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Blood Flow and Reactivity to Noradrenaline in Induced and Autotransplanted DMBA Rat Mammary Neoplasia*

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Abstract—*Blood flow during the 'resting condition' and during noradrenaline infusion was compared by means of a microsphere tracer technique in induced DMBA-tumours along the mammary ridge and in autotransplanted tumours, as well as in abdominal skin, skeletal muscle, liver and kidneys. During 'resting conditions' the blood flow of tumours transplanted into skeletal muscle of the hindlimb, liver and kidney was similar to that of the non-transplanted tumours present along the mammary ridges. A pronounced decrease of blood flow upon moderate noradrenaline infusion was characteristic of autotransplanted as well as non-transplanted tumours, which contrasted to the insignificant reaction of skeletal muscle. The striking similarities in blood flow and reactivity of non-transplanted DMBA tumours and autotransplanted ones, and the dissimilarities of the transplanted tumours and the hosting tissues, favour the idea that these tumours regulate their blood supply irrespective of host tissue.*

INTRODUCTION

DUE TO an increasing awareness of the clinical relevance of tumour vascular supply in radiotherapy, hyperthermia and chemotherapy, data on morphological and functional properties of the blood suply to various experimental tumours are accumulating. However, tumour models and analysis of blood flow are varying and hence results may be confusing. Most work has been done on transplanted tumours (cf. [1]), and such studies indicate a decreased tumour blood flow upon noradrenaline administration. Whether tumour blood flow is regulated by intrinsic mechanisms or by the vascular network of the surrounding tissue is not clear, but is of basic and clinical relevance pertaining to treatment of primary tumours and their secondaries. Recently, blood flow studies on 7,12 dimethylbenz-α-anthracene (DMBA)-induced, non-transplanted mammary tumours of the rat were published [2]. A high 'resting' blood flow as well as a profound decrease upon noradrenaline infusion were found. In this study blood flow and reactivity were measured in the above-mentioned tumours *in situ* along the mammary ridge and also in autotransplanted ones.

MATERIALS AND METHODS

Female Sprague–Dawley rats (Anticimex, Stockholm, Sweden), 50–55 days old, were fed DMBA (16 mg dissolved in 1 ml olive oil) by gavage while under brief ether anaesthesia [3]. After 8–10 weeks the tumours were surgically removed during Brietal anaesthesia and tiny pieces (1–2 mm^3) of the tumours were dissected free and transplanted deep into the quadriceps muscle, underneath the capsule of the left kidney and into one liver lobe. At the time when the transplanted tumours had become of suitable size (6–8 weeks later) there were also new, recently developed, induced mammary tumours present along the mammary ridges. Cardiac output and regional blood flow were measured by means of a double isotope microsphere technique [2, 4] during 'resting conditions' and during constant noradrenaline infusion in the same animal.

The procedure was as follows: the rats were

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anaesthetised with Nembutal (50 mg/kg body wt) i.p. The right carotid artery was cannulated to allow injection of microspheres (3MCo St Paul, Minn, diam. 15 μm labelled with ^{141}Ce and ^{85}Sr) into the left ventricle. One femoral artery and the caudal artery were cannulated to obtain reference blood samples (0.6 ml/min during 90 sec) and monitor blood pressure and heart rate. The right brachial artery was cannulated for the infusion of NaCl solution (9 g/l), simulating 'resting conditions', or 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 (20–40 mm Hg). Temperature was kept at 38°C by means of a heating lamp servo-controlled by a rectal thermistor. Attempts were made to keep the depth of anaesthesia, the preparation time, the blood loss and the respiratory status as constant as possible.

Tumours (transplanted ones and along the mammary ridges) as well as samples from abdominal skin, muscle, kidney and liver were dissected out, weighed fresh, fixed in formaldehyde solution and processed further for activity measurements in a Packard well-type autogamma spectrometer to produce regional blood flow data. Whole-body gamma-detection, including dissected tissues and reference samples, was performed by use of a Packard Armac scintillation counter to determine cardiac output. Perfusion was calculated according to the equation:

$$\frac{\text{organ flow}}{\text{organ activity}} = \frac{\text{reference sample flow}}{\text{reference sample activity}}.$$

Histopathological examination of the original tumours, pieces of which were implanted, as well as of the transplantation progeny were made together with the newly developed tumours along the mammary ridges at the time of blood flow analysis.

Statistical analysis

Statistical analysis was performed after natural logarithmic transformation of the blood flow data [5]. Student's t test group comparison and, where appropriate, pairing design was used. All values are given as the mean ± S.E.M. P values less than 0.05 were considered significant.

RESULTS

Tumour yield

All rats induced with DMBA developed multiple tumours along the mammary ridges. The tumours were of varying degrees of differentiation, not known at the time of transplantation. In the majority of experimental animals ($n = 58$) transplantation was performed from one or two tumours only to skeletal muscle of the hindlimbs (78 implantations). Tumour take was low (19%) which, together with technical failures of the blood flow experimental set-up, resulted in nine rats with a total of 12 intramuscularly developed tumours. Tumour weights ranged from 0.08 to 8.60 g, with a mean of 1.47 g. Ten of the 12 tumours were within the range 0.08–0.96 g, with a mean of 0.44 g. Two rats developed successfully measured liver tumour transplants and two animals renal tumour transplants. After removal of the tumours along the mammary ridges 'new' ones developed in cicatricae, as well as in undisturbed regions. Only the latter (14 tumours in 11 animals, weights ranging from 0.08 to 4.30 g, with a mean of 1.25 g) were subject to analysis.

Morphology

All tumours investigated were of adenoma/adenofibroma type of varying differentiation. According to epithelial architecture and nuclear appearance they were divided into three classes of differentiation [2]. Tumours resulting in a subsequent tumour take when transplanted were more poorly differentiated than those tumours not producing a progressive transplant ($P < 0.01$). The general appearance of the transplants could not be distinguished from the tumours along the mammary ridge and differentiation as related to their origins was higher, the same or poorer. This finding is probably due to the great heterogeneity of these tumours. The tumours present along the mammary ridges at the time of flow analysis were of the same general appearance and of moderate differentiation not different from the transplanted counterparts.

Blood flow

Data on blood flow are summarized in Fig. 1.

During rest the animals ($n = 9$) maintain a blood pressure of 130 ± 6 mm Hg and a cardiac output of 19.2 ± 2.5 ml/min × 100 g, resulting in a total systemic resistance of 6.77 mm Hg × min × 100 g/ml. Noradrenaline infusion caused a mean pressure elevation of 25 mm Hg and an increase of cardiac output to 22.8 ± 2.9 ml/min × 100 g, i.e. total systemic resistance remained unchanged under the prevailing experimental conditions.

The induced tumours ($n = 14$) along the mammary ridges had a high perfusion at resting conditions, 49.1 ± 6.2 ml/min × 100 g, with a substantial decrease to 8.2 ± 1.4 ml/min × 100 g during noradrenaline infusion. Resting blood flow and vascular resistance as well as reponse to noradrenaline infusion of i.m. transplanted tumours ($n = 12$) are essentially the same as for the

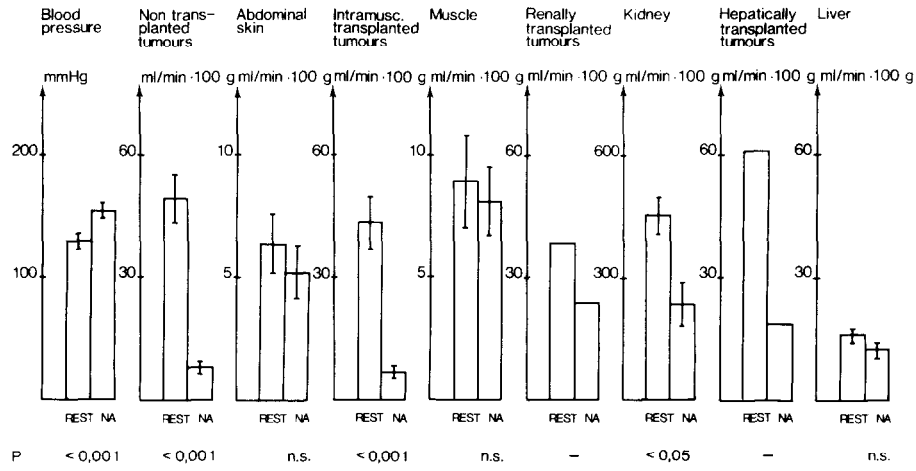

Fig. 1. Blood pressure and blood flow data at resting conditions and during noradrenaline infusion.

non-transplanted ones, i.e. 43.2 ± 8.7 ml/min × 100 g at rest and 7.0 ± 1.6 ml/min × 100 g during noradrenalien infusion. Corresponding values for the more homogenous weight group were 45.6 ± 10.5 ml/min × 100 g at rest and 6.4 ± 1.8 ml/min × 100 g during noradrenaline infusion. In contrast, perfusion of the skeletal muscle ($n = 11$) during rest is very low, 8.9 ± 1.9 ml/min × 100 g, and the reaction to noradrenaline is minimal under the prevailing conditions. Blood flow at rest in two renally transplanted tumours was similar to non-transplanted ones and approximately 1/10 of the flow of the kidneys per unit weight. In response to noradrenaline the flow of the kidneys and renally transplanted tumours was halved. The arterial blood supply to the two intrahepatically transplanted tumours was approximately four-fold the arterial supply of normal liver during rest. Noradrenaline caused a substantial decrease in perfusion only in the tumours.

DISCUSSION

Since most studies on tumour circulation have been performed on transplanted tumours, it was considered to be of importance to investigate whether any major differences in blood flow characteristics are induced by the transplantation procedure. In this study, where induced non-transplanted DMBA-tumours and autotransplanted ones were studied simultaneously in the same rats, no evidence of major alterations could be demonstrated. This result supports the relevance of studies on transplanted tumours, where different tumour models have been implanted intramuscularly. Young *et al.* [6] found a direct relation between blood flow in transplanted V2 carcinoma and in the hosting tissue, whereas our results seem to be contradictory. However, intramuscular transplantation was not done in their work and also their tumour was rapidly growing, with a marked tendency to necrosis, in contrast to the one used in this work. Edlich *et al.* [7] also used the V2 carcinoma but they could not find a relation between blood flow and implantation site as measured with the ^{86}Rb technique. A question often raised is whether blood perfusion is regulated from the surrounding vascular network of the host, vessels being external to the tumour or incorporated within the tumour mass, or if perfusion is regulated by intrinsic mechanisms. The data presented in this work show that the characteristics of resting blood flow and response to noradrenaline is retained in the transplanted tumours. Thus these similarities to the original non-transplanted tumours and dissimilarities to hosting tissues favour the concept that blood perfusion is regulated by intrinsic mechanisms of the tumour. Whether this regulation is accomplished by newly formed vessels or by transformed vasculature of the surrounding tissue incorporated within the tumour is not clear, but it is of little importance in the search of specific means of intervention of tumour blood perfusion.

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