Manipulation of Experimental Rat and Rabbit Liver Tumor Blood Flow with Angiotensin II

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ABSTRACT
The effects of angiotensin II on the distribution of blood flow to experimental hepatic tumors in ten rats and rabbits were examined using blood flow tracer microspheres. The ratio of arterially introduced microspheres lodging in tumor tissue compared to the surrounding normal hepatic parenchyma was measured before and after i.v. infusion of angiotensin II-inducing incremental systemic responses. A significant elevation ($P < 0.05$) in this ratio was described for both rats (3.0-fold) and rabbits (3.2-fold) following the drug infusion. Ratio elevation occurred in 37 of 40 tumors examined despite the lack of a clear dose-response relationship. In addition, angiotensin II was found to significantly ($P < 0.05$) increase the number of microspheres gaining arterial access to the central portions of the tumors. In terms of internal radiation therapy, these results would indicate a substantially enhanced radiation dose reaching tumor tissue after angiotensin II infusion, while relatively sparing the surrounding normal tissue.

INTRODUCTION
The liver is by far the most significant site of dissemination of colorectal cancer where approximately 50% of deaths are due solely to hepatic metastases (1). Contention exists as to what treatment modality provides the best means of management, but the technique of localized internal radiation therapy is slowly gaining interest (2-7). The method requires an intrahepatic arterially introduced suspension of $^{90}$Y-saturated polystyrene microspheres to become preferentially entrapped in the metastases (8). The subsequent radiation dose to the tumor tissue is required to be substantially greater than to normal tissue for optimal therapeutic benefit. The isotope $^{90}$Y has been utilized mainly for its pure $\beta$ radiation and because its mean penetration of 0.25 cm in liver tissue will confine radiation damage to the immediate area of greatest microsphere accumulation.

Arterial hypervascularity in the growing edge of the tumor has been demonstrated by the preferential embolization of 15 $\mu$m-diameter microspheres at a rate almost 4 times that in normal liver parenchyma (9, 10). Several studies have suggested that neoplastic vessels may lack the ability to react to vasoactive agents, thus implying that the tumor blood supply is maintained during the influence of vasoactive agents acting on normal vessels (11-15). As a result of this phenomenon, the ratio of tumor blood supply to normal tissue blood supply, which is normally greater than one, may be even further enhanced by the introduction of vasoconstrictor agents.

Adrenergic agents have been used to alter the distribution of blood flow to tumor tissue with varying success (16, 17). This is probably associated with different receptor populations in different organ systems producing antagonistic effects (18). The vasoconstrictor peptides, vasopressin and angiotensin II, have also been assessed during angiography of renal neoplasms (19, 20). Vasopressin evokes hepatic arterial vasoconstriction but is only of short duration. However, angiotensin II is one of the most potent hepatic arterial constrictors (18, 21).

In conjunction with research into the therapeutic effectiveness of internal radiation therapy utilizing $^{90}$Y-labeled microspheres, we have studied the influence of angiotensin II on the hepatic distribution of microspheres in animals with implanted liver tumors. Two animal models, rats and rabbits, were examined to determine the ratio of injected microspheres embolizing in the tumor vasculature compared to normal hepatic vasculature (namely, the therapeutic ratio) in both a control situation and following venous infusion of angiotensin II.

MATERIALS AND METHODS

Animals. Ten inbred DA rats with an average body weight of 216 ± 20 (SD) g had small segments (1 mm$^3$) of mammary adenocarcinoma implanted into both the left and right medial lobes of the liver 7–9 days prior to experimentation. The tumor originated in the Monash University, Melbourne, with the DA rat colony arising from an immunosuppressed animal. Ten New Zealand white rabbits with a mean body weight of 2.44 ± 0.36 kg were similarly implanted with VX2 carcinoma 11–14 days prior to experimentation. This tumor, from the Australian National University, Canberra, has been used extensively by our group (8). All resultant tumor growths were tested individually. However, one tumor from each of four rabbits failed to develop and so was not included in the study. At the time of final operation, tumors in the rat livers were 5–10 mm in diameter, while those in the rabbits were 5–20 mm in diameter.

Radioactive Microspheres. Commercially produced (Nenterac; New England Nuclear Co.) polystyrene copolymer tracer microspheres with a diameter of 15 $\mu$m and labeled with either $^{60}$Co, $^{103}$Ru, or $^{113}$Sn were used to mimic the distribution of the similar $^{90}$Y therapeutic spheres produced in our laboratory. In the rats, each injection contained approximately 8 × 10$^5$ spheres, while the rabbits were given injections of approximately 4 × 10$^6$ spheres in heparinized 10% dextran. Tissue samples were counted in a 2-channel well-type $\gamma$-counter with sample size maintained constant to minimize geometrical errors. The therapeutic ratio was determined under control conditions from radioactive counts of embolized spheres labeled with $^{113}$Sn and after infusion of angiotensin II, using the counts of spheres associated with either of the contrasting isotopic labels. The ratio was described by the mean activity per unit of tumor tissue compared to normal liver tissue under the two conditions in each individual animal following sacrifice.

Procedure. Under sodium pentobarbital anaesthesia in rats and halothane-nitrous oxide in rabbits, polyethylene catheters (A, 1 mm; inside diameter, 0.5 mm) were introduced into the ascending aorta via the right carotid artery and into the femoral vein of each animal. The carotid cannula allowed both injection of the microspheres and provided a means
of monitoring mean arterial blood pressure. The femoral cannula was used for saline control infusions and angiotensin II infusions.

At the commencement of each experiment, normal saline was infused for the control measurement. When the blood pressure was shown to be constant for at least 5 min, the first injection of spheres was pulsed into the aorta over a period of approximately 30 s. The blood pressure was again monitored following the injection, and the angiotensin II was then infused to produce a measured increase in systemic pressure. When the desired blood pressure was steadily maintained, the second sphere injection was made in a similar manner. After each injection, the catheter and stopcock were flushed twice with 0.2 ml of normal saline in rats and 0.4 ml in rabbits.

The normal saline infusion rate was 0.2 ml/min in both animal models, while in the rat, angiotensin II solution was infused at rates of 0.9–2.25 μg/min determined by the desired response. In rabbits, the infusion of angiotensin II was at rates of 2–5 μg/min. Fifteen min after the final injection, the animals were sacrificed, and the liver and kidneys were removed and fixed in 10% buffered formalin. Samples of renal cortex from each kidney were taken to compare the relative magnitude of counts in each kidney to provide a measure of sphere mixing in the blood. In the rats, 60 liver samples weighing 0.10–0.15 g were taken and 70 samples in the rabbits. These samples were taken from (a) the central portions of the tumors in rabbits (but not in the smaller rat tumors), (b) from the growing edge of the tumor, (c) the normal liver tissue from the lobe in which the tumor was implanted, and (d) the rest of the normal liver lobes. The specific activity of each sample was measured, and the mean ± SD for each liver compartment was calculated for determination of the control and the drug-induced therapeutic ratio.

The response of the liver vasculature to angiotensin II was examined under systemic pressure elevations principally within the range of a 20–40% increase above control pressure. In addition, a small number of animals were exposed to pressures above and below this range.

Statistics. The difference between the therapeutic ratio, determined from tumor and normal tissue samples, under control conditions compared to the ratio after drug infusion was measured with Student’s t test for paired observations. This test was also used to determine differences in the ratio of spheres lodged in the tumor center compared to normal tissue. Variations in the percentage coefficient of variation for normal tissue before and after drug infusion were tested with a 2-tailed t test. Tests were carried out on the results for each animal.

RESULTS

The initial control injection of microspheres was found not to influence the cardiovascular responses of the animals prior to angiotensin II infusion or the introduction of the second population of microspheres. Animals receiving doses of angiotensin II that induced blood pressure responses less than approximately 50% above control levels survived the surgical manipulation with no visible signs of distress. However, larger doses of angiotensin II produced both cardiac and respiratory anomalies.

Rats. There was a statistically significant (P < 0.025) increase in the therapeutic ratio in 17 of the 20 tumors examined in rats after the introduction of angiotensin II. Chart 1 describes the magnitude of increases in the ratio of microspheres lodged in the tumor compared to those lodged in the normal hepatic vasculature during infusion of angiotensin II at various doses inducing elevations in mean systemic blood pressure between 10 and 60 mm of Hg. The variation in the therapeutic ratio was found to be only marginally dose dependent.

The initial control therapeutic ratio (i.e., before drug infusion) for all rats averaged 3:1 (range, 1.1–6.8:1), while the mean therapeutic ratio after a blood pressure elevation of approximately 25% was 9:1. This resulted in a 3-fold increase in the ratio for that blood pressure change. There was a large degree of variation in the drug-induced therapeutic ratio ranging from 2:1 to 23:1, but this was not obviously related to drug dose. The therapeutic ratio in two tumors did not increase significantly above control levels, and in one tumor, the increase (20%) was also not statistically significant (P > 0.05).

Rabbits. In all cases the therapeutic ratio was significantly (P < 0.05) enhanced above that of the control ratio. Chart 2 describes the extent of the increase in the ratio of microspheres embolized in the tumor vasculature compared to normal tissue from control to angiotensin II levels over a range of blood pressure increments.

There was no obvious relationship between the ratio response and either angiotensin II dose or change in blood pressure. In contrast to the results in the rat model, the control therapeutic ratios were high with a mean ratio of 48:1 and a range of 3:1 to 222:1. After administration of angiotensin II to increase the blood pressure by approximately 25% over control levels, the mean ratio was increased to 155:1. This was equivalent to an enhancement of the therapeutic ratio to more than 3 times the control.

The central portion of most of the large tumors in rabbits was found to be relatively avascular and necrotic. Under control conditions, this area of central necrosis received a relatively small number (20%) of microspheres compared to the growing edge of the tumor and, in some cases, received less than the normal tissue surrounding the tumor. Table 1 demonstrates an increase in the relative proportion of microspheres reaching the central portions of tumors after angiotensin II. The ratio of spheres lodged in the tumor center compared to the normal tissue vasculature was enhanced in all animals examined, and this enhancement in each case was statistically significant (P < 0.05). However, the mean ratio of center to tumor edge did not significantly change from control (0.16:1) to drug infusion (0.23:1).

The coefficient of variation (standard deviation divided by the mean) of distribution of microspheres into the normal liver lobes in rats was 45.4 ± 9.2% under control conditions. After angiotensin II infusion, the coefficient of variation for animals with a pressure elevation of approximately 25% was 55.1 ± 12.2%. In rabbits, the corresponding coefficients were 74.3 ± 15.1% and 77.8 ± 17.0%, respectively. In both models, the change in pattern of microsphere distribution to normal liver parenchyma was not significant (P > 0.05).

There was no significant difference in the number of microspheres lodging in the cortex of either kidney in the two animal groups. Under control conditions, the ratio of counts in left kidney to right was 1.05 ± 0.24 in rats and 0.90 ± 0.15 in rabbits. Infusion of angiotensin II did not significantly alter this distribution pattern (1.05 ± 0.15 and 1.02 ± 0.30 in rats and rabbits, respectively), thus indicating good mixing of microspheres in the aortic bloodstream under both control conditions and after drug infusion.

DISCUSSION

During the progressive growth of a tumor, the host vessels are replaced by new nutrient vessels under stimulation by angiogenetic factors. These neoplastic vessels may develop anatomically, but they do not retain normal physiological function. Reg-
ulation of blood flow velocity, direction, pressure, and capacity is lost (22). However, although the tumor vascular bed exhibits little blood flow regulation, the arteriolar supply to the tumor residing in adjacent normal tissue is subject to normal vasomotor control (23, 24). Therefore, vasoconstrictor agents can exert an indirect influence on the tumor blood flow.

Angiotensin II has been assessed in renal pharmaecoangiography to reduce blood flow in normal tissue, while allowing pooling of contrast medium in tumor vessels (25). This drug acts more peripherally in the vascular bed than other vasoconstrictors do and therefore should have less effect on the arteries feeding the tumor while still providing a net decrease in flow to the smaller vessels of the normal tissue (26).

To ensure that internal radiation therapy can provide the greatest benefit, the optimal therapeutic ratio must be obtained. We have described a significant improvement in the number of microspheres delivered to both the growing edge of tumor tissue and in the center of the tumor compared to the unaffected hepatic vasculature, following the infusion of angiotensin II.

In both animal models, the relationship between the dose of angiotensin II administered and the response in terms of the therapeutic ratio was unpredictable. This was primarily a result of the large degree of individual variation encountered from tumor to tumor, both in the control ratio and the drug-enhanced ratio over a wide range of responses. This variation probably resulted from individual fluctuation in the size and maturity of the tumors influencing their vascular morphology. Despite the high degree of variability in the magnitude of the ratio, in almost all cases, angiotensin II improved the proportion of spheres delivered to tumor tissue. These results are supported by a number of studies.

In 1981, Tveite et al. (19) demonstrated that angiotensin II infusion had a lower blood flow-reducing effect on tumors (16%) than on intact renal tissue (41%). Similarly, Suzuki et al. (27)
described an approximate 6-fold selective increase in blood flow to subcutaneous tumors without increasing blood flow to normal tissue. Similar results have also been reported in rat liver tumors using noradrenaline (16), rat kidney neoplasms using oxytocin, noradrenaline, and vasopressin (20), and mouse mammary tumors with adrenaline, noradrenaline, and isoproterenol (15). The use of vasoconstrictors in conjunction with internal radiation therapy for hepatic metastases in humans was described in a qualitative report by Grady et al. (28). Utilizing adrenergic agents, they were able to produce a vasoconstricting effect on normal vasculature and provided evidence of increased concentration of microspheres in tumor tissue.

Under control conditions, microspheres were found to lodge preferentially around the growing edge of the tumor. This would provide access of radiation to the area of greatest neoplastic activity. However, the central portions of the tumor which, though relatively dormant, harbor the potential for further tumor growth received proportionately low numbers of microspheres and, therefore, possibly suboptimal radiation doses. The effect of the angiotensin II was not only to potentiate the tumor edge:liver ratio but to potentiate the ratio of microspheres in the tumor center to normal liver, thus enhancing the therapeutic ratio of the total fraction of tumor tissue. This effect has also been described by Krylova (22) in implanted tumors in rats using adrenaline, bradykinin, and glucagon (29).

Homogeneity of distribution of spheres within the liver is described as a coefficient of variation. A low coefficient is synonymous with homogeneous distribution. In both animal models, the coefficient of variation was relatively high in comparison with those described by Chamberlain et al. (8), resulting from dissimilar sphere injection techniques. The important point to be taken from the present results is that the coefficient of variation was not significantly altered by the infusion of angiotensin II.

We conclude that beneficial manipulation of the blood supply to hepatic tumors may be mediated through the infusion of angiotensin II. Though the magnitude of the improvement in the therapeutic ratio is not a constant function of drug dose, it is a significant reproducible phenomenon within two species. The increase in the net proportion of arterial blood flow to tumor tissue will provide enhanced access of cytotoxic agents to both the central portions and the growing edge of the tumor, while relatively sparing the normal liver tissue.

REFERENCES


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