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Response of Neoplastic and Normal Vasculature to Acetylcholine

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Abstract—*Intravascular acetylcholine causes vasodilatation and an increase in normal tissue blood flow and could reasonably be expected to increase blood flow in malignant neoplasms. In this study using the microsphere reference sample technique, however, blood flow of V2 carcinoma, implanted in several organ sites in rabbits, was found to decrease after both an intra-arterial bolus and infusion of acetylcholine chloride. Cardiac output was not significantly changed. These observations may explain the recent report by Marincek* et al. *of a decrease in CT X-ray absorption in renal neoplasms after acetylcholine and previous reports of the lack of utility of vasodilators in improving the diagnosis of malignant neoplasms after arteriography.*

INTRODUCTION

THE UTILITY of using vasoconstrictors to enhance the diagnostic capability of arteriography has been described by several investigators [1–3]. This methodology has been especially useful in evaluating renal cell carcinomas. Observed cancer blood vessels do not vasoconstrict and it is reasonable to suggest that neoplastic blood flow is maintained, or perhaps even increased, relative to the surrounding normal tissue. If neoplastic blood flow was maintained or increased relative to the surrounding normal tissue, the delivery of intravascular chemotherapeutic agents or perhaps radioactive microspheres to the malignancy would be improved. However, in all cases where the blood flow of malignant neoplasms has been directly measured, blood flow decreases after vasoconstrictor administration [4–6]. In addition, there is some evidence that the higher the resting blood flow, the greater the reduction in both neoplastic and normal tissue blood flow after vasoconstrictors such as norepinephrine [4].

Vasodilators generally cause an increase in tissue blood flow and vascular dilatation. Since neoplastic blood flow decreases after vasoconstrictors, it seemed reasonable to expect that after vasodilators, an increase in blood flow should be observed. In addition, if the increase was proportional to the resting flow, then the use of vasodilators could be a useful technique to increase the blood flow in malignant neoplasms for a diagnostic and therapeutic advantage.

In this study intra-arterial acetylcholine was administered to rabbits with previously implanted V2 carcinomas in several organ sites. Blood flow was measured using the microsphere reference sample technique [7] in both neoplastic and normal tissue. The results were somewhat unexpected in that in all neoplasms studied the blood flow within the V2 carcinoma decreased. This suggests that vasodilators would not be useful in facilitating the delivery of intra-arterial chemotherapeutic agents or radioactive microspheres to neoplastic tissue.

MATERIALS AND METHODS

Twelve male New Zealand white rabbits, weighing approximately 2.5 kg each, were used in this study; they were housed in individual cages and fed a standard diet and water *ad libidum*.

A V2 carcinoma cell suspension was prepared by aspirating the viscous fluid through a 16-gauge needle from the intramuscular site of the carrier rabbits. The fluid was centrifuged at 2000 rev/min for 10 min, the supernatant decanted and the cell pellets resuspended in MEM 199. The viable cell number was microscopically estimated by the trypan blue vital dye exclusion method using a

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conventional hemocytometer. The cell concentration was then adjusted to yield at least 10^6 cells/ml.

Test rabbits were anesthetized intravenously with a mixture of 35 mg/kg ketamine and 5 mg/kg rompun. An abdominal midline incision was made and 0.3 ml of V2 carcinoma cell suspension was injected into each of the following organs: psoas muscle, kidney, spleen and liver (right lateral lobe, right medial lobe). Bleeding around the injection sites was controlled with topical thrombin. After closure of the abdominal cavity in two layers, the rabbits were injected with 150,000 I.U. Bicillin® i.m.

After an interval appropriate for tumor growth (12–24 days after inoculation), the rabbits were anesthetized and a catheter (Formocath 90) was advanced from the right carotid artery into the left ventricle of the heart for microsphere injection. Blood pressure was monitored in the contralateral carotid artery with a Statham pressure transducer (Type P 23 AC) and recorded on a Grass Polygraph. Two additional catheters (PE 100) were placed in the abdominal aorta via both femoral arteries, one proximally just below the diaphragm and the other distally above the aortic bifurcation. The former was used for acetylcholine administration and the latter for microsphere reference sample blood collection.

Microspheres (15 ± 5 μm; 3M Co.), labeled with ^{141}Ce, ^{51}Cr or ^{85}Sr respectively, were used in sequential order for tissue blood flow and cardiac output determinations. The microspheres, suspended in 10% dextran, were sonicated for 10 min and subsequently shaken vigorously for 5–10 sec before injection. Three 0.01-ml standards for each isotope were taken and placed in counting tubes. Subsequently, 1×10^6 microspheres were injected over the period of 1 min into the left ventricle. Simultaneously, an arterial reference blood sample was drawn from the femoral catheter with a Harvard pump calibrated at a rate of 4.026 ml/min. Blood withdrawal continued for 15 sec after termination of the microsphere injection. This was recorded as the control blood flow observation. Subsequently, a bolus injection of 20 μg of acetylcholine chloride,* dissolved in normal saline, was injected at a concentration of 20 μg/ml into the proximal abdominal aorta and flushed with heparinized saline; immediately following this the second labeled isotope was injected into the left ventricle. After sufficient time had elapsed for acetylcholine metabolism, and when the anesthetic level was stable, 10 μg/ml acetylcholine was infused with a Harvard pump at a rate of 0.57 ml/min. As soon as the effect (tachycardia) of acetylcholine infusion had reached a steady state the third isotope was injected. The acetylcholine concentrations were chosen, after initial dose–response studies, as the highest levels which could be given and not significantly affect cardiac output.

The rabbit was then killed by an overdose of sodium pentobarbital and an autopsy performed. The entire V2 carcinoma of each tumor site was excised and dissected free from surrounding normal parenchyma. The central grossly necrotic portion of the V2 was separated from the intact, grossly viable tumor periphery. Samples of normal host tissue, contralateral when possible (psoas muscle and kidney), were also taken. The specimens were weighed and divided among several counting tubes. The reference blood samples were also transferred to counting tubes.

Using pulse height analysis, the radioactivity was assayed in a three-channel Packard Auto Gamma Scintillation Counter Model No. 5360. The counts/min for each test tube containing the radionuclide, blood, tumor and normal tissue were calculated and fed into an Informatek, Simis 4 computer, which calculated the blood flow to each organ, expressed in ml/min/g, the percent of cardiac output to each organ and the total cardiac output. Cardiac output and blood flow before and after acetylcholine of both neoplastic and normal tissue were compared statistically using the unpaired Student's t test.

RESULTS

Neoplastic tissue

After intra-arterial administration of acetylcholine chloride, regional blood flow fell in all V2 carcinomas. Control blood flow in the grossly viable capsule of the V2 carcinoma varied with implantation site (Fig. 1). The highest V2 perfusion rate was found in the kidney (0.78 ± 0.18 ml/g/min, $\bar{x}$ + S.E.M.) and the lowest in the spleen (0.27 ± 0.09 ml/g/min). A bolus injection caused a relatively greater blood flow reduction than that seen during the infusion. Successful observations were made in twelve rabbits. Blood flow data from the two liver V2 neoplasms (right medial and lateral lobe) were not significantly different and were pooled.

The bolus injection of 20 μg acetylcholine produced a significant decrease in neoplastic blood flow in psoas muscle ($P < 0.025$), spleen ($P < 0.05$) and liver ($P < 0.025$) implantation sites when compared to resting flow. Blood flow also fell in the V2 kidney carcinoma, but not significantly ($P > 0.1$).

After the infusion of 10 μg/ml acetylcholine, at the rate of 5.7 μg acetylcholine/min, a reduction

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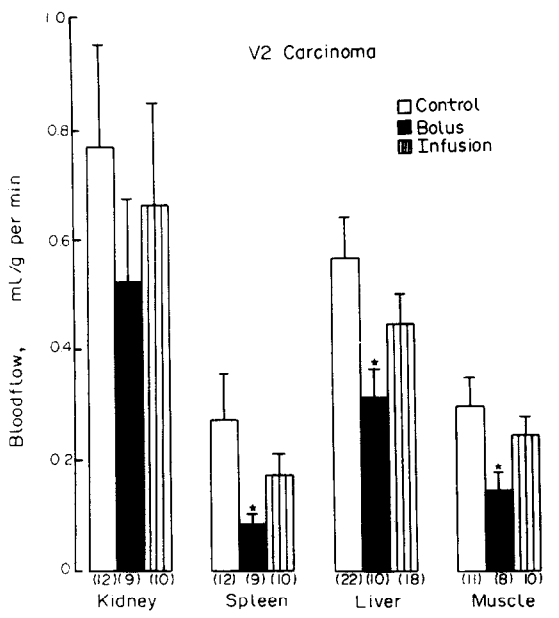

Fig. 1. Blood flow (ml/g/min) *in V2 carcinoma implants at rest (open column); after an intra-arterial bolus injection of* 20 μg *acetylcholine (solid column); and after an intra-arterial infusion of acetylcholine at* 5.7 μg/min *(hatched column). Bars: mean* ± *S.E.M.* ** = $P < 0.05$. *Number of tissue samples for each organ shown in parentheses. Data from both liver V2 sites (medial and lateral lobe) were not significantly different and were pooled.*

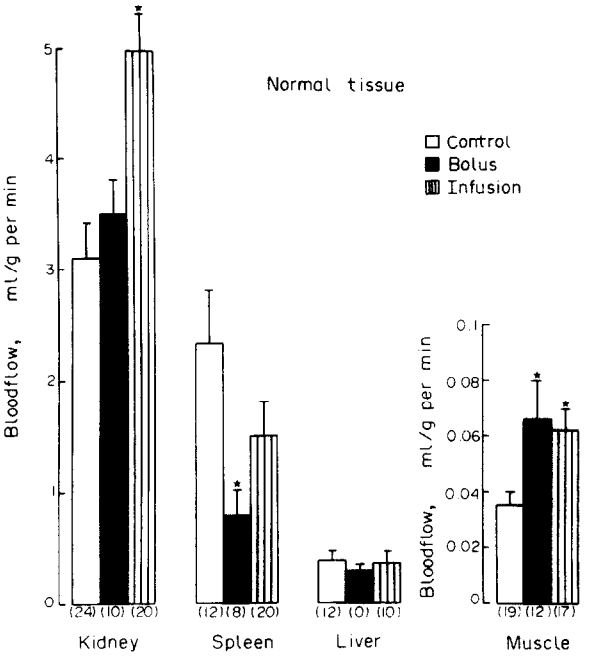

Fig. 2. Blood flow (ml/g/min) *of normal organs at rest (open column); after an intra-arterial injection of* 20 μg *acetylcholine (solid column); and after an intra-arterial infusion of acetylcholine at* 5.7 μg/min *(hatched column). Bars: mean* ± *S.E.M.* ** = $P < 0.05$. *Number of tissue samples for each organ shown in parentheses. Data from paired organs were not significantly different and were pooled.*

in mean neoplastic blood flow of kidney, spleen, liver and psoas muscle was noted, but none was of statistical significance ($P > 0.1$).

Normal tissue

An intra-arterial bolus injection of 20 μg acetylcholine caused a significant ($P < 0.025$) rise in psoas muscle blood flow from 0.04 ± 0.006 to 0.07 ± 0.02 ml/g/min; perfusion rate to the kidney also increased from 3.10 ± 0.28 ml/g/min to 3.51 ± 0.29 ml/g/min, but not significantly ($P > 0.1$) (Fig. 2). The significant ($P < 0.025$) decrease in splenic blood flow was probably due to the very large neoplasms which nearly replaced the normal tissue in this organ. The rapid tumor growth, however, was only noted in the spleen. The weight calculated from 12 rabbits yielded a mean of 3.83 g of V2 carcinoma and 0.65 g of remaining normal spleen. The decrease in liver blood flow was not significant ($P > 0.1$).

When 10 μg/ml acetylcholine was infused intra-arterially, at the rate of 5.7 μg/min, a significant increase in blood flow compared to control values was seen in the kidney ($P < 0.005$) and psoas muscle ($P < 0.025$). Blood flow in the kidney rose from 3.10 ± 0.28 to 5.08 ± 0.55 ml/g/min, while blood flow in the muscle increased from 0.04 ± 0.006 to 0.06 ± 0.01 ml/g/min. Regional blood flow in the spleen and liver fell when compared to resting flow, but not significantly ($P > 0.1$). Cardiac output in these experiments revealed no significant changes after either bolus or infusion of acetylcholine chloride.

DISCUSSION

The ability to selectively alter neoplastic blood flow with vasoactive agents would have important diagnostic and therapeutic implications. For example, radiographic contrast media and chemotherapeutic agents could be more selectively delivered to neoplastic tissue by increasing the regional blood flow to the neoplasm as opposed to surrounding normal tissue. Following intravascular norepinephrine or epinephrine, the diagnostic quality of the arteriograms of renal neoplasms is improved and, because the neoplastic blood vessels did not constrict, these images suggested that the neoplastic blood flow was maintained or perhaps even increased. However, in all studies where neoplastic blood flow has been measured directly after vasoconstrictor agents (norepinephrine, epinephrine, angiotensin, etc.), neoplastic blood flow fell [4–6, 8]. Some evidence even suggests that the magnitude in blood flow reduction is directly proportional to resting flow [4].

If the blood flow in neoplastic tissue can be decreased with vasoconstrictors, it seems reasonable to expect that neoplastic blood flow would perhaps increase after vasodilator agents. If so, this characteristic could be exploited to deliver

more selectively either diagnostic or therapeutic intravascular agents. However, in this study the administration of acetylcholine uniformly caused a decrease in neoplastic blood flow. Acetylcholine causes vasodilation by stimulating vascular innervation and subsequent relaxation of smooth muscle. Other vasoactive agents with different receptor sites might be expected to produce vasodilatation. However, prostaglandin E_2, which acts at the endothelial level, has also been shown by Rankin *et al.* [8] to decrease neoplastic blood flow. Similarly, isoproterenol has been shown to decrease neoplastic blood flow, although this effect is probably as much mediated by systemic changes in cardiac output as by the beta stimulation of the vasculature by this agent [9]. Thus it seems that across the broad spectrum of vasodilator agents a uniform decrease in neoplastic blood flow occurs after their administration.

A possible explanation for the blood flow decrease after acetylcholine in the different tumor loci evaluated could be a steal phenomenon in that the neoplastic vessels are insensitive to this drug and are maximally dilated at rest. The dilatation and blood flow increase in surrounding normal tissues would divert blood away from the tumor, leaving the neoplasm with a decreased blood flow. This hypothesis has also been suggested by Jirtle *et al.* [9], who reported unchanged tumor vascular resistance after isoproterenol.

The possibility that acetylcholine produced more A–V shunting within the V2 neoplasms and, as a result, microsphere estimations underestimated neoplastic blood flow could also explain these observations. This possibility seems unlikely in that normal liver blood flow actually declined slightly after the administration of acetylcholine, indicating that very few, if any, microspheres were shunting via the portal system. In addition, pulmonary blood flow did not suggest systemic shunting. The generally low level of blood flow in these studies is presumably due to the large tumor burden of the animal [10].

Vasodilatation and increased tissue blood flow are well-known effects of intravascular acetylcholine on normal tissue [11, 12]. In this study significant increases in normal renal and muscle blood flow were observed. The spleen actually showed a decrease in blood flow; however, this is, in all likelihood, related to the large neoplastic burden within the spleen and the fact that very little normal splenic tissue remained in any of these animals. Actually, splenic flow was found to be quite variable, an observation previously made in the case of normal rabbits by Neutze *et al.* [13]. A slight decrease in liver blood flow was also identified, but this decrease was not significant. In this context it should be emphasized that the microsphere method only measures the blood flow in the hepatic artery. Although the portal venous vasculature has been reported to be relatively insensitive to acetylcholine [14], no definite conclusions can be drawn from these studies about the effect on overall hepatic blood flow since portal blood flow was not measured.

The differences in blood flow observed between bolus and infusion administration in this study are interesting. However, the reason for the larger decrease in blood flow in V2 carcinomas after bolus as opposed to infusion administration and, conversely, the generally larger increase in blood flow in normal tissues after infusion as opposed to bolus administration is not readily apparent. Although a variable response between bolus and infusion administration has been noted by other authors [15, 16], the decrease in neoplastic blood flow after acetylcholine observed in this study probably explains our previous CT observation [17] of a decrease in the X-ray absorption of renal neoplasms after acetylcholine administration. These observations may also, in part, explain the lack of utility of acetylcholine in the angiographic evaluation of neoplasms [18]. There are, however, studies of the use of acetylcholine in renal pharmacoangiography whose results appear to differ from those reported here [19, 20].

The results in this study, using acetylcholine, and in previous studies, using prostaglandin E_2 [8] and isoproterenol [9], suggest that vasodilator agents will not be useful in improving the diagnostic quality of angiography or CT scanning or in more selectively delivering intravascular agents to neoplastic tissue. However, the ability to decrease blood flow in neoplastic tissue and increase blood flow in surrounding normal tissue might be very useful in the thermal treatment of malignant neoplasms, using hyperthermia. A decrease in blood flow within the neoplastic tissue would tend to create a heat sink in that heat would not be conducted away from the neoplasm by the perfusing blood and, conversely, normal tissue in the field of heating would be cooled by the increased blood flow and conduction of the heat away from the normal tissues.

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