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Effect of Angiotensin II on Blood Flow in the Transplanted Sheep Squamous Cell Carcinoma*

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Abstract—*The effects of systemic infusion of angiotensin II on the distribution of blood flow in the sheep squamous cell carcinoma after transplantation to the liver were measured using tracer microspheres. The ratio of arterially introduced radioactive microspheres embolizing in tumour tissue compared to normal hepatic parenchyma was measured before and after infusion of angiotensin II. Doses of angiotensin II inducing increases in mean arterial blood pressure of* 26 mmHg *produced significant increases in the embolization ratio from* 2.8 *to* 4.1:1. *In addition, the ratio of microspheres gaining access to the necrotic centres of the tumours compared to the normal liver tissue significantly increased from* 1.6 *to* 2.3:1. *In terms of the technique of internal radiation therapy for hepatic metastases, the concurrent infusion of angiotensin II with injection of radioactive microspheres would result in a substantially enhanced radiation dose to liver tumours. At the same time the dose to normal tissue can be minimized.*

INTRODUCTION

METASTATIC hepatic cancer is presently one of the most common and recalcitrant forms of human malignancy. However, the prognosis for treated hepatic metastases is little different to that of the untreated disease with a median survival time of approx. 7 months from the time of diagnosis [1, 2]. Various treatment modalities have been assessed with minimal success, but internal radiation therapy has recently been the subject of clinical and experimental investigation [3, 4].

Internal radiation therapy requires an intrahepatic arterially introduced bolus of yttrium-90 impregnated microspheres to become entrapped in the microvasculature of liver tumour tissue. Yttrium-90 is a pure β emitting isotope with a mean tissue penetration of 0.25 cm which will confine radiation damage to the immediate area of microsphere accumulation. The greatest therapeutic benefit will be obtained when the subsequent radiation dose is substantially greater in tumour tissue rather than in normal tissue. For this to occur the microspheres must be actively shunted into tumour tissue.

Several publications have recently shown that the administration of vasoactive drugs prior to the introduction of microspheres into the hepatic artery causes preferential tumour embolization [5, 6]. This occurs in addition to normal tumour arterial hypervascularity described for the liver [7] and results from the relatively physiologically unreactive neovasculature of hepatic metastases [8]. Vasoconstrictors act on normal liver vasculature with generalized vasoconstriction but not on the resident tumour tissue, with a net blood flow response favouring the tumour supply. Blood borne microspheres will also embolize in the tumour tissue in proportion to that blood flow.

In 1986, Harker *et al.* [9] described the suitability of the sheep epidermal squamous cell carcinoma as a solid tumour model. We have further examined the suitability of this tumour for utilization in assessing the action of vasoactive drugs after transplantation to the liver. This study was designed to determine the effect of the potent vasoconstrictor peptide angiotensin II on the distribution of microspheres in transplanted tumour and normal liver tissue in the sheep model.

MATERIALS AND METHODS

Animals

Five crossbred merino sheep with an average body weight of 42.3 ± 5.2 kg (S.D.) were utilized for these experiments. The animals each had a spontaneous tumour occurring on one of either the ear, nose or vulva. The epidermal squamous cell carcinoma which arises spontaneously in approx.

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1% of sheep in Australia has been described in detail by Harker *et al.* [9]. Small segments (1 mm^3) of healthy tumour tissue were transplanted from the primary site from each animal into both the left and right lobes of the liver. The primary tumour was then completely resected and after recovery the animals were maintained for 12 weeks prior to experimentation.

Radioactive microspheres

Polystyrene copolymer tracer microspheres (Nentrac, New England Nuclear) with a diameter of 15 μm labelled with either cobalt-57 or tin-113 were used to measure the ratio of blood supplying the implanted tumour tissue and the surrounding normal hepatic parenchyma. Each injection of microspheres contained approx. 3×10^6 microspheres carried in heparinized 10% dextran. Activity was determined in tissue samples using a 3 channel Gamma counter ensuring that the sample size was maintained constant to minimize geometrical errors. The activities thus measured were used to determine the ratio of microspheres embolizing in tumour tissue compared to normal hepatic tissue (*T/N*) under control conditions and after the systemic infusion of angiotensin II. The control *T/N* ratio, during the infusion of saline, was measured using one set of labelled microspheres (e.g. cobalt-57) while the *T/N* ratio associated with the blood flow after angiotensin II was measured with the contrasting labelled microspheres.

Procedure

Under halothane–nitrous oxide anaesthesia a laparotomy was performed and the hepatic artery of the sheep exposed. Gastric or duodenal vessels leading from the hepatic artery, distal to the injection site at the junction with the gastroduodenal artery, were ligated. A 1 mm (outside diameter) polyethylene catheter was tied into the gastroduodenal artery with the tip of the catheter placed at the junction with the hepatic artery. At this point the common hepatic artery generally divides into the left and right hepatic arteries. This catheter was used for the introduction of the microspheres.

Similar catheters were introduced into the femoral vein and femoral artery of the sheep, the former for infusion of saline as a control and for the angiotensin II and the latter for the measurement of the animal's mean systemic blood pressure.

At the commencement of each experiment, normal saline was infused and the blood pressure monitored. The first population of microspheres was pulsed into the hepatic artery over 30 s when the pressure was constant for at least 5 min. The angiotensin II was then infused at an increasing dose until the blood pressure was maintained at 25% above the control level. When this pressure was constant the second population of microspheres was then injected. After each injection the catheter and stopcock were flushed twice with 0.4 ml of normal saline. The infusion rates were 0.6–1.0 ml/min and the concentration of angiotensin II was 20 μg/ml.

Fifteen minutes after the final injection, the animals were sacrificed and the liver removed and fixed in 10% formalin. The liver of each animal was then divided into approx. 200 samples weighing 1.0–1.5 g. These samples were taken from (a) the central portions of the tumour if areas of macroscopic necrosis were present, (b) from the growing edge of the tumour, and (c) from the rest of the surrounding normal liver. The specific activity of each sample was measured, and the mean ± S.D. for each of the above liver compartments was calculated for determination of the *T/N* ratio and the ratio of microsphere embolization in the central portions of the tumour compared to the normal parenchyma (*C/N*).

Statistics

The difference between the *T/N* and the *C/N* ratios under control conditions compared to those after angiotensin II infusion was measured with Student's *t* test for paired observations. This test was also used to determine variations in the percentage coefficient of variation for normal tissue samples before and after drug infusion.

RESULTS

The initial control injection was found not to influence the cardiovascular responses of the sheep prior to angiotensin II infusion or the introduction of the second population of microspheres. There were no adverse reactions to the doses of angiotensin II used throughout the experiment. From the five sheep examined there was a total of 28 tumours with a mean size of 10.7 ± 5.3 mm (S.D.). Of these, 26 had central portions of tumour necrosis. The mean increase in blood pressure induced by the angiotensin II in the sheep was 26.8 ± 2.9% above each individual control measurement.

There was an increase in the *T/N* ratio (tumour growing edge) for 27 of the 28 tumours examined (Fig. 1). The *T/N* ratio for one tumour dropped by 7%. The mean control *T/N* ratio for all tumours was 2.8:1 (2.5 S.D.) which was increased after the angiotensin II infusion to 4.4:1 (3.4 S.D.). The average increase was 106 ± 89% from control to drug induced change which was significant at $P < 0.001$.

The C/N ratio (central necrosis to normal tissue) increased in 22 of the 26 tumours from a control of 1.6:1 (2.5 S.D.) to 2.3:1 (3.3 S.D.) after the angiotensin II (Fig. 2). This was equivalent to

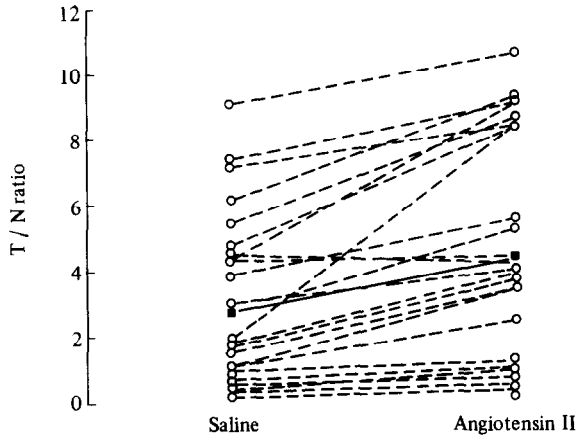

Fig.1. Changes in the tumour growing edge to normal liver tissue ratio (T/N) *in sheep after the infusion of angiotensin II. Boxes indicate group means.*

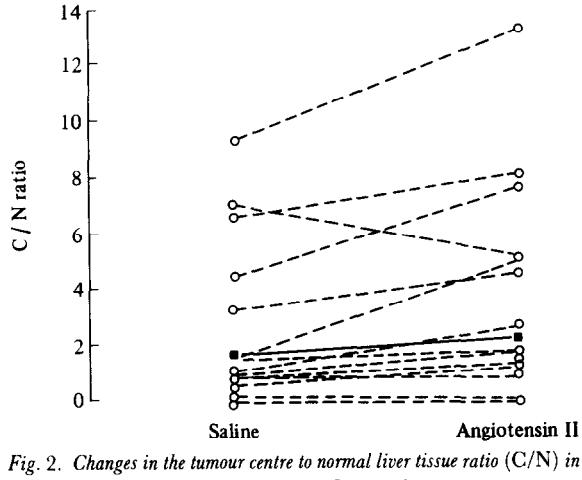

Fig. 2. Changes in the tumour centre to normal liver tissue ratio (C/N) *in sheep after infusion of angiotensin II. Boxes indicate group means.*

an average increase of 84 ± 131%, significant at $P < 0.01$.

The percentage coefficient of variation for the samples of normal liver was determined as a measure of the homogeneity of distribution of the microspheres on embolization. The mean coefficient for the control measurement was 52.4 ± 29.3% which decreased after the infusion of angiotensin II to 39.4 ± 13.4%. The decrease, in two of the five sheep, was not statistically significant ($P > 0.05$).

DISCUSSION

Neoplastic vessels have been shown to develop anatomically under stimulation by angiogenetic factors but they do not retain normal physiological function. They do not have the ability to regulate blood flow, direction or capacitance and they do not have a normal neural innervation [10]. Because these vessels are relatively physiological inert, a degree of blood flow control in tumour tissue can be exerted through the vasoactive manipulation of the surrounding normal hepatic vasculature. Angiotensin II as a potent hepatic arterial vasoconstrictor [11] will cause generalized vessel constriction in the normal tissue but not in tumour tissue, resulting in a net favouring of blood flow to tumour tissue. Blood borne microspheres will therefore favour the tumour vasculature when used in conjunction with angiotensin II.

The efficacy of internal radiation therapy is dependent on the preferential delivery of radioactive microspheres to tumour tissue rather than to normal liver tissue. We have described a significant improvement in the number of microspheres delivered to both the growing edge of tumour tissue and to the centre of the tumour compared to the unaffected hepatic vasculature, following the infusion of angiotensin II. We have previously reported a similar response in tumours in rats and rabbits after systemic angiotensin II [5] but this large animal model has allowed the determination of *T/N* and *C/N* ratios after infusion of the drug directly into the hepatic artery.

Angiotensin II has been used in renal and muscle pharmacoangiography to highlight tumour blood flow in humans [12, 13]. More recently Sasaki *et al.* [14] have advocated the use of angiotensin II with intrahepatic arterial infusion chemotherapy to provide better accessibility of chemotherapeutic drugs to tumours. They described significant increases in the *T/N* ratio in humans from a mean of 1.5:1 to 4.3:1 using hepatic scintigraphy. The degree of increase in the *T/N* ratio agrees favourably with the results recorded for the sheep, though with this model it was also possible to measure changes in the blood supply to the tumour centre. In this region of relative avascularity and necrosis, but still with the potential for further tumour growth, the *C/N* ratio also increased significantly with angiotensin II. This means that the total fraction of liver tissue can be induced to receive disproportionate numbers of radioactive microspheres, rather than just the growing edge.

The homogeneity of the distribution of the microspheres within the liver was described by the percentage coefficient of variation, a relatively low coefficient being synonymous with even distribution. The coefficients for the normal liver compared favourably with those reported for rats and rabbits [4, 15]. The infusion of angiotensin II had no significant influence on the distribution patterns of the microspheres in the normal liver tissue. Thus the potential for the occurrence of local accumulations of microspheres and therefore radiation was not affected by the vasoconstrictor.

CONCLUSION

We would conclude that the vasoconstrictive effects of infusion of angiotensin II concurrent with the injection of radioactive microspheres into the

hepatic artery will provide preferential delivery of the microspheres to tumour tissue. The increase in the net proportion of blood supply to the tumour tissue provides greater access to cytotoxic agents to both the central portions of the tumour and the growing edge of the tumour, while relatively sparing the normal tissue. The transplantable sheep squamous cell carcinoma provides an excellent model for tumour blood flow studies in a preparation of similar size and morphology to the human situation.

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