Inhibition of Nitric Oxide Synthase Induces a Selective Reduction in Tumor Blood Flow That Is Reversible with L-Arginine

Gillian M. Tozer, Vivien E. Prise, and David J. Chaplin

Tumour Microcirculation Group, Gray Laboratory Cancer Research Trust, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, United Kingdom

ABSTRACT

The effect of i.v. administration of the nitric oxide synthase (NOS) inhibitor N\textsuperscript{\textdagger}monomethyl-L-arginine (l-NMMA) on tumor blood flow compared with normal tissue blood flow was studied in anesthetized BD9 rats bearing subcutaneous P22 carcinosarcomas. Blood flow was measured by the uptake of radiolabeled iodoantipyrine. The reversibility of blood flow changes was tested by subsequent administration of l-arginine, the natural substrate for NOS. The effect of l-NMA was compared to that of the imidazolineoxyl N-oxide C-PTIO, a carboxyl derivative of 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide and a nitric oxide scavenger. Drug-induced changes in mean arterial blood pressure (MABP) were measured and used to calculate relative drug-induced changes in tissue vascular resistance. Heart rate was measured from blood pressure traces.

l-NMA significantly decreased heart rate and increased MABP in a dose-dependent manner. Significant dose-dependent reductions in blood flow with l-NMA were observed in tumor, skeletal muscle, spleen, and skin overlying the tumor. No significant effect was found for normal skin, brain, heart, kidney, and small intestine. At 1 mg/kg, the effect of l-NMA was selective for the tumor, with a significant decrease in tumor blood flow to 0.45 of the control level and no significant effect in any of the normal tissues. Higher doses did not produce any further reduction in tumor blood flow, presumably due to an increase in tumor perfusion pressure arising from the increase in MABP at these doses. Vascular resistance was increased to some extent in all of the tissues studied but, overall, was greatest in the tumor. At 1 mg/kg, there was a 2-2.5-fold increase in tumor vascular resistance but no significant increase in any of the normal tissues. At the highest dose used (10 mg/kg), the increases in vascular resistance in the skeletal muscle and spleen were equivalent to that in the tumor. Administration of L-arginine 15 min after l-NMA completely reversed the decrease in tumor blood flow observed for 1 mg/kg l-NMA alone. In contrast to the effect of l-NMA, constant i.v. infusion of C-PTIO had no effect on tumor or normal tissue blood flow. These results indicate that nitric oxide is important for maintaining a vasodilatory tone in tumors and that inhibition of NOS may provide a means for enhancing therapeutic regimens that would benefit from a selective reduction in tumor blood flow.

INTRODUCTION

Methods for selective modification of tumor blood flow have potential for enhancing a wide variety of cancer treatments (1). The vasodilator hydralazine has been used in experimental tumor systems as a means of selectively reducing tumor blood flow. This approach has led to improved tumor heating during hyperthermia (2) and an increased cytotoxicity of drugs that are reduced to their active form under hypoxic conditions (the so-called bioreductive drugs such as the nitroimidazoles; Refs. 3 and 4). The selective reduction in tumor blood flow using hydralazine is normally put down to a "steal" of blood away from the tumor. This is where a disproportionately large decrease in tumor blood flow, compared with the drug-induced decrease in systemic blood pressure, occurs as a result of minimal arteriolar vasodilation in the tumor compared with the adjacent normal tissue. Alternatively, the decrease in blood pressure induced by hydralazine, besides reducing perfusion pressure, could set up an acute imbalance between intravascular and extravascular pressures within the tumor, which would result in a reduction in capillary/venular diameters and therefore a disproportionately large decrease in tumor blood flow (5). Whatever the cause, hydralazine administration produces a consistent decrease in tumor blood flow in animal models (5), which is sometimes, but not always, the case in clinical studies (6, 7). Horsman et al. (7) have suggested that a blood pressure reduction of =\textsuperscript{\textdagger}15% may be required for an effective reduction in tumor blood flow and that the variable clinical results are most likely due to different levels of induced hypotension. This degree of hypotension is an obvious disadvantage for the clinical application of hydralazine and other vasodilators as a means of reducing tumor blood flow. Therefore, other approaches need to be investigated.

Methods for selective modification of tumor blood flow have potential for enhancing a wide variety of cancer treatments (1). The vasodilator hydralazine has been used in experimental tumor systems as a means of selectively reducing tumor blood flow. This approach has led to improved tumor heating during hyperthermia (2) and an increased cytotoxicity of drugs that are reduced to their active form under hypoxic conditions (the so-called bioreductive drugs such as the nitroimidazoles; Refs. 3 and 4). The selective reduction in tumor blood flow using hydralazine is normally put down to a "steal" of blood away from the tumor. This is where a disproportionately large decrease in tumor blood flow, compared with the drug-induced decrease in systemic blood pressure, occurs as a result of minimal arteriolar vasodilation in the tumor compared with the adjacent normal tissue. Alternatively, the decrease in blood pressure induced by hydralazine, besides reducing perfusion pressure, could set up an acute imbalance between intravascular and extravascular pressures within the tumor, which would result in a reduction in capillary/venular diameters and therefore a disproportionately large decrease in tumor blood flow (5). Whatever the cause, hydralazine administration produces a consistent decrease in tumor blood flow in animal models (5), which is sometimes, but not always, the case in clinical studies (6, 7). Horsman et al. (7) have suggested that a blood pressure reduction of =\textsuperscript{\textdagger}15% may be required for an effective reduction in tumor blood flow and that the variable clinical results are most likely due to different levels of induced hypotension. This degree of hypotension is an obvious disadvantage for the clinical application of hydralazine and other vasodilators as a means of reducing tumor blood flow. Therefore, other approaches need to be investigated.

A minimal response of tumor blood vessels to vasodilators such as hydralazine has led to the suggestion that these vessels are near-maximally dilated in their resting state (8). The cause and the full extent of this vasodilatory tone in tumors is unknown. A possible vasodilatory mediator is the free radical NO, which is generated primarily from the conversion of l-arginine to l-citrulline in the presence of the enzyme NOS. Both cNOS and iNOS have been identified in experimental and human tumors by immunohistochemical techniques, suggesting that NO levels in tumors are high (9–13). Inhibition of NOS, leading to a reduction in the vasodilatory stimulus of NO, is therefore a possible strategy for reducing tumor blood flow.

There is some evidence that competitive inhibition of NOS using analogues of l-arginine results in a decrease in blood flow to experimental tumors. Andrade et al. (14) used tumors growing in s.c. sponge implants in mice to determine the effects of systemic administration of L-NAME and L-NMMA on tumor and granulation tissue blood flow. Both inhibitors slowed the washout rate of 133Xe injected into the sponge implants hosting tumor tissue, but only minimally affected the washout rate from sponges hosting normal granulation tissue. l-Arginine administration reversed the effect of the NOS inhibitors. Wood et al. (15–17) found that l-NNA decreased the energy status of several murine tumors as measured by 31P-magnetic resonance spectroscopy, whereas the spectra from normal skin were unaffected. Moreover, they found evidence for a decrease in tumor radiosensitivity and an increase in tumor sensitivity to the cytotoxic effect of the bioreductive drug RB6145 with l-NNA. Meyer et al. (18) found that L-NMMA directly administered to tumors growing in dorsal "window chambers" reduced the diameter of central tumor venules by 13% and reduced the velocity of red cells flowing through central venules by 25%. However, in contrast to the other studies, the effect of L-NMMA in control tissue, in this case granulation tissue, was greater than that in the tumor. l-Arginine reversed the effects of L-NMMA in the granulation tissue but not in the tumor.

Further assessment of NOS inhibitors as tumor blood flow modi-

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An alternative approach to NOS inhibition is direct scavenging of NO via a radical-radical reaction, and one of them, NO after its formation. The imidazoline oxyl N-oxides have been found to scavenge NO via a radical-radical reaction, and one of them, C-PTIO on tumor blood flow is unknown.

The aims of the present study were: (a) to obtain a dose response for the effect of L-NNA on tumor blood flow in vivo; (b) to compare the tumor response to L-NNA with those from a range of normal tissues; (c) to relate L-NNA-induced blood flow changes to changes in MABP; (d) to determine the reversibility of any observed changes in blood flow induced by L-NNA; and (e) to compare the L-NNA-induced blood flow changes with those induced by C-PTIO.

MATERIALS AND METHODS

Tumors. Early generations (up to 12 away from the primary tumor) of the P22 transplanted rat carcinosarcoma were used for these experiments (23). Tumors were grown s.c. in the left flank of 7—9-week-old male BD9 rats and were used for experimentation at 2—3 weeks postimplantation when all three orthogonal diameters measured 10—15 mm, including skin thickness. Mean tumor weight ± 1 SE for all tumors used was 1.29 ± 0.04 g.

Blood Flow. Tumor and normal tissue blood flow was measured using uptake of 125I-labeled IAP (Institute of Cancer Research, Sutton, United Kingdom). This method has been described previously (23). Briefly, animals were anesthetized with Hypnorm (fentanyl—fluanisone) and midazolam, and two tail veins and one tail artery were catheterized using polyethylene catheters (external diameter, 0.96 mm; internal diameter, 0.58 mm). MABP was monitored via the tail artery catheter up to the point of blood flow measurement. Animals were heparinized with 0.2 ml of 1000 units/ml heparin (CP Pharmaceuticals, Wrexham, Wales, United Kingdom) via a catheterized tail vein.

Drugs were obtained from Sigma Chemical Co Ltd. (Poole, United Kingdom) except for C-PTIO, which was obtained from Cambridge Bioscience (Cambridge, United Kingdom).

Three experimental protocols were used:

(a) L-NNA (0.1—10 mg/kg) or the vehicle for the drug (water acidified with a few drops of 1 N HCl) was administered by i.v. bolus injection in a volume of 1.7 ml/kg into a catheterized tail vein. Blood flow was measured 20 min after drug or vehicle administration.

(b) L-NNA (1 mg/kg) or acidified water was administered by i.v. bolus injection via a catheterized tail vein in a volume of 0.9 ml/kg. L-arginine (200 mg/kg) or the vehicle for the drug (0.9% saline) was injected 15 min later in the same volume. Blood flow was measured 15 min after L-arginine or 0.9% saline administration.

(c) C-PTIO (1.7 mg/kg/min) or the vehicle for the drug (0.9% saline) was infused into a catheterized tail vein at a rate of 0.3 ml/kg/min. Blood flow was measured 15 min after the start of infusion.

At the appropriate time, blood flow was measured by infusing 0.3 MBq (8 μCi) of 125I-labeled IAP dissolved in 0.8 ml of saline into a tail vein catheter over 30 s. During the 30-s period, free-flowing arterial blood from the tail artery was collected into preweighed vials at 1-s intervals. At the end of the 30 s, the rat was killed by i.v. injection of Euthatal. Tumor, overlying skin, skin from the contralateral flank, gastrocnemius muscle, spleen, left kidney, small intestine, heart, and brain were excised, weighed, and counted on a Wallac Autogamma counter. Blood samples were similarly weighed and counted.

Blood flow rate to tumor and normal tissues was calculated from tissue counts, the equilibrium partition coefficient of IAP in the different tissues (24), and the arterial input function derived from the arterial blood counts. This method is based on principles derived by Kety (25) and is described in detail elsewhere (26). Blood flow rate was calculated in milliliters per gram per minute. Perfusion pressure for all tissue was assumed to change in direct proportion to MABP, and changes in tissue vascular resistance for tumor and normal tissues were calculated from MABP divided by blood flow rate. Results were generally expressed as drug-treated values relative to values obtained from a control group of animals given only drug vehicle.

Statistics. All data were tested for normality of distribution and equal variances between groups using a Shapiro-Wilk W-test and an O'Brien's F-test, respectively. In the majority of cases, these conditions were fulfilled, and differences between groups were tested for significance using either a Student's t test for unpaired data (comparison of two groups) or a standard ANOVA followed by the Tukey-Kramer honest significant difference test (comparison of more than two groups). The Wilcoxon rank-sum test for nonparametric data was used for groups that failed the test for normality, and a Student's t test for unequal variances was used where appropriate. JMP statistics for the Apple Macintosh was used for all analyses.

RESULTS

Fig. 1. Absolute blood flow rate to the P22 tumor compared with selected normal tissues from untreated, tumor-bearing rats pooled from the control groups of all of the experiments in this series. MABP and heart rate for the whole group were 93 ± 1 mmHg and 354 ± 7 beats/min, respectively. Tumor blood flow rate was 0.47 ± 0.03 ml/g/min, which was approximately twice than that to the overlying skin and about 30% higher than that to resting skeletal muscle (P < 0.01). Tumor blood flow rate was significantly lower than that to brain, heart, kidney, spleen, and small intestine. Kidney had the highest blood flow rate, which was approximately 10 times that to the tumor (4.81 ± 0.10 ml/g/min). Blood flow rate to the skin overlying the tumor was significantly higher than that to the skin from the contralateral flank (P < 0.01), suggesting that growth of the tumor has local hemodynamic effects beyond the tumor periphery.

Fig. 2, a and b, shows that MABP was significantly increased and heart rate was significantly decreased in a dose-dependent manner at 20 min after L-NNA administration. At the higher doses, MABP increased over the first few minutes after L-NNA administration and then remained relatively constant until the 20-min end point time (results not shown). Tumor blood flow rate was decreased in a dose-dependent manner (Fig. 3a). Comparison of Figs. 2a and 3a shows that tumor blood flow rate was significantly reduced at a dose
normal tissues compared with that for tumor using MABP as a relative measure of perfusion pressure in all cases. There was a small but significant rise in vascular resistance at 3 and 10 mg/kg l-NNA for brain, heart, kidney, and small intestine and at 10 mg/kg only for contralateral skin. A larger effect was observed for skin over the tumor (a 2-fold increase at 10 mg/kg), spleen (a 3-fold increase at 10 mg/kg), and skeletal muscle. The effect in skeletal muscle was of the same order as that in the tumor, rising to a 3.5-fold increase at 10 mg/kg.

Fig. 6 shows the effect of 1.7 mg/kg/min C-PTIO on tumor and normal tissue blood flow. This dose had no effect on blood flow to any of the tissues investigated, including the tumor. C-PTIO also had no effect on MABP, but there was a small reduction in heart rate to 0.88 of control ($P < 0.05$). Previously, we have shown that this dose of C-PTIO results in a plasma C-PTIO concentration of more than 100 $\mu$M (27).

Fig. 7 shows the effect of attempting to reverse the decrease in tumor blood flow elicited by 1 mg/kg L-NNA by subsequent administration of high-dose L-arginine, the natural substrate for NOS. L-arginine alone (200 mg/kg) had no significant effect on tumor blood flow, as shown by comparison of the first two columns from the left in Fig. 7. However, the decrease in tumor blood flow induced by L-NNA, which was significant at the 1% level, was completely reversed by L-arginine. This is shown in Fig. 7 by a significant difference between tumor blood flow measured after L-NNA alone and that measured after L-NNA plus L-arginine ($P < 0.05$), and by an insignificant difference at the 5% level between the L-NNA plus L-arginine group and the control group. The decrease in tumor blood flow

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**Fig. 2.** Effect of i.v. bolus administration of L-NNA on MABP (a) and heart rate (b) of anesthetized BD9 rats. Bars, mean ±1 SE. Each point represents data from at least seven control and eight treated animals. Asterisks, significant differences between the control group and the drug-treated group (*, $P < 0.05$; **, $P < 0.01$).

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that caused no significant change in MABP; i.e., tumor blood flow rate was significantly decreased below control levels at L-NNA doses of $\geq 1$ mg/kg, whereas MABP was only increased significantly at $\geq 3$ mg/kg. At 1 mg/kg, tumor blood flow rate was reduced to 0.45 of control levels. No further reduction was achieved by increasing the L-NNA dose to 3 and 10 mg/kg. Fig. 3b shows the effect of L-NNA on tumor vascular resistance, using MABP as a relative measure of perfusion pressure. There is a good dose response throughout the dose range, with a 3–3.5-fold increase in vascular resistance at 10 mg/kg. This is a result of the constant blood flow measured between 1 and 10 mg/kg (Fig. 3a) in the face of a rising MABP between these doses (Fig. 2a).

Fig. 4 shows the effect of L-NNA on blood flow rate to the normal tissues compared with that to the tumor. The effect of L-NNA was small for most normal tissues. Doses up to 10 mg/kg had no significant effect on blood flow rate to contralateral skin, brain, heart, or small intestine. There was a significant reduction in flow to skin overlaying the tumor and spleen at the highest dose used (10 mg/kg). The most sensitive normal tissue was skeletal muscle, in which significant reductions in blood flow rate were observed for 3 and 10 mg/kg L-NNA. At these doses, the effect was similar to that observed in the tumor. At 1 mg/kg, there was no significant difference between the flow reduction in skeletal muscle and that in tumor ($P > 0.05$), although the decrease from control values was only significant in the tumor ($P < 0.01$). No normal tissue showed a greater reduction in blood flow rate than that observed in the tumor.

Fig. 5 shows the effect of L-NNA on tissue vascular resistance for the most sensitive normal tissues investigated, including the tumor. C-PTIO also had no effect on MABP, but there was a small reduction in heart rate to 0.88 of control ($P < 0.05$). Previously, we have shown that this dose of C-PTIO results in a plasma C-PTIO concentration of more than 100 $\mu$M (27).

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**Fig. 3.** Effect of i.v. bolus administration of L-NNA on tumor blood flow (a) and tumor vascular resistance (b). Values are plotted as fractions of values obtained in control, untreated rats. Bars, mean ±1 SE. Each point represents data from at least seven control and eight treated animals. Asterisks, significant differences between the control group and the drug-treated group (*, $P < 0.05$; **, $P < 0.01$).
Fig. 4. Effect of i.v. bolus administration of L-NNa on normal tissue blood flow (-----) compared with the effect in tumor (- - - -). Values are plotted as fractions of values obtained in control, untreated rats. Bars, mean ± 1 SE. Each point represents data from at least seven control and eight treated animals. Asterisks, significant differences between the control group and the drug-treated group (*, P < 0.05; **, P < 0.01).
Fig. 5. Effect of i.v. bolus administration of L-NNA on normal tissue vascular resistance (—) compared with the effect in tumor (- - - -). Values are plotted as fractions of values obtained in control, untreated rats. Bars, mean ± 1 SE. Each point represents data from at least seven control and eight treated animals. Asterisks, significant differences between the control group and the drug-treated group (*, P < 0.05; **, P < 0.01).
Twelve treated and thirteen untreated rats were used. Asterisks, significant differences between the control group and the drug-treated group.

**DISCUSSION**

The increase in MABP, observed in the anesthetized BD9 rat with escalating doses of L-NNA, was similar to that described previously for several other analogues of L-arginine in different rat systems (19, 28). Hypertension would follow from an increase in total peripheral vascular resistance, which is indicated, in our experiments, by the large decrease in blood flow and the increase in vascular resistance in skeletal muscle, this tissue constituting the largest bulk of tissue in the body. The vasoconstrictive effect of L-NNA in skeletal muscle may not be due to a local inhibition of the endothelial cell form of cNOS, because we have found that administration of L-NNA to isolated perfused rat hind limb has very little effect on vascular resistance (29).

Recently, it has been found that intracerebral injection of L-NAME, the pro-drug for L-NNA, induces a hypertensive effect in rats, which may result from inhibition of NO as a neurotransmitter or from an indirect increase in sympathetic activity (30). Therefore, a combination of local and central effects of L-NNA could account for the hypertension observed in our experiments.

The decrease in heart rate observed in the BD9 rat with L-NNA is also consistent with previous reports (19, 28). NO is known to act as a negative inotrope in cardiac muscle (31) such that L-NNA might be expected to increase heart rate. The observed decrease is probably the result of a reflex bradycardia in response to increased blood pressure. The significant decrease in heart rate at 1 mg/kg L-NNA, in the absence of any rise in MABP, probably reflects this homeostatic mechanism. Alternatively, the decrease in heart may be due to inhibition of NO as a neurotransmitter, as suggested by Rees et al. (19).

The large L-NNA-induced decrease in blood flow in the P22 rat carcinoma represents a broadly consistent with previous reports of the effects of competitive inhibitors of NOS on tumor vascular parameters (14-18). However, our investigation of a full dose response for tumor and normal tissue blood flow modification, combined with measure-ments of systemic cardiovascular parameters, provides additional quantitative and mechanistic information. The maximum decrease in tumor blood flow was achieved at an L-NNA dose of 1 mg/kg, with doses greater than this having no further effect. Doses of L-NNA >1 mg/kg significantly increased MABP such that tumor perfusion pressure would also be increased. This would account for the plateau in the dose-response curve for tumor blood flow by counteracting the vasoconstrictive effect of L-NNA in the tumor (Blood flow rate = Perfusion pressure - Vascular resistance).

The decrease in blood flow observed in skeletal muscle with L-NNA is consistent with the hypertensive action of the drug, as outlined above. However, skeletal muscle was the only normal tissue that showed a decrease in blood flow approaching that of the tumor. At the highest L-NNA dose used (10 mg/kg), the effect in the spleen was equivalent to that in the tumor, but the effects at lower doses were insignificant. The reason for this sudden decrease in spleen blood flow is unknown. Skin overlaying the tumor also showed a significant decrease at 10 mg/kg that was not observed in skin excised from the contralateral flank. This suggests that the tumor exerts an influence on vascular tone beyond the tumor periphery that can be reversed by L-NNA. The maximum decrease in tumor blood flow occurred at an L-NNA dose (1 mg/kg) that had no effect on MABP and an insignificant effect on skeletal muscle blood flow.

MABP is unlikely to represent the absolute perfusion pressure for the tumor due to the parallel arrangement of blood vessels feeding the tumor and its host normal tissue, in this case, skin (32). Therefore, calculations of tissue vascular resistance using MABP were confined to relative changes. It is recognized that an error may be introduced into these calculations if the increase in MABP induced by L-NNA caused a disproportionately large or small increase in pressure within the tumor feeding vessels (probably at the arteriolar level). However, in the absence of such measurements, the assumption of a proportionate change appears reasonable. Tumor vascular resistance increased over the range of L-NNA doses used, with an indication that the effect was beginning to plateau at the highest dose (10 mg/kg), at which point tumor vascular resistance was increased more than 3-fold. Skeletal muscle and spleen were the only tissues that showed effects approaching that in the tumor. However, even in these tissues, sig-
significant increases in vascular resistance were observed only at the highest L-NNA doses used (see Fig. 5), whereas in the tumor, there was a significant increase at 1 mg/kg, a dose that produced no significant increase in MABP. The increases in vascular resistance observed in tumor, skeletal muscle, and spleen most likely represent vasoconstriction, although a contribution from an increase in blood viscosity or modification of vascular permeability cannot be ruled out.

Administration of 200 mg/kg L-arginine 15 min after 1 mg/kg L-NNA completely reversed the decrease in tumor blood flow observed following administration of L-NNA alone. This is in contrast to the study by Meyer et al. (18), in which 60 min of exposure to L-arginine did not reverse the decrease in red blood cell velocity and venule diameter, observed in tumors grown in cutaneous window chambers in the rat, after 60 min of exposure to L-NMMA. Our result suggests that the decrease in tumor blood flow observed with 1 mg/kg L-NNA was due to a vasoconstrictive effect, which is reversible on restoration of NO levels with L-arginine administration. It was suggested by Meyer et al. (18) that the irreversibility of the vascular effects they observed in tumors could be due to endothelial damage and development of microthrombi resulting from platelet aggregation and leukocyte adhesion, both known effects of reduction in vascular NO (33, 34). The difference between the two sets of results may be due to differences in dose, routes of administration, and, particularly, drug exposure times. Different L-arginine analogues have also been reported to have somewhat variable biological effects (35). Our results suggest that systemic NOS inhibition, followed by L-arginine administration, does not produce a greater decrease in tumor blood flow relative to normal tissue blood flow than NOS inhibition alone. In fact, tumor blood flow is completely restored to control levels using L-arginine. However, the capacity for reversal of L-NNA effects in solid tumors and normal tissues at higher doses and longer exposure times would be worth investigating to test fully the hypothesis that tumor NOS inhibition, followed by normal tissue rescue using L-arginine, could be used to produce a selective reduction in tumor blood flow (18).

I.v. infusion of the NO scavenger C-PTIO at a dose of 1.7 mg/kg/min had no effect on MABP or blood flow to tumor and normal tissue. The negative effect on MABP is consistent with a previous report using the same dose of C-PTIO in a different strain of rats (21). However, scavenging NO by C-PTIO has been found to inhibit endothelium-dependent vascular relaxation ex vivo, reverse the acute hypotensive response associated with bacterial polysaccharide-induced endotoxic shock, and decrease solid tumor vascular permeability to Evans blue (20–22). Plasma levels of C-PTIO in our study were comparable to the concentrations required to produce these effects. The reason for the difference in effect between C-PTIO and L-NNA in our experiments is unclear. C-PTIO is susceptible to nonspecific reduction by a range of reducing agents, and it is possible that the concentration of active C-PTIO in vascular smooth muscle cells is too low to reduce NO levels sufficiently to induce vasoconstriction. Indeed, the use of liposome-encapsulated PTIO to circumvent problems with nonspecific reduction has been reported recently (36). The ability of C-PTIO to cross the blood-brain barrier and thus interfere with the neurotransmitter function of NO has not been reported to our knowledge. Because some of the vascular effects of NOS inhibitors may be mediated by effects on the central nervous system, a failure of C-PTIO to scavenge NO in the brain could partly explain the lack of vascular response. However, this is unlikely to be the case for the P22 tumor, in which local production of NO appears to be important for maintenance of vasodilator tone. Evidence for this comes from ex vivo perfusions of isolated tumors in which administration of L-NNA significantly increased vascular resistance (29). The radical product of NO scavenging by C-PTIO, C-PTI, has been reported to vasodilate in its own right (37), such that this compound could be reversing the vasoactive effect of NO scavenging. However, it is not clear whether the lifetime of this compound in biological tissue would be sufficient to explain our results with C-PTIO. Finally, if an adduct of NO, such as a nitrosothiol, rather than NO itself, is responsible for maintenance of vasodilator tone, this would explain the lack of effect of C-PTIO compared with L-NNA in our experiments. This possibility has great biological significance and is the subject of intense research (38).

L-NNA produced a reduction in tumor blood flow to 45% of control at a dose that caused no significant change in MABP or blood flow to normal tissues. Therefore, this approach is promising for enhancing therapeutic regimes that rely on an excessive tumor hypoxic fraction, such as chemotherapy using bioreductive drugs. It is also consistent with a tumor growth-retarding effect of chronically administered L-NAME, the pro-drug for L-NNA (39, 40). The tumor selectivity of blood flow modification is consistent with various reports of up-regulation of NO synthesis pathways in solid tumors of both experimental animals and humans. Typically, experiments used immunohistochemistry (9, 11–13, 41), Western blotting for different NOS isoforms (11, 12), and electron paramagnetic resonance (42, 43). It is also possible that differences in uptake rates of L-NNA into tumor versus normal tissue vasculature could contribute to the observed differential between blood flow changes in tumor and normal tissues at the time point chosen.

There is some evidence that the inducible isoform of NOS may be particularly important in tumors (9, 43), although the primary stimulus for iNOS production in tumors is unknown. There have been various reports of localization of iNOS in tumor cells (41), tumor-associated macrophages (44), and tumor neovascularature (9, 11), the latter suggesting a vascular role for NO derived from iNOS. If NO generated by iNOS controls vascular tone in tumors, systemic administration of a specific iNOS inhibitor could enhance the tumor-selective decrease in blood flow that we observed for L-NNA, the latter compound being an inhibitor that affects all NOS isoforms with some selectivity for the constitutive forms. This could occur via a retention of the vasoconstrictive effect in the tumor and avoidance of the systemic hypertensive effect of L-NNA, thus producing a larger decrease in tumor blood flow for the same degree of vasoconstriction. Our results suggest that a 3-fold reduction in tumor blood flow could be possible in the absence of an increase in MABP.

In summary, NOS inhibition using L-NNA, but not direct scavenging of NO using C-PTIO, produced a selective reduction in tumor blood flow that could be completely reversed using L-arginine. These results confirm the importance of NO production in tumors and suggest that NO is an important factor in maintaining a vasodilatory tone in tumors. NOS inhibition has potential for enhancing therapeutic regimens that would benefit from a selective reduction in tumor blood flow.

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