma Chemical Co) was freshly dissolved in water to give an iso-volume of 0.5 ml for various doses and injected intravenously in bolus doses of 0.5, 1.0 and 1.5 mg kg$^{-1}$ rat body weight.

Rat calcitonin gene-related peptide 'CGRP' (Peninsula Lab) was dissolved in distilled, deionised water containing 0.001% (v/v) acetic acid and 0.1% (w/v) bovine serum albumin, and stored at -70°C (Zaidi et al., 1989). For intravenous injection, the mixture was re-dissolved in saline, to give a constant injection volume of 0.5 ml for various doses. CGRP was injected in bolus doses of 30 pmol (5.8 μg kg$^{-1}$), 600 pmol (10.8 μg kg$^{-1}$), 1 nmol (20.2 μg kg$^{-1}$) and 2 nmol (38.8 μg kg$^{-1}$).

Spectroscopy

$^{31}$P spectra were obtained on a 1.89T Oxford Research System 32MR-32 spectrometer. A range of radiofrequency surface coils was used, the size (1–2 cm diameter) being chosen to fit closely around the tumour. The magnetic field was shimmed to obtain a $H_2O$ resonance with a maximum linewidth of 40 Hz. Experimental parameters included a spectral width of 2 KHz, 90° pulse with a pulse length of 10 μs, pulse repetition time of 2 s, 2048 data points and 300 or 600 averaged free induction decays. Data processing involved exponential line broadening of 15 Hz. Broad spectral lines were removed by spectral deconvolution (Tozer et al., 1989). Peak areas were calculated by a computer program which allowed operator definition of the baseline and peak limits. No assumptions were made about the peak shapes in the analysis of spectra. However there could be a systematic error from small signals due to peak overlap. This error would be significant only if the area of the peak were considerably different from that of neighbouring peaks. For example if the area the inorganic phosphate peak were 2-fold greater than that of the neighbouring peaks, it would be underestimated by approximately 10%. Consequently changes observed in the spectra following treatment with vasodilators would be slightly underestimated. Non-systematic error in estimating the peak area would have been of the order of 10%, given the signal-to-noise ratio obtained in the collection of spectra.

Blood pressure measurements

Mean arterial blood pressure was monitored using a Gould P23XL physiological pressure transducer connected to a Gould RS3200 recorder (see below).

Experimental protocol

The rats were anaesthetised and polythene catheters containing heparinised normal saline were implanted into a tail vein and tail artery. The tail artery catheter was connected to the pressure transducer by a sufficient length of pressure tubing to monitor the changes whilst the rat was within the bore of the magnet. The rat was placed on two plastic tissue culture flasks containing recirculating warm water to maintain core temperature at 37°C. It was positioned so that its tumour hung vertically downwards between the two culture flasks and rested on the surface coil. A baseline spectrum was accumulated over a period of 20 min, following which either prazosin or CGRP was injected via the tail vein catheter without disturbing the position of the rat in the magnet. Control rats were injected with 0.5 ml of saline. Spectra were accumulated for at least 80 min following prazosin or water and for up to 40 min following CGRP. Blood pressure was monitored throughout.

Data analysis

The results given in both the text and figures are means and standard errors for the given number of rats.

Results

A typical $^{31}$P NMR spectrum from a tumour is shown in Figure 1a together with sequential spectra following an intravenous bolus injection of 600 pmol CGRP. The MABP recorded simultaneously is shown in Figure 1b. The principal change in NMR spectrum was an increase in the amplitude of the Pi peak, which was maximal in the first 10 min period, whilst the blood pressure was at its minimum. With CGRP there was a fairly rapid recovery of blood pressure, with Pi, PCr and NTP peaks returning to control values more slowly. Following prazosin, the changes in MRS parameters and reduction in MABP were not reversed during the 80 min period of study, consistent with the long half life of this drug.

With both prazosin and CGRP, the reduction in MABP and corresponding increase in Pi/EP were dose dependent. Thus an inverse relationship was found between decreasing MABP and increasing Pi/EP with escalating doses of vasodilator, as shown in Figure 2 for CGRP. A similar result was found for prazosin.

NMR parameters other than Pi/EP, i.e. βNTP/Pi, PCr/Pi and PME/Pi were also recorded. βNTP/Pi and PCr/Pi showed a good correlation with Pi/EP as shown in Figure 3a and b. Tables I and II show that the parameters Pi/EP, βNTP/Pi, PCr/Pi and PME/Pi were all correspondingly altered as a

![Figure 1](image-url)
function of dose of prazosin or CGRP. No significant change was observed in these parameters in control animals treated with saline. Since these parameters are all interdependent, it was decided to concentrate the analysis of Pi/\Sigma P for convenience.

The time course of changes of Pi/\Sigma P and blood pressure following an injection of 1 mg kg\(^{-1}\) prazosin is shown in Figure 4. It is seen that following injection, there was a steady fall in MABP with a corresponding increase in Pi/\Sigma P. At 70 min both were still changing. The response of Pi/\Sigma P and MABP following administration of a bolus dose of 2 nmol CGRP is shown in Figure 5. In this case it can be seen that there was a rapid fall in MABP with a corresponding rapid increase in Pi/\Sigma P. However, the return towards normal of MABP began after a few minutes, whereas Pi/\Sigma P recovered more slowly. In all these studies the rats were anaesthetised by a combination of hypnorm and hypnovel. This anaesthetic slightly reduces the blood pressure (Field & Burney, unpublished). However, any interactions between the vasodilators and the anaesthetic are not known.

**Discussion**

There is a strong evidence that \(^{31}\)P MRS can detect changes in tumour bioenergetics brought about by changes in tumour oxygenation and perfusion. For example, Evelhoch et al. (1986), using in situ photon activation-\(^{15}\)O decay measurements and \(^{31}\)P NMR spectroscopy, showed a positive correlation between \(^{15}\)O perfusion and NTP/Pi and PCr/NTP ratios. A positive correlation was also observed between declining mean tissue \(pO_2\) and NTP/Pi, PCr/Pi, PME/Pi, PDE/Pi and \(pH_{K_1}\) with increasing tumour size in a murine fibrosar-
correlation reduction al., coma doses rodents), recently tumour effective 1990). These results show that among other factors producing
tumour flow and stress reduction in tumour perfusion is a given reduction in blood pressure.

Hydralazine can cause almost complete radiobiological hypoxia in tumours but this requires high doses of drugs resulting in a massive and clinically unacceptable reduction in blood pressure (Okunieff et al., 1989; Tozer et al., 1990). At lower doses of hydralazine ($\approx0.1\text{ mg kg}^{-1}$ in rodents), resulting in a clinically acceptable reduction in blood pressure (up to $\approx25$%), laser doppler flow studies show that tumour blood flow may actually be improved (Kalmus et al., 1990). Some NMR studies also show changes consistent with improved tumour blood flow following lower doses of hydralazine (Okunieff et al., 1989; Tozer et al., 1990). These experimental results are consistent with the clinical studies of Rowell et al. (1990) who showed an increase in tumour blood flow measured by Single Photon Emission Computed Tomography (SPECT) in patients given oral hydralazine.

Taken together these results show that at a clinically acceptable reduction in blood pressure, hydralazine may even cause an improved blood flow and only with a clinically unacceptably large reduction in blood pressure is blood flow reduced. In addition, hydralazine has a fairly long half life (Gross, 1977). Clearly hydralazine is unlikely to be of clinical use in reducing tumour blood flow and more effective vasodilators are needed for this purpose.

Prazosin and CGRP both produced changes in NMR spectra consistent with a reduction in tumour blood flow for much smaller reduction in blood pressure than with hydralazine. The relative effectiveness of these compounds is demonstrated in Figure 6, in which an injection resulting in a 20% drop in MABP, causes at least a 15% increase in Pi/ΣP. In contrast, a similar drop in blood pressure with hydralazine leads to a 15% decrease in Pi/ΣP, consistent with an increase in blood flow. With hydralazine it is necessary to give sufficient dose to cause at least a 30–40% reduction in blood pressure to achieve a significant increase in Pi/ΣP.

In control animals treated with saline, the variation in the NMR parameters was relatively small. As seen in Tables I and II, the variation in the ratios Pi/ΣP, βNTP/Pi, PCR/Pi and PME/Pi was of the order of 2–8%. The variability in the area of the same individual peak in spectra collected from the same control tumour ranged between 4–12%. The experimental design, such that the rat was not moved within the magnet throughout the course of the experiment, the drug being administered remotely, ensured that any change in spectrum was due solely to the administration of the drug.

The use of anaesthetics to immobilise the rats during the procedures is almost certainly less perturbing than the methods to forcibly restrain them. The anaesthetic used was a combination of a morphine type analgesic, fentanyl; a neuroleptic of butyrophenone group, fluanisone; and a benzodiazepine, midazolam; all mediating their effect through the centra nervous system. This combination anaesthetic was chosen because it has the advantage over other commonly used anaesthetics (such as pentobarbitol, ketamine, morphine, halothane and enflurane), of not increasing the peripheral vascular resistance, conferring better stress protection and preserving tissue perfusion (Skolleborg et al., 1990). Although no interaction between this combination anaesthetic and the different vasodilators used in this study has been documented, it seems unlikely that any such interaction would occur between such centrally acting anaesthetic agents and the tested vasoactive compounds which mediate their effect through specific receptors. Furthermore, this combination anaesthetic has little or no effect on the release of corticosteroids, which play an important role in central haemodynamics and an interaction with the hypothalamic-hypophysial-cortical axis also seems unlikely.

Whereas, midazolam has little or no effect on various haemodynamic parameters, fentanyl and fluanisone do decrease the mean aortic blood pressure and increase the cardiac output, although the reduction in blood pressure is less than that produced by the other commonly used anaesthetics e.g. pentobarbitone, ketamine or diazepam (Cullen & Walker, 1986). The aim of the study was to relate changes in NMR parameters to changes in blood pressure. If there were any effect of the anaesthetic it would almost certainly be confined to causing a small additional reduction in blood pressure and would be a common factor throughout, including all similar studies with hydralazine, which have been used for comparison to the effects of CGRP and prazosin. Any effect of the anaesthetic would therefore not alter the conclusions.
Prazosin may not be suitable for clinical use as a modifier of tumour blood flow because of its long half life (see Table III). The hypotensive effects may last for up to 10–12 h (Stanaszek et al., 1983). Faintness and dizziness have been reported to occur in 50% of patients receiving this drug. Severe orthostatic hypotension is less common and is attributed to the 'first dose effect' (Rudd & Blaschke, 1985).

One of the limitations for the clinical use of CGRP might be the sudden reduction in blood pressure during the first few minutes following the injection of a bolus dose. This may be particularly harmful to elderly patients. However, it has been shown that CGRP can be administered safely by infusion to achieve controlled hypotension (Struthers et al., 1986).

CGRP has a short plasma and biological half life in man (see Table III). This may be a very effective method of administration since the effects on metabolism are more prolonged than the reduction in blood pressure (Figure 5). Lack of tachyphylaxis and other side effects associated with prazosin and hydralazine, make CGRP the more favourable for clinical use.

The increase in tumour blood flow following low doses of hydralazine suggested by a decrease in Pi/EP and by other techniques (Kalmus et al., 1990; Rowell et al., 1990) can be explained by increased perfusion pressure occurring as a result of reflex sympathetic stimulation in the presence of little vasodilatation (Maekawa et al., 1984). Prazosin blocks the action of nor-epinephrine by post-synaptic receptor blockade and subsequently decreases its further release through negative feed back control (Stanaszek et al., 1983). CGRP causes sympathetic stimulation (Fisher et al., 1983), increasing the nor-epinephrine release by almost 2-fold. Although the mechanism of action of these drugs is far from fully understood, there appears to be a similarity in the mode of action of prazosin and CGRP, both being receptor mediated, in contrast to hydralazine which is known to cause arteriolar vasodilatation by direct relaxation of smooth muscle (Ablad, 1963). Whether this difference in the mode of action is the cause of prazosin and CGRP being substantially more effective than hydralazine in modifying tumour blood flow for a given reduction in MABP, remains speculative. It is plausible however, that compounds with a receptor mediated mode of action, because of sensitive vasomotor control, preferentially cause vascular collapse and flow stasis, thus rendering tumours hypoxic for small reduction in blood pressure. In contrast, diversion of blood by the 'steal' phenomenon to cause hypoxia probably requires a greater reduction in systemic blood pressure.

In conclusion, two vasodilators, prazosin and CGRP, have been shown to produce changes in MRS parameters consistent with a reduction in tumour blood flow at a substantially smaller reduction in mean arterial blood pressure than hydralazine. CGRP has the greater potential for clinical use because it has a short half life and a lower probability of causing side effects. Studies are in progress using PET to monitor changes in human tumour blood flow following administration of CGRP. There is however a need to understand better the mechanism of action of these drugs in the context of pathophysiology of tumour vasculature, if they are to be used to maximal effect.

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Table III Plasma and biological half-lives of three vasodilators in man

| Drug   | Plasma Half-life in man | Biological Half-life |
|--------|------------------------|---------------------|
| Hydralazine | 4 h | 30 h |
| Prazosin    | 2–3 h | 10–12 h |
| CGRP       | 9 min | 19 min |

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