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Influence of Noradrenaline on Local Tumour Blood Flow

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Abstract—*The local blood flow in a transplantable rat sarcoma and in normal rat muscle and subcutaneous tissue was studied by local ^{133}Xe clearance technique. Noradrenaline, mixed and injected with the isotope, reduced significantly the local blood flow in both tumour and normal tissues. This suggests that the tumour vascular bed, opposite to what has earlier mostly been stated on the basis of lack of adrenergic innervation, might be directly influenced by vasoactive drugs.*

INTRODUCTION

THE BLOOD supply of malignant tumours is based mainly on a neovascularization, but pre-existing normal blood vessels from the host might also be incorporated in the growing tumour cell mass. A study of three different rat tumours by microangiography showed a muscular wall in both veins and arteries of one tumour, while no obvious muscle layer was found related to the vessels of the two other tumours [1].

In a study on two transplantable rat tumours, a 20-methylcholanthrene induced sarcoma and a hepatoma, no adrenergic innervation of tumour vessels was found [2]. A similar observation was later made in a human renal adenocarcinoma, in which no adrenergic innervation was found in the main part of the tumour, while large arteries infiltrated by the tumour had a scarce adrenergic innervation. Occasionally nerve terminals could be seen close to these arteries, innervating small tumour vessels [3].

This probably means that the main part of the tumour vascular bed has no adrenergic innervation, which does, however, not exclude that it might still be influenced by vasoactive drugs. Thus, in a previous study on the above mentioned rat tumours, the intratumour blood flow distribution, recorded by isotope technique, was significantly changed towards low blood flow values after i.v. administration of noradrenaline [4]. A similar effect of noradrenaline was seen in normal muscle. Due to the i.v. administration of the vasoconstrictor the changed intra-tumour blood flow distribution could be explained both by a direct effect of noradrenaline on the tumour vascular bed or by an indirect effect on normal afferent vessels supplying the transplantation area. To differentiate between these two possible explanations, noradrenaline might be applied locally to the tumour vessels or the vascular area, where the tumour is growing, might be subjected to sympathetic nerve stimulation.

The aim of the present study was to investigate the influence of noradrenaline on the local tumour blood flow by a local isotope clearance technique, in order to differentiate between a direct or an indirect effect of noradrenaline on the tumour vascular bed.

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MATERIALS AND METHODS

Animals and tumour

Inbred rats from a Lister strain of animals with a mean body weight of 150 g were studied. They were transplanted s.c. into one hindpaw with a 20-methylcholanthrene induced sarcoma in its 140th transfer generation. Animals, tumour and transplantation technique has been described in detail recently [5]. Studies were performed 8 days after tumour transplantation with a mean tumour diameter of 5 mm.

Isotope

Radioactive xenon [^{133}Xe] dissolved in saline (40 MBq/ml) was obtained from AB Kabi

Diagnostica, Studsvik, Sweden. It was injected into tumour or normal tissue in a volume of 0.02 ml through a fine bore injection needle with a diameter of 0.3 mm. At repeated injections into the same tissue, an attempt was made to inject the isotope into the same limited tissue area, by standardizing the direction and introduction of the injection needle.

Noradrenaline

Inj-noradrenaline 1 mg/ml (Apoteksbolaget, Sweden). Noradrenaline was added to the xenon solution in a concentration 0.01 mg/ml of xenon in saline.

Experimental technique

Recording of local blood flow by ^{133}Xe *disappearance rate.* Animals were studied under light ether anaesthesia. The xenon solution with or without noradrenaline was injected. Radiation from ^{133}Xe at the injection site was detected with a 1.5 in. NaI crystal in a cylindrical thick lead collimator directly over the tumour or normal tissue to be studied. The detector signals were registered in a pulse height analyzer and a ratemeter. The disappearance curve was written on a linear recorder. Activity was plotted in a semilogarithmic diagram vs time and the $t_{\frac{1}{2}}$ for disappearance was calculated. The local capillary blood flow in ml/min/g tissue $= \lambda \times \ln 2/t_{\frac{1}{2}}$, where λ is the partition coefficient for xenon between tissue and blood.

Recording of tissue–blood partition coefficient for ^{133}Xe. The tissue–blood partition coefficients for the tumor investigated in this study and for normal rat muscle and subcutaneous tissue were determined separately according to a technique modified from Veall and Mallett [6]. Biopsies with a weight of 0.5 g were taken from tumour, muscle and subcutaneous tissue in 3 animals. Each biopsy was homogenized in 2 ml saline and 1 ml specimens were transferred to 5 mm wide glass tubes. ^{133}Xe solution in a volume of 0.1 ml was added and the tubes were rapidly sealed in a gas-flame. Double specimens of 1 ml heparinized blood from each animal were also treated in the same way. All tubes were slowly agitated at 37°C for 1 hr and were thereafter centrifuged (except blood specimens) and submitted for xenon activity measurement at the same temperature.

The radiation from ^{133}Xe was detected by a 2 × 2 in. NaI (Tl) crystal which was well shielded from background radiation by surrounding lead layers. An inner layer of brass (4 mm) depressed the influence of characteristic X-rays produced in lead.

The detector signals were stored as spectra in a multi-channel analyzer. The 80.9 keV gamma-radiation as well as X-rays in the 30–35 keV interval produced in the ^{133}Xe-decay were measured. Intensities were calculated as the integrated number of counts in the peaks of the spectra with background subtraction.

To obtain a sufficient spatial resolution a 35 mm thick brass collimator was placed between the detector and the sample. A 6 × 10 mm^2 opening defined the field viewed by the detector. A special sample holder made a reproducible positioning possible.

Each sample was measured for 200 sec in two positions to get the relative amounts of ^{133}Xe activity in gas, blood and centrifuged tissue homogenate. One set of tissue and blood samples was discarded due to low ^{133}Xe activity.

The tissue–blood partition coefficients were calculated as the ratios of xenon uptake in per cent between tissue and blood. The mean coefficients ± maximal errors (5 specimens of each tissue) were for tumour 0.56 ± 0.10, for muscle 0.56 ± 0.02 and for subcutaneous tissue 1.2 ± 0.6. The large spread of values for subcutaneous tissue is probably explained by heterogeneities in the homogenates of this rather tough and fatty tissue.

RESULTS

The recorded semilogarithmic disappearance curves were all linear except for the first 1–3 min after local xenon injection. The slopes of the curves were constant throughout the recordings with exception for three noradrenaline recordings, one in muscle and two in tumours. In these recordings the curve became steeper 8–14 min after xenon–noradrenaline injection. The calculated blood flow values are presented in Fig. 1. The local blood flow in 15 tumours and in normal muscle and subcutaneous tissue was significantly reduced by noradrenaline. The mean normal blood flow (mean ± S.D.) in tumour ($n = 29$), muscle ($n = 12$) and subcutaneous tissue ($n = 12$) was 25 ± 11, 39 ± 20 and 48 ± 15 ml/min/100 g tissue, respectively. The corresponding values after local administration of noradrenaline were 3 ± 2 ($n = 15$), 7 ± 4 ($n = 7$) and 7 ± 4 ($n = 12$) ml/min/100 g tissue. The differences between control and noradrenaline recordings were statistically significant ($P < 0.01$).

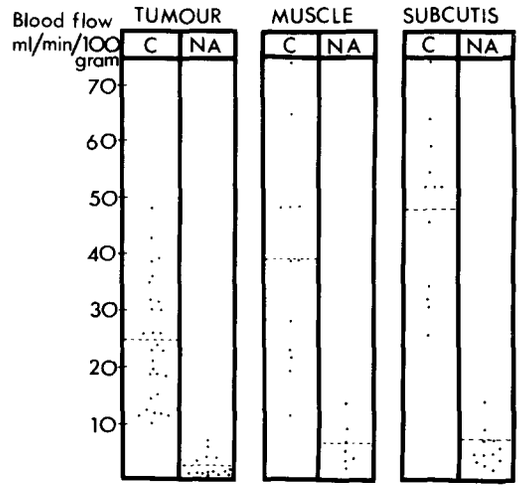

Fig. 1. Blood flow in ml/min/100 g *tissue recorded by local* ^{133}Xe *clearance technique in tumour and in normal muscle and subcutaneous tissue. Flow recordings without (C) or with (NA) noradrenaline mixed with the injected* ^{133}Xe. *Note the significantly* ($P<0.01$) *reduced blood flow in tumour and normal tissue after local administration of noradrenaline.*

Two recordings of the normal blood flow were made in tumours before studying the influence of noradrenaline, and the difference in flow between double recordings in the same tumour area was studied. A mean $\pm$ S.D. difference of 4 ± 3 ml/min/100 g tissue between such double recordings was found, which was considerably below the spread of xenon clearances from injection sites in different tumours.

DISCUSSION

In the present study the local blood flow in a transplantable rat tumour and in normal muscle and subcutaneous tissue of the rat was studied by a local isotope clearance technique. A significant reduction of the local blood flow in tumour and normal tissues was observed when noradrenaline was injected mixed with the isotope.

The intra-tumour distribution of blood flow is characterized by a wide heterogeneity [5]. This means that single recordings of tumour blood flow by local isotope clearance technique, which reflect the blood flow of very limited tissue areas, will most probably not give a good description of the mean tumour blood flow. However, with the aim of studying the influence of vasoactive drugs on tumour blood flow, the method has the advantage that the active substance could be injected mixed with the isotope into the tumour tissue. This technique might thus reflect the direct influence of the injected drug on the local tumour vascular bed. Such a suggestion is supported by the constant slopes of the radiodisappearance curves throughout most recordings in noradrenaline injected tumours. In two muscle and one tumour recording with noradrenaline the curves became steeper 8–14 min after noradrenaline injection, which was interpreted as an escape from the noradrenaline effect [7]. No disappearance curve indicated simultaneous isotope injection into two or more different types of tissues with considerate differences of xenon clearance values. This should have resulted in non-linear curves with slope decreasing with time from injection.

An attempt was made to study the same limited tumour area in repeated local injections of isotope. The mean difference between double normal tumour flow recordings of 4 ± 3 ml/min/100 g tissue does indicate that this attempt was not quite successful. However, it includes several difficulties. The injection needle and syringe have to be withdrawn before activity recording, since this might be disturbed by remaining isotope activity in the syringe. A new local injection under identical conditions does probably mean a further slight tissue damage and the risk that the isotope will not be injected into exactly the same tissue area. However, the large difference between control recordings and noradrenaline recordings does probably mean that the difference between the separate control recordings is of minor importance. The present results suggest that tumour blood flow might be significantly influenced by vasoactive drugs, which is opposite to what has earlier been concluded based on the very primitive appearance of newly formed tumour vessels and their lack of adrenergic innervation [3]. The technique used in the present study might also support the conclusion that the influence of noradrenaline on tumour blood flow is based on a direct influence on the tumour vascular bed.

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