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Distribution of Different Sized Microspheres in Experimental Hepatic Tumours*

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Abstract—*The extent of embolization of different sized radioactive microspheres in experimental tumours and the homogeneity of their distribution in normal liver was examined in* 25 *rats. The ratio of arterially introduced microspheres lodging in tumour tissue compared to the surrounding normal hepatic parenchyma was measured for* 15, 32.5 *and* 50 μm *diameter tracer microspheres. The mean tumour to liver arterial perfusion ratio (T/L) for* 15 *and* 32.5 μm *spheres was approximately* 3 : 1 *in both cases and there was no significant difference between these values* ($P > 0.05$). *However,* 50 μm *microspheres did not preferentially lodge in malignant tissue (mean T/L ratio* 1 : 1). *The homogeneity of distribution of microspheres embolizing in the normal liver tissue was assessed for each microsphere size. As microsphere diameter increased from* 15 *to* 50 μm, *microspheres lodged more evenly throughout the liver substance. For* 15 μm *microspheres the coefficient of variation was* 55.5% ± 8.3 *and* 32.5 μm *microspheres distributed with a coefficient of* 35% ± 16.8 *while* 50 μm *spheres distributed most evenly with a coefficient of* 19.7% ± 6.8.

INTRODUCTION

INTERNAL hepatic radiation therapy is an encouraging new treatment modality for metastatic liver cancer. It utilizes intra-arterially administered microspheres labelled with a high energy, short range isotope capable of destroying cancer cells e.g. Yttrium 90 or Phosphorus 32. Microspheres are injected into the hepatic artery to flow in blood vessels which feed the malignant tissue and because hepatic metastases are supplied almost exclusively by the hepatic artery as opposed to portal vein [1–6] microspheres become preferentially trapped in tumour tissue.

Two basic criteria for the success of this treatment are:

(1) a preferentially greater arterial blood flow to the tumour is necessary if microspheres are to concentrate in malignant tissue.
(2) homogeneous distribution of microspheres in normal liver is required to minimize liver necrosis due to local accumulation of radioactive microspheres.

These parameters have been assessed for conventional 15 μm blood flow tracer microspheres. Previous studies have shown that the concentration of microspheres lodged in tumour compared to normal liver is consistently of the order of 4 : 1 [5] and their embolization in normal liver is homogeneous [7].

Tumour vasculature is characteristically aberrant, both morphologically and functionally [1–3, 8] and the individual variation between tumours is marked. Histologically, blood vessels within metastatic carcinoma of the liver have an afferent supply from the hepatic artery and show a morphologic pattern dependent on the type of differentiation and rate of growth of the tumour.

Capillaries within rapidly growing tumours have an average diameter up to five times greater than normal, appearing as sinusoid-like vessels with little tendency to differentiate into arterioles and venules. Ackerman and co-workers [8] assessed the dimensions of the new vessels in a Walker 256 carcinoma as ranging from 25–75 μm in diameter; compared to normal capillaries of 1–8 μm and arterioles 20–30 μm in diameter.

It may be expected that the pattern of embolization of intra-arterially administered microspheres in the liver and tumour may vary with the size of microsphere employed in a manner dependant on the anatomy of the tumour microvasculature. It has also been reported that larger microspheres do not mix well with blood and thus

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distribute poorly throughout the circulation [9, 10]. However, the relevance of this to intra-organ distribution has not been investigated.

This study was designed to examine the pattern of distribution and the extent of lodgement of microspheres in tumour and normal tissue as a function of microsphere size.

MATERIALS AND METHODS

Animals

Twenty-five inbred, adult male DA rats (200–350g) had small segments (1 mm^3) of salivary adenocarcinoma [11] implanted into both the left and right medial lobes of the liver, 7–10 days prior to systemic injection of microspheres. All resultant tumour growths were tested individually after achieving diameters of 4–10 mm. None of the tumours had developed central region necrosis and the substance of the whole tumour was richly vascularized. Stribley and co-workers [5] have reported in detail on the vascular supply to the salivary adenocarcinoma.

Commercially produced (Nentrac, New England Nuclear Co.) radioactive blood flow tracer microspheres of 15 μm and 50 μm in diameter labelled with Cobalt 57 and Tin 113 respectively were employed. Intermediate 32.5 μm diameter ion exchange beads labelled with Yttrium 90 were also used in conjunction with the gamma labelled microspheres. Microspheres were suspended in normal saline and 0.1% Tween 80 (Polysorbate 80) for injection into animals.

The number of microspheres injected into each animal were approx. 28×10^4 15 μm microspheres/100 g body wt (BW), 28×10^3 32.5 μm microspheres or 7×10^3 50 μm microspheres. This was done either in the form of a single isotope injection or the injection of two contrasting microsphere isotopes (e.g. 15 μm followed by 50 μm microspheres). These numbers were determined from a calibration procedure estimating the least number of microspheres required to provide maximum homogeneity within the normal liver substance. For each microsphere size, 10 rats were injected with incremental increases in numbers of microspheres. The percentage coefficient of variation (%CV) of microspheres from 80 tissue samples from the liver was calculated for each animal. This was plotted against the number of microspheres per unit body weight injected into the animal for the three sphere sizes. The specific number of microspheres was then estimated as the lowest number of that diameter required to produce the best homogeneity of distribution.

Tissue samples

Eighty liver tissue samples per animal were counted for gamma or beta radiation following arterial injection of microspheres. The tumour/liver arterial perfusion ratio (T/L) was determined firstly from the radioactive counts of one of the embolized sphere populations and secondly from the counts of spheres of a different size associated with a contrasting isotopic label. The tumour/liver ratio for each tumour described the ratio of the number of microspheres per gram tumour tissue to the number per gram normal tissue, as measured by specific activity (cpm/g) in each tissue compartment. Homogeneity of distribution of microspheres was described by the %CV (standard deviation/mean) of the tissue samples taken from the normal liver lobes unaffected by tumour presence.

Procedure

Rats were anaesthetized using sodium pentobarbital (6 mg/100 g BW) given intraperitoneally and a catheter (I.D. 0.15 cm) was introduced into the ascending aorta via a right carotid cannulation. Radioactive tracer microspheres were mixed rapidly with heparin (1000 I.U.) via a 3-way tap and injected into the aorta over a period of approx. 10 sec, while the syringe was rotated constantly to avoid settling of the microspheres. Following administration of the microspheres the catheter and syringe were flushed twice with 0.1 ml of blood withdrawn via the cannula and finally with 0.1 ml heparinized saline.

Each animal was sacrificed a minimum of 5 min after the final injection of microspheres to ensure total trapping of the spheres within the tissues. Liver and kidneys were subsequently removed and fixed in 10% buffered formalin. Five samples of renal cortex were taken from each kidney to compare the relative magnitude of specific activities in each, which provided a standard index of microsphere mixing in the blood. The 80 liver samples, of approx. 0.1 g each, were selected in a random fashion from both the normal tissue of the liver and from tumour tissue (growing edge and tumour centre inclusive).

In cases where an animal received two contrasting injections, the first injection of 15 μm microspheres did not alter the heart rate, blood pressure or breathing pattern of the animals prior to the introduction of the second set of larger microspheres. Nor did the first injection effect the pattern of embolization of the second microsphere population in liver or tumour.

Statistics

The specific activity of each sample was recorded and the mean and standard deviation for tumour and normal tissue were calculated for determination of the T/L ratios and %CV for the three microsphere sizes.

The means of the T/L perfusion ratio and %CV for each sphere size group were compared using a 2-tailed Student's *t*-test.

RESULTS

Microspheres lodged in tumour

Table 1 describes the magnitude of the ratios of microspheres lodged in the tumour compared to those lodged in the normal hepatic vasculature for 15, 32.5 and 50 μm microspheres.

There was no statistically significant difference ($P > 0.05$) between the mean T/L ratios for 15 and 32.5 μm microspheres accumulated from all experiments where these were administered. However, comparison of the mean T/L ratio between 15 and 50 and 32.5 and 50 μm experiments did show a significant difference. In the majority of cases 50 μm spheres did not penetrate the tumour circulation as well as 15 or 32.5 μm microspheres.

Distribution of microspheres in normal liver

The mean coefficient of variation of distribution of 15 μm microspheres within the normal liver lobes was 55.5% ± 8.3. For 32.5 μm microspheres the %CV was 35.0% ± 16.8 and for 50 μm spheres was 19.7% ± 6.8. A lower %CV is synonymous with more homogeneous spread of microspheres trapped in normal liver microvessels. There was a significant difference between each mean %CV. Table 1 summarizes the T/L ratio and the accompanying %CV for all three microsphere populations.

Renal embolization of microspheres

Comparison of the specific activity of each kidney relative to its pair describes the degree of mixing of microspheres within the bloodstream. There was a small decrease in the percentage difference (counts in the least active kidney divided by counts in its pair as a percentage) of counts between the kidneys as the diameter of the microsphere increased. The mean percentage difference with 15 μm microspheres was 84.3% ± 12.7 (S.D.) and for 32.5 and 50 μm the values were 74.4% ± 14.3 and 69.7% ± 21.8 respectively. There was a strong correlation between the %CV for the liver and the percentage difference in each animal for each microsphere size. The respective correlation coefficients were 0.93 for 15 μm, 0.90 for 32.5 μm and 0.81 for 50 μm.

DISCUSSION

Results obtained here confirm the increased arterial supply to experimental hepatic tumours. The overall mean figure of T/L ratio was 3 : 1 for 15 μm microspheres which compares well with the findings of other investigations using similar animal models [2, 3, 5, 11]. The present study has also described an analogous T/L ratio for 32.5 μm spheres. Fifty-micrometre diameter microspheres were not found to embolize in tumour to the same degree as their smaller counterparts and generally concentrated in tumour tissue and in normal tissue to the same extent (mean T/L 1 : 1).

Vessels within a tumour are frequently immature and normal physiological function, including blood flow, pressure and capacity, are lost [12]. There is variation between tumours with respect to the size of vessels within them as well as the number of vessels, i.e. vascularity, but from these results it can be concluded that for salivary adenocarcinoma implants in rat liver the vast majority of vessels within the tumour are 32.5 μm ± 2.5 or less. Arterioles large enough to allow penetration of 50 μm ± 5 microspheres appear to be restricted to the normal hepatic vasculature outside the tumour boundaries.

Even within a histological class, each tumour showed individual vascularity. This may account for the wide range of T/L ratios found, although the size of implant and its period of growth was kept as constant as possible. Stribley and coworkers [5] reported that when the tumour diameter of the salivary adenocarcinoma exceeded

Table 1. Comparison of the distribution characteristics of 15, 32.5 and 50 μm microspheres in tumour and normal liver tissue

Microsphere size	*n*	T/L Ratio	Difference*	%CV	Difference
15 μm	27	3.18 ± 3.4+	NS ($p>0.05$)	55.5 ± 8.3	S ($p<0.005$)
32.5 μm	13	3.21 ± 3.2		35.0 ± 16.8	
15 μm	27	3.18 ± 3.4	S ($p<0.05$)	55.0 ± 8.3	S ($p<0.001$)
50 μm	18	0.88 ± 0.83		19.7 ± 6.8	
32.5 μm	13	3.21 ± 3.24	S ($p<0.005$)	35.0 ± 16.8	S ($p<0.025$)
50 μm	18	0.88 ± 0.83		19.7 ± 6.8	

n = number of tumours.
+ = mean ± standard deviation.
* = two-tailed Student's *t*-test.

6 mm there was a decrease in the T/L ratio related to central tumour necrosis. Although no macroscopic tumour necrosis was observed in this study there may have been some variability induced by this effect on tumours over that size.

Homogeneity of distribution of microspheres within the liver has been characterised by measurement of the coefficient of variation of distribution of microspheres. Lower %CVs are synonymous with more even distribution of the microspheres throughout the normal tissue of the organ.

Results show marked improvement in homogeneity of distribution as the microspheres increase in diameter from 15 to 50 μm. It has been suggested that 50 μm microspheres would distribute in the liver less evenly, followed by 32.5 μm and then 15 μm—for reasons of imperfect mixing of larger microspheres within the blood stream [9, 10]. In fact, the distribution of 50 μm microspheres was significantly better than either 32.5 or 15 μm microspheres.

One possible explanation for this is based on the functional behaviour of the microcirculation. It is proposed that normal autoregulatory processes (most pronounced at the precapillary levels) cause an uneven trapping of microspheres due to oscillatory opening and closing of terminal arterioles. Proximal to the capillaries and terminal vessels the inherent opening and closing mechanisms taper off and microspheres might lodge in a more even manner. Local autoregulatory mechanisms have a similar effect on normal blood cells but as these are not trapped the uneveness of perfusion is only transient.

The coefficient of variation for 15 μm microsphere distribution was relatively high in comparison to those obtained by Chamberlain *et al.* [11], owing to dissimilar sphere injection techniques. Thus the low %CVs described here for 32.5 and 50 μm spheres may be improved upon. Our results indicate that good mixing of microspheres with aortic blood, as determined by relative kidney counts, is a prerequisite for good mixing in the hepatic artery prior to embolization within the liver. The distribution characteristics of microspheres in the liver is directly correlated with their mixing in the aorta within any one population of microsphere sizes. However, this does not influence the comparative distribution patterns within different sized microspheres on embolization within the liver. Although the degree of mixing of 50 μm microspheres was less than 15 μm microspheres in the aorta, their distribution within the liver substance was superior and 32.5 μm was intermediate. The presence of vessel junctions within the hepatic arterial system probably allows continual remixing of microspheres before their eventual embolization thereby enabling good distribution.

CONCLUSION

It has been demonstrated that arterially embolized 15 and 32.5 μm microspheres share equal tumour perfusion properties in that they both lodge in tumour tissue in the order of three times that of the ambient normal liver tissue. In contrast, 50 μm microspheres concentrate less in tumour than in normal tissue. Homogeneity of distribution improves as microspheres increase in diameter from 15 to 50 μm and this has wider implications in tissue perfusion studies. In view of the good distribution of 32.5 μm spheres and their ability to preferentially lodge in tumour tissue, they would be recommended as the optimal microsphere size for this animal model in order to study tumour blood flow.

Based on these experiments, the use of large microspheres (> 15 μm) in clinical research trials for intrahepatic radiotherapy warrants investigation. The significance of these results in terms of internal hepatic radiotherapy is that the optimum therapeutic microspheres size is 32.5 μm as they are most likely to distribute homogeneously within the normal liver substance, yet still provide a concentrated dose of radiation to tumour tissue. This would potentiate preferential irradiation of malignant tissue while relatively sparing the normal hepatic parenchyma in patients with metastatic liver cancer.

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