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Letter to the Editor

Blood Flow Measurements by Means of Radioactive Microspheres. A Useful Technique in Malignant Tumors?

BERNHARD ENDRICH, RUDOLF SCHOSSER and KONRAD MESSMER
Institute for Surgical Research, Klinikum Grosshadern, University of Munich, Marchioninistraße 15, 8000 *Muenchen* 70, *Federal Republic of Germany*

MEASUREMENTS of blood flow in lymphatic metastases of the SMT-2A mammary adenocarcinoma in W/Fu rats were recently reported by R. L. Jirtle [1]. Twenty-five μm microspheres (MS), labelled with either ^{57}Co or ^{113}Sn, were used to differentiate between flow to the outer and central regions of tumor and tumor metastases. Even though such studies should be essential for planning and evaluating tumor treatment, there are three main drawbacks in the above study concerning the technique of utilizing radioactively labelled MS:

(1) For this method to determine blood flow, MS should all be trapped in the terminal vascular bed without deteriorating the circulation. Consequently, to assess the proportion of trapped and still circulating MS, simultaneous reference sampling from the arterial and venous line is inevitable [2]. With a venous reference sample, one could distinguish between nutritional and non-nutritional flow in a malignant tumor. This differentiation has not been performed in the above study, although effective tumor treatment depends on nutritional blood flow only.

(2) 240 g rats received approximately 70,000 MS. Cardiac output (*CO*) was calculated according to

$$CO = \frac{I_{inj}\, Q_{ar}}{I_{ar}}, \qquad (1)$$

where Q_{ar} is the rate at which the arterial reference sample was withdrawn, I_{ar} the number of MS in the blood sample and I_{inj} the total number of MS injected into the animal. With a *CO* of 100 ml/min (see result section), I_{ar} can be calculated:

$$I_{ar} = \frac{I_{inj}\, Q_{ar}}{CO}, \qquad (2)$$

indicating that an average of 357 MS was present in the arterial reference sample. This number is about 10% lower than the minimal number of spheres recommended by Buckberg *et al.* [3] for 90% precision on the 95% confidence limit.

Furthermore, the number of MS in the given tissue specimen can be established:

$$I_{cap} = \frac{Q_{cap}\, I_{ar}\, W}{Q_{ar}}, \qquad (3)$$

with I_{cap} being the number of MS in the given specimen, Q_{cap} the capillary blood flow and W the weight of the tissue sample. The lowest number of MS can be calculated by assuming a sample weight of 0.07 g and a mean blood flow of 0.262 ml/min/g, as reported for the central region of inguinal glands; the same analysis for the largest number of MS made with a sample weight of 0.86 g and 0.495 ml/min/g yields a number of MS in the tissue specimen ranging from 13 to 298 MS. Even if one does this calculation with maximum values (i.e., 0.86 g, 0.563 ml/min/g), only 339 MS are found.

Since reliable estimates of cardiac output and regional blood flow can only be made with at least 400 MS in both the arterial reference sample and the tissue specimen [1, 3, 4], the above calculations strongly suggest that the MS technique was not adequately used in the study of Jirtle.

(3) The size of MS was randomly assessed by referring to experiments published earlier [5].

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Diameters of microcirculatory blood vessels in malignant tumors are not uniform but vary to a significant extent, with values reported up to 200 μm [6]. Even though quantitative estimates were not given, dilation of microcirculatory blood vessels has also been reported for a DMBA mammary adenocarcinoma in rats [7].

These results are supported by our own recent findings: using a transparent chamber technique [8], intravital microscopy and quantitative videotechniques [9], we analyzed capillary diameters daily in the amelanotic melanoma A-Mel-3 of hamsters (Fig. 1). The mean tumor weight was 0.12 g on day 12 with a mean capillary diameter of 14.4 ± 0.9 μm, 33% of the values being above 15 μm and 8.5% above 25 μm respectively. Moreover, the frequency distribution of capillary diameters shifts to the right, indicating a further increase of capillary diameter with tumor growth. For 25 μm MS the errors appear small, but might be of significance as a tumor increases in size.

Further evidence is given by Hilmas and Gilette [10], who measured vessel diameter in C3H/Bi mouse mammary carcinoma. At a tumor volume of 35 mm^3 the mean diameter was 10.5 μm, a value which corresponds well to our numbers at identical tumor volumes. During continuous growth, however, the mean vascular diameter increases to a value of approximately 30 μm at a tumor volume of 870 mm^3.

As a consequence, we would like to forward the following conclusions:

(1) Radioactively labelled MS should only be used for measurements of blood flow in malignant tumors if the appropriate size of MS has been established by measuring microvessel diameters at the desired tumor weight.

(2) As demonstrated in Fig. 1, one has to be aware that a considerable number of both 15 μm and 25 μm MS could sneak through the network of nutritional capillaries, causing an underestimation of nutritional blood flow, while a possible a–v shunting or non-nutritional blood flow in the tumor is significantly overestimated.

(3) If the capillary diameter would require MS with a size of 50 μm or more, the method will reveal doubtful results due to the preferential axial streaming of larger MS causing severe inhomogeneities in the MS distribution [11].

(4) Special care is needed to provide the necessary number of at least 400 MS per tissue specimen. Based on the numbers provided by Jirtle [1], this can be accomplished by:

(a) increasing the total number of MS to be injected into the animal
(b) dissecting larger tissue samples ($\geq$1 g) at the end of the experiment
(c) a combination of the two.

The method can certainly be utilized for quantitative studies of blood flow in malignomas. But, before using this 'indirect' microcirculatory method, the criteria listed above should be taken into account for *each* tumor under study.

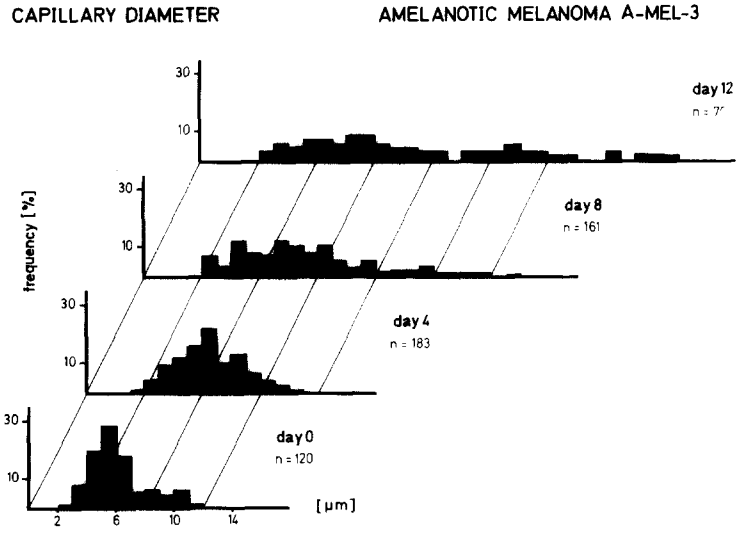

Fig. 1. Frequency distribution of capillary diameters in the amelanotic melanoma A-Mel-3 of the hamster prior to and 4, 8 and 12 days after transplantation of 4×10^4 *tumor cells.*

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