Quantitative Study of Vascularity in Walker Carcinoma 256

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SUMMARY

Vascularity in rat Walker carcinoma 256 was studied quantitatively by a radioisotopic method. Functional intravascular volume and extravasation of plasma protein per unit of tumor mass decreased as the tumors grew. The intravascular volume that we obtained with 51Cr-labeled RBC's was 0.0079 ml/g of tumor mass, which was less than 10% of the value obtained by other workers using dextran. A possible reason for this discrepancy and evidence in support of our results are discussed.

INTRODUCTION

Information regarding tumor vasculature is important not only for the understanding of tumor cell proliferation and tumor growth but also for the planning of tumor radiotherapy and chemotherapy; the supply of oxygen, an authenticated modifier of the radiosensitivity of living cells, as well as that of therapeutic drugs, depends on the blood circulation. In this work, we studied quantitatively the functional state of the blood vessels of rat Walker carcinoma 256, using a radioisotopic technique.

MATERIALS AND METHODS

A small piece of Walker carcinoma 256 (4 to 10 mg) was injected s.c. by a trocar needle into 1 thigh of male Sprague-Dawley rats weighing 300 to 350 g. When the tumors had grown to appropriate sizes, vascular volume and vascular permeability were studied as described by us previously (8). Briefly, a mixture of 51Cr-labeled rat RBC's and 125I-labeled human serum albumin was injected into the rats through the jugular vein under light anesthesia with sodium pentobarbital. At various time intervals thereafter, about 1 ml of blood was withdrawn by cardiac puncture, the rats were killed by intracardiac injection of 1 ml of saturated KCl solution, and the tumors were removed immediately. The 51Cr and 125I radioactivity of the tumors, blood, and plasma was counted with a γ-scintillation spectrometer. The dry weight of the tumors was obtained by drying overnight at 110°, and vascular volume and vascular permeability were calculated by the formulas shown below.

**Vascular Volume.** Blood (ml)/g of tissue = (51Cr activity/ml of blood)/([51Cr activity/g of tissue] - [51Cr activity/ml of blood]).

**Vascular Permeability.** Plasma (ml) extravasated in 1 hr/g of tissue = total plasma in g of tissue - intravascular plasma in g of tissue, where total plasma = ([125I activity/g of tissue]/[125I activity/ml of plasma]) - intravascular plasma = vascular volume X [(1 - hematocrit/100)].

RESULTS AND DISCUSSION

Chart 1 shows that the complete mixing of newly injected RBC's with nonradioactive RBC's in the tumor and host circulation was achieved in 15 min and that the ratio of the amount of 51Cr-labeled RBC's in tumors to that in the blood 2 hr after injection was essentially the same as that at 15 min. This suggested that a possible exchange of newly injected labeled RBC's with host RBC's trapped in the static vessels did not occur in Walker carcinoma 256 or that the exchange was not extensive enough to be noticed if it ever occurred within the 2-hr interval. This may appear to be at variance with the observation of Tannock and Steel (9), who reported that such an exchange occurred slowly in a transplantable rat mammary adenocarcinoma BICR/M1. However, their observation was made over a 3-day interval, whereas ours was for 2 hr.

As shown in Chart 2, the amount of extravasated 125I-labeled plasma protein continuously increased during 2 hr after injection, indicating that the intra-extravascular equilibrium of newly injected plasma protein was not accomplished. Dewey (1) also observed similar results in Walker carcinoma 256 using 131I-labeled rabbit γ-globulin. Accordingly, we killed the animals 1 hr after injection of labeled RBC's and labeled plasma protein in the subsequent experiments to study whether any change in vascular volume and vascular permeability takes place as the tumors grow. As shown in Table 1, the relative vascular volume in the larger tumors, which usually contained a considerable amount of necrotic tissue, was significantly less than that in the smaller tumors, suggesting that larger tumors have a poorer blood supply than smaller tumors. The conclusion is in accordance with the results of other histological or angiographic studies, indicating that tumors overgrow their vasculature and that necrosis develops as the tumors grow owing to an inadequate supply of nutrients, including oxygen (2, 4, 6, 7, 10).

The average vascular volume of the 68 tumors we used in this study was 0.0480 ± 0.0035 ml/g, dry weight. Since the average dry weight of this tumor was 16.4% of the wet weight, the 0.0480 ml/g, dry weight, would be equivalent to about 0.0079 ml/g, wet weight. Gullino and Grantham (3) reported that the vascular space of Walker carcinoma 256 was 10.6% of

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the wet weight, i.e., about 0.106 ml/g, wet weight, which is more than 10 times the value that we obtained. The cause of this profound discrepancy is not clear, but it appears to be due to the difference in the methods used. Gullino and Grantham injected dextran with an average molecular weight of 375,000 into the tumor-bearing rats i.v. and calculated vascular volume by comparing the dextran content in the tumors with that in the blood 1 hr after the injection, considering that no leakage of dextran occurred during this time interval. Mayerson et al. (5) and Wasserman et al. (11) reported, however, that dextran with a molecular weight as high as 412,000 diffuses through capillary walls rather freely. It would be quite possible that with a molecular weight as high as 375,000 occurs also in Walker carcinoma 256, which contains highly permeable vessels as is described below. Thus, it is likely that the large vascular volume Gullino and Grantham obtained by using dextran resulted from, at least in part, the extravasation of dextran during the 1-hr interval, and we conclude that the intravascular volume of Walker carcinoma 256 we obtained using undiffusible $^{51}$Cr-labeled RBC's should be closer to the true value.

The amount of plasma protein extravasated during 1 hr in Walker carcinoma 256 was $0.5931 \pm 0.0492$ ml/g, dry weight (Table 1). This is about 20 to 30 times that in muscle and skin of rats as we reported elsewhere (8). The extravasation of plasma protein in the larger tumors was less than that in the smaller tumors, as was the vascular volume. The decreased extravasation of plasma protein in the larger tumors may be merely a reflection of decreased vascularity in the larger tumors. The possibility could not be excluded, however, that a structural change of vessel wall was responsible for the decrease in the extravasation of plasma protein as the tumors grow; that is, the newer tumor vessel wall, which is made of patchy endothelium, and the lack of elastic tissue or smooth muscle offer little resistance to substances such as plasma protein and allow efficient extravasation, whereas the older vessels in the larger tumors are more firm and less permeable.

The radioisotopic technique we used in this study, which has the advantage of being truely quantitative as compared with other histological methods, could be used to study the vascularity of other solid experimental tumors. This method would also be useful to reveal the sequential changes in vascularity of experimental tumors under chemotherapy or radiotherapy, in which the early change in vascularity would inevitably influence the supply of therapeutic drugs or oxygen to tumor cells and the response of tumors to subsequent treatments.

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**REFERENCES**


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