Vascular Reactivity and Perfusion Characteristics in 7,12-Dimethylbenz(a)anthracene-induced Rat Mammary Neoplasia

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ABSTRACT

Blood flow during "resting conditions" and during noradrenaline infusion were studied by the labeled microsphere technique in dimethylbenz(a)anthracene-induced mammary tumors, skin, muscle, and lung in the rat. Intratumoral distribution of flow was studied by autoradiography of spheres trapped in the vascular beds of the tumors. Histological examination was performed and correlated to the blood flow data. Mean blood flow to the tumors during "resting conditions" was relatively high (49 ml/min/100 g tissue) but was substantially decreased (5 ml/min/100 g tissue) during noradrenaline infusion which produced a pressure elevation of 35 mm Hg. Thus, vascular resistance of the tumor tissue increased dramatically. Cardiac output increased, but total systemic resistance was unchanged. Vascular resistance in muscle was unchanged in contrast to an increase seen in skin. Shunted systemic blood flow to the lungs and bronchial arterial flow decreased indicating reactivity of abnormally large arteriovenous passages in the tumors. Poorly differentiated tumors had a higher vascular resistance than did well-differentiated tumors. Autoradiography revealed a nodular flow distribution with a slight tendency of higher perfusion in the periphery of these tumors.

INTRODUCTION

Knowledge of regulatory mechanisms for tumor blood supply may potentially improve results of irradiation and cytotoxic drug therapy and contribute to the understanding of neoplastic progression (3, 13). Most studies of tumor vascularity have been performed on fast-growing transplanted tumors using morphological methods. The tumors seem to have an irregular vascular network comprised of large vessels with poorly developed smooth muscle which are not differentiated into arterioles and venules (14). Whether or not tumor vessels react to humoral vasoactive stimulants is a matter of debate. Studies using pharmacocangiographic methods indicate that tumor vessels are not able to constrict (2), while other studies using isotope techniques have shown that blood vessels of various transplanted tumors have this ability (1, 4, 7, 9, 11). This discrepancy of results obtained for tumor blood flow and vascular reactivity may be ascribed to a number of factors, such as different tumor models, different methods of analysis, and variable physiological status of the vascular beds at the actual moment of investigation. Studies of tumor angiogenesis suggest that tumor vessels are derived from the surrounding normal tissue (3). This would indicate that the vascular reactivity of tumor vessels is similar to that of the surrounding host tissue.

Thus, with this as a background, this study of the vascular hemodynamics of a slowly growing nontransplanted rat mammary adenomatous neoplasm induced by DMBA3 (Sigma Chemical Co., St. Louis, Mo.) was undertaken. The histopathological type of tumor was correlated to resting blood flow and blood flow during noradrenaline infusion, which was measured by means of a double-isotope microsphere technique. The distribution of blood perfusion within the tumors was also studied by autoradiography.

MATERIALS AND METHODS

Tumor Model. Female Sprague-Dawley rats (Anticimex, Stockholm, Sweden), 50 to 55 days old, were fed DMBA (16 mg dissolved in 1 ml olive oil) by gavage while under brief ether anesthesia (5, 12). Six to 8 weeks later, nodules could be palpated along the mammary ridge. Experiments were performed 10 to 12 weeks after induction.

Cardiac Output and Regional Blood Flow Measurement. Cardiac output and regional blood flow were measured by means of a double-isotope microsphere technique (10) during "resting conditions" and during constant noradrenaline infusion. The radioactivity of the tumors was not only studied in a well-type auto-y-spectrometer but also by autoradiography to illustrate the intratumoral distribution of blood flow (6).

The procedure was as follows. The rats were anesthetized with Nembutal (50 mg/kg body weight) i.p. The right carotid artery was cannulated to allow injection of microspheres into the left ventricle. One femoral artery and the caudal artery were cannulated for obtaining reference blood samples and for the respective monitoring of blood pressure and heart rate. The right brachial artery was cannulated for the infusion of NaCl solution (9 g/liter) simulating "resting conditions" or of noradrenaline (0.005 mg/ml) added to this solution. The rate of noradrenaline infusion was adjusted individually to produce a substantial increase of arterial blood pressure (30 to 40 mm Hg). Attempts were made to maintain the body temperature around 37° and to keep the depth of anesthesia, the preparation time, the blood loss, and the respiratory status as constant as possible. Polystyrene spheres (3M Co., St. Paul, Minn.), diameter 15 ± 3 (S.D.) µm, were labeled with 144Ce and 85Sr, respectively. The number of spheres injected per animal was approximately 600,000 per isotope. The microspheres, which were suspended in 1 ml NaCl solution (9 g/liter) including dextran and Tween, were injected within 45 sec. Reference blood samples were withdrawn at 0.6 ml/min during 90 sec. Two measurements were made in each animal. Thus, microspheres were injected first during "resting" steady state conditions and then while infusing noradrenaline solution.

The rats were killed, and the tumors and samples from abdominal skin, muscle, and lung were dissected out, wet weighed, fixed in formaldehyde solution, and processed further for activity measurements in a well-type y-spectrometer.

Whole-body y detection, including dissected tissues and reference samples, was performed by use of a Packard Armac scintillation counter. Cardiac output and regional perfusion could then be calcu-

1 This investigation was supported by the Swedish Cancer Society (1193-B82-04X), Konung V:s Research Foundation, and Syskonen Svenssons Foundation.
2 To whom requests for reprints should be addressed.
3 The abbreviation used is: DMBA, 7,12-dimethylbenz(a)anthracene.
RESULTS

Tumor Yield. Experiments were performed 10 to 12 weeks after tumor induction. The mean weight of the 10 rats at this age (17 to 19 weeks) was about 300 g. The total number of tumors was 62, i.e., an average of 6.2 per animal. The tumors weighed between 0.02 and 15 g, with a mean of 1.95 g and a median of 0.78 g. The majority of tumors were located in the cranial halves of the mammary ridges. Skin ulcerations were present above 4 of the larger tumors.

Histopathology. All tumors were subjected to histological analysis using van Gieson staining. Two of them were fibromas. The others were adenomatous neoplasms, which were further subdivided into well-differentiated (n = 18), poorly differentiated (n = 16), and intermediate (n = 27) groups. Tumors with different degrees of differentiation were found to be present in the same animal. The mean and median weights of the well-differentiated tumors were 2.12 and 0.75 g as compared to 2.75 and 1.47 g for the poorly differentiated ones. The histopathological pattern of the large tumors showed a marked heterogeneity within each tumor. Necrotic areas were seen especially in large and poorly differentiated tumors. Skin ulceration was not related to poor differentiation.

Cardiac Output, Regional Blood Flow, and Vascular Resistance. Blood pressure and cardiac output were increased to the same extent during the noradrenaline infusion. Blood flow to muscle did not change while blood flow to abdominal skin showed a moderate decrease. Tumor blood flow sharply dropped during the infusion of noradrenaline. The flow to the lungs (bronchial arterial and shunted flow) also decreased during noradrenaline infusion (Chart 1). Changes in total systemic resistance per 100 g body weight and organ vascular resistance per 100 g tissue (Chart 2) were related to the changes in resistance vessel radius or total cross-sectional area of the resistance vessels and were calculated by dividing blood pressure with blood flow. Thus, as seen in Chart 2, the total systemic resistance did not change during noradrenaline infusion while that of abdominal skin increased. The tumors showed a pronounced increase in vascular resistance. Blood flow and vascular resistance were compared between the different tumor groups at various stages of differentiation. No difference was found between matched-weight groups of tumors with wet weights below 2.0 g at resting conditions. However, larger poorly differentiated tumors had a significantly lower blood flow (30.8 ± 5.0 ml/min/100 g) and higher vascular resistance (4.03 ± 0.43 mm Hg/min/100 g/ml) than did well-differentiated tumors (59.8 ± 7.9 and 1.90 ± 0.21, respectively). There was no significant difference in the ratio of vascular resistance at rest:vascular resistance during noradrenaline infusion between tumors with different degrees of differentiation. There was a positive correlation between weight and vascular resistance at resting conditions for poorly differentiated tumors but not for intermediately and well-differentiated tumors. Vascular resistance during noradrenaline infusion was not related to tumor weight. The 2 fibromas had a higher vascular resistance at rest (6.5 and 8.0 mm Hg/min/

![Chart 1](chart1.jpg)
Hemodynamic Characteristics in DMBA Tumors

Chart 2. Histogram illustrating the mean of the vascular resistance units (mm Hg per min per 100 g tissue per ml) in abdominal skin, muscle, and tumors and the total systemic resistance (mm Hg per min per 100 g body weight per ml) during rest and noradrenaline (NA). ns, not significant; bars, S.E.

100 g/ml) than did adenomatous neoplasms but reacted similarly to noradrenaline.

The amount of radioactivity trapped in lung tissue increased with increasing total tumor mass (range, 2 to 40 g; mean, 12 g) during resting conditions and noradrenaline infusion. A nominal lung flow comprised of bronchial perfusion and shunted flow from normal tissues was calculated by extrapolation of the tumor weight to zero and found to be 165 ml/min/100 g at rest. This result corresponded well with values obtained in normal non-tumor-bearing rats. There was found to be a significant decrease in the amount of trapped radioactivity in the lungs by one-third during noradrenaline infusion as compared to resting conditions. Blood flow through the tumors was decreased by one-tenth, which indicated that blood flow through arteriovenous passages larger than 15 μm was decreased.

Analysis of "shunting" in relation to tumor differentiation is impossible to make in this study due to the presence of tumors of several types within the same animal. No correlation between total tumor weight and cardiac output could be detected in this limited material. A central and a peripheral part from the tumors weighing more than 3 g were dissected out in a standardized fashion. The mean weight of the central part was 0.42 g and of the peripheral anulus was 1.21 g. No statistical difference in blood flow could be obtained between the central and peripheral parts of the tumors. This could probably be explained by the fact that well-perfused regions were not strictly peripheral but were distributed across the section of the tumors, which was also illustrated by the autoradiograms.

 Autoradiography. The autoradiograms of coronar brain sections showed a homogenous distribution of spheres and a resolution capable of visualizing the well-perfused cortical and central gray substances which were in contrast to the poorer perfusion of the white matter. Sections from kidneys produced dense and homogenous blackening in the cortical areas. Sections of 15 tumors with weights over 3 g produced a great variety in the patterns of blackening (Fig. 1). In general, there was a heterogenous "nodular" blackening with a tendency to appear at the periphery. Regions of low blackening corresponded well to areas of necrosis as judged from histological analysis. So far, no direct correlation between intratumoral perfusion pattern and histology can be found.

DISCUSSION

Several studies have demonstrated the presence of vascular reactivity to noradrenaline and other pressor drugs in various transplanted tumors (1, 4, 7, 9, 11). Very few hemodynamic studies have been performed on induced tumors. DMBA-induced mammary neoplasia was chosen as the model of the present study since these tumors seem to be closely related to mammary tumors in man from both morphological and functional points of view (5, 12). The tumors are derived from mammary epithelium, i.e., they are of epidermal origin. The histological appearance of the tumor is known to be both of a benign adenoma-adenofibroma type as well as of a malignant adenocarcinoma type. Thus, the possibility of relating functional characteristics to morphology is offered. The microsphere tracer technique was chosen for blood flow analysis because flow at resting conditions and during noradrenaline infusion could be studied in various tissues of the same animal. This reduction of error due to biological variation is of special importance because of the heterogeneity of tumor morphology. Furthermore, autoradiographic analysis of intratumoral flow distribution is made possible.

The microsphere tracer technique measures only flow through vessels with dimensions less than the sphere diameter, which in this case was 15 μm. Thus, blood flow data is anatomically determined in contrast to results obtained from blood...
flow analysis with diffusible tracer substances. Arteriovenous passages larger than 15 \( \mu \)m are known to exist in the skin as part of the thermoregulatory system and probably in tumor tissue. The blood flow through these passages in this study may be only indirectly estimated by the amount of activity trapped in the lungs. Results are presented both as blood flow and vascular resistance. The former gives direct information as to the access of nutrients to the tissues, but the latter parameter is more informative concerning reactivity in the vascular bed and provides an indirect measure of the cross-sectional area of the vascular bed under study. Blood flow in tumors at resting conditions is 49 \( \pm \) 2 ml/min/100 g. Similar values were found in s.c., i.m., and intrahepatically autotransplanted tumors of this type. However, autoradiography reveals a considerable heterogeneity of flow within the tumors. The background of this heterogeneity is unknown but may represent areas with differences in growth pattern and cellularity. The vast heterogeneity of flow in the adenomatous neoplasms may involve several factors such as cellularity, follicles, connective tissue, and necrosis. Further studies to elucidate the importance of these factors for blood flow are under way. The finding of a decreased perfusion in larger tumors is confirmed here but only for poorly differentiated tumors weighing more than 1 g. This fits well with the concept of angiogenesis and tumor progression in which hypoperfused areas occur in tumors weighing more than 1 g. The lack of this relation for well- and intermediately differentiated tumors indicates the importance of growth characteristics. The significantly lower blood flow (higher vascular resistance) of poorly differentiated as compared to well-differentiated tumors might be even more marked if blood flow were related to cell number instead of to wet weight. An increased interstitial pressure in tumors has been demonstrated to increase with tumor weight in other studies. This finding may imply that vascular compression compromises perfusion of larger tumors.

It is evident that blood flow through tumor tissue is drastically reduced and vascular resistance is increased during a moderate infusion of noradrenaline. The vascular bed of tumors reacts more than those of skin and muscle, which might indicate a higher sensitivity to noradrenaline. Such a conclusion may be erroneous since the degree of smooth muscle contraction at ”resting conditions” may vary from tissue to tissue. Smooth muscle of the tumor vascular bed might be more relaxed than that of skin and muscle at rest for metabolic reasons and therefore may react more to a particular vasoactive drug. Dose-response curves determined for various organs in preliminary in vitro experiments have confirmed the hypersensitivity of the tumor vascular bed to noradrenaline (16). An abnormally high number of spheres were trapped in the lungs of tumor-bearing rats as compared to controls. A positive correlation between the amount of radioactivity and the total tumor mass was found. The amount of spheres in the lungs was reduced by approximately one-third during noradrenaline infusion irrespective of the tumor burden. Values obtained for bronchial flow and for shunted flow from normal tissues by extrapolation of the tumor weight to zero in a tumor mass-lung activity graph were well in accordance with those results found for normal rat. Thus, the fraction of lung activity derived from tumor ”shunting” can be determined both at rest and during noradrenaline infusion. It is clear from these calculations that arteriovenous passages larger than 15 \( \mu \)m react but to a lesser degree than do the smaller ones. At least 40% of tumor blood was demonstrated to pass through arteriovenous connections larger than 15 \( \mu \)m in experiments using larger spheres (25 \( \mu \)m).

In conclusion, resting blood flow of DMBA-induced mammary tumors is relatively high. Vascular resistance is higher in poorly differentiated tumors than in well-differentiated ones and increases with increasing tumor weight in poorly differentiated tumors. There is also an abnormal passage of blood through arteriovenous passages larger than 15 \( \mu \)m. Noradrenaline drastically increases vascular resistance of tumors with the most profound reduction of flow through passages less than 15 \( \mu \)m in diameter. Perfusion of blood in tumors is heterogeneous, with a tendency to be greater at the periphery of the tumor.

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