Perfusion Characteristics and Norepinephrine Reactivity of Human Renal Carcinoma

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ABSTRACT

Kidneys, surgically removed due to carcinoma, were subjected to perfusion in vitro. The perfusion distribution was studied by means of labeled microspheres injected during maximal vascular dilation and during two different norepinephrine concentrations. The perfusion concluded with injection of barium sulfate. Two-mm-thick slices of tissue were autoradiographed and microangiographed for visualization of perfusion and distribution of vascular density, respectively. Multiple specimens from tumor and cortical tissues were subjected to quantitative perfusate flow analysis.

In spite of regionally high vascular density, perfusion through "normalized" capillaries was very low in tumor tissue as compared to cortex (during maximal dilation, one-tenth of the cortical flow). During moderate norepinephrine infusion, the perfusate flow decreased, and the resistance of the cortex increased. The flow to tumor tissue increased while the vascular resistance remained constant.

During higher norepinephrine concentrations, the flow was redistributed; i.e., the cortical flow increased while that of the tumor decreased, due to a marked increase in tumor vascular resistance while the cortical tissue showed a very moderate rise in resistance. The thin-walled tumor vessels might be collapsed under a high tissue pressure at low perfusion pressures. At higher perfusion pressure, the vessels might open up, and contractile activity may not be expressed until then. The tumor vascular resistance increased 3 to 4 times, while that of cortex showed a 7-fold increase. Indications that a considerable fraction of the perfusate passes arteriovenous passages larger than 15 μm were obtained in individual experiments, this fraction increasing upon norepinephrine infusion.

INTRODUCTION

The vascular supply to tumor tissue is often insufficient, resulting in hypoxic and nutritionally deprived areas with dormant tumor cells resistant to therapy. The sequence of events from an avascular early preinvasive phase to a richly capillarized state has been described by Folkman and Cotran (1). The seemingly contrary process of vascular rarification, or rather vascular hypoperfusion, in more advanced tumor growth has been more difficult to study and interpret. Interest has been focused on the endothelial proliferation rate relative to that of tumor cells (2, 3). The tissue pressure within tumors has been found to be high, and poor perfusion has been ascribed to this factor (4–7).

The pharmacological possibilities of influencing vascular perfusion in tumors have also attracted interest. Widely different results have been published, probably due to the use of different tumor models and methods of analysis (8). Most studies on experimental tumors in animals, using functional measurements, have shown a considerable influence on tumor perfusion upon exposure to various vasoactive agents (9–13). In contrast, radiographic observations of the response of the vasculature of human renal carcinoma and normal renal tissue to exposure to vasoconstrictors have indicated absence of vascular contractility in the tumor vessels (14–16).

It was therefore considered of interest to study the behavior of the vascular bed of human renal adenocarcinoma, a tumor growing in a restricted volume and exhibiting extensive necrosis, by a perfusion technique permitting detailed analysis of the perfusion distribution and vascular resistance at various controlled states of vasoconstriction in histologically and microangiographically defined tissue specimens.

MATERIALS AND METHODS

Tumor System. Kidneys were obtained from patients with angiographically diagnosed renal carcinoma. Kidneys with multiple renal arteries, tumor growth into the renal vein, or infiltration into the renal fascia were excluded. Transperitoneal perifascial nephrectomy was performed. Within a few minutes of division of the renal vessels, the artery was cannulated, and the kidney was flushed with 5% low-molecular-weight dextran (Perfadex; Pharmacia, Uppsala, Sweden) at room temperature until the venous effluent was clear.

Perfusion Technique. Via an arterial cannula the kidney and tumor, together with adjacent fat, were connected to rubber tubing (Fig. 1) and a peristaltic constant flow pump (Ismatec MP4) and perfused with an oxygenated perfusate, kept at 37°C, consisting of 4% dextran (mean M, 70,000; Macrodex; Pharmacia) and 100 ml of horse serum (normal serum; SBL, Stockholm, Sweden) in 1,000 ml of a salt solution with 143 mM Na+, 4.3 mM K+, 2.5 mM Ca++, 0.83 mM Mg++, 141 mM Cl−, 13.3 mM HCO3−, 0.46 mM H2PO4−, and 5.6 mM glucose. The whole preparation was immersed in the perfusate to avoid gravitational pressure artifacts. The venous effluent was drained into this perfusate. The afferent rubber tubing supplied the specimen with perfusate, and also accommodated two thin catheters (PE 50) introduced via a T-connection and ending approximately 1 cm within the renal artery, i.e., protruding through the nipple. One of the catheters was used to draw a reference perfusate sample during microsphere injection, while the other was used for continuous pressure recording (Fig. 1).

Papaverine was given as a bolus dose (median dose, 120 mg; range, 80 to 160 mg) to induce maximal dilation. The pump flow was gradually increased to produce a pressure of approximately 30 to 40 mm of Hg in the renal artery, and the flow was set at that rate for the rest of the experiment.

The first injection of microspheres (New England Nuclear, 15 μm) labeled with 125I consisted of approximately 300,000 spheres in 1 ml of saline with Tween. The injection was given with a fine needle through the latex tubing during 45 s. Fifteen s before, during, and 30 s after microsphere injection a reference sample was drawn, at a rate of 2 ml/min (Model 351 sage infusion pump). Norepinephrine (0.02 mg/ml) was then infused via a T-tube, and the final concentration in the perfusate could be calculated from the rate of infusion. The arterial pressure was recorded continuously. After a certain increase in pressure under steady-state conditions, the second injection of microspheres was given in the same way as the first one but labeled with 141Ce. The norepinephrine dose was then increased stepwise, and at a certain level the third injection of microspheres, labeled with 109Ru, was given.
mm-thick slices using a household cutting machine. The slices were used for the following analyses.

(a) The thinnest section, approximately 1 mm, was used for X-ray microangiography. The section, between plastic films, was placed on the envelope of Kodak X-omax film. Exposure was made at 27 kV and 63 mA in a CGR mammograph with conventional film development. The result provided information on vascular density and showed whether, for technical reasons, any part of the specimen was inadequately perfused.

(b) A slightly thicker section, approximately 2 mm, was used for autoradiography. It was placed between plastic films on the envelopes of Kodak X-omax films placed on either side of the section. One film was exposed for 2 to 3 wk and, depending upon the degree of blackening of this film, the other was exposed for up to 2 to 3 mo.

The result provided information on the average perfusate flow distribution for the 3 different microspheres, where the iodine-labeled ones dominated film blackening.

(c) A thick section, approximately 3 to 4 mm, was used for cutting pieces, 0.1 to 1 g, for quantitative radioactivity measurement to provide perfusate flow data at each microsphere injection (7, 8). Five to 10 pieces were taken from cortical tissue, and 10 to 20 pieces were from tumor tissue. After the radioactivity measurements these pieces were prepared for routine histology to ensure tissue representation and make correlation between morphology and physiology possible.

Data Processing. Perfusate flow data and resistance units, obtained by dividing the flow value for each biopsy by the perfusate pressure for each individual experiment, were in normalized (17). Only pieces with more than 50 spheres of each type were included in the analyses.

From each experiment, three mean flow or resistance values were obtained (in three experiments only two values, due to technical problems), at two different norepinephrine levels, and one at maximal dilation. The mean flow and resistance values were pooled at the following four dose levels of norepinephrine: (a) maximal dilation (without norepinephrine); (b) low dose (0.015 to 0.02 μg/ml); (c) intermediate dose (0.02 to 0.4 μg/ml); and (d) maximal dose (>1.6 μg/ml).

Statistics. All flow and resistance data were in normalized before analysis of significance (17). Student's t test, pairing design, was used throughout the study. P values less than 0.05 were considered significant. The means ± SE is given in the text and figures.

In the figures, * = P < 0.05, ** = P < 0.01, and *** = P < 0.001.

RESULTS

Twenty-eight kidneys with carcinoma were subjected to perfusion but, due to technical failures such as leakage and failure of reference sample drawing, only 14 specimens could be evaluated. The mean weight, including perirenal fat, was 475 ± 58 g. All tumors were adenocarcinomas of varying type and differentiation. Measured upon a midsection assuming an ovoid geometry, the mean tumor weight was 105 g. The tumors showed extensive patchy areas of necrosis macroscopically and microscopically. On X-ray angiography of the tissue slices, resolving vessels at the glomerular level, a dense irregular network of tumor vessels, frequently much denser than in cortical tissue, could be visualized (Fig. 2). In contrast, the autoradiograms showed a very poor perfusion in vessels trapping the 15-μm spheres, even in densely vascularized tumor areas, compared to the well and uniformly perfused cortex. Owing to the anatomically "secondary" capillary network of the medulla, few spheres were trapped there (Fig. 3).

During perfusion, a mean flow of 216 ml/min, or 45 ml/min × 100 g, yielded a pressure of 37 mm of Hg at maximal dilation. Upon infusion of norepinephrine, a mean dose/resistance curve based upon approximately 9 measurements for each of the 14 experiments (Fig. 4) disclosed that the whole specimen was capable of a 6-fold resistance increase from dilation to maximal contraction. No increase of resistance after microsphere injections could be seen in individual experiments indicating a negligible influence of the vascular plugging upon fluid dynamics.

Quantitative analyses of perfusate flow passing vessels less than 15 μm in diameter was performed on 72 cortical and 134 tumor samples in these 14 experiments. There was a very small intraexperimental variation for cortical tissue (10%). The perfusate flow for the different doses of norepinephrine is shown in Fig. 5. A redistribution occurs from cortical tissue to tumor tissue from maximal dilation to moderate norepinephrine concentrations. At higher norepinephrine dose levels,
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Fig. 3. Autoradiogram of an adjacent slice to that of Fig. 2, showing homogeneous blackening from cortical tissue, while that from tumor tissue is more patchy and of lower intensity.

the distribution is reversed, and the tumor flow decreases while the cortical flow seems to increase. However, in some individual experiments, the perfusate flow decreased both in cortex and tumor tissue at high norepinephrine concentrations, which seems impossible since the bulk flow delivered to the preparation is constant. Obviously, a fraction of the bulk flow escapes detection, presumably via arteriovenous passages larger than 15 μm in diameter.

The mean vascular resistance data for the corresponding norepinephrine dose intervals are given in Fig. 6. The resistance of the renal cortex steadily increased with increasing norepinephrine doses, while that of the tumor tissue remained unchanged at low doses, but increased more rapidly than did that of the cortical tissue at higher norepinephrine concentrations.

The quotients between tumor and cortical vascular resistance are shown in Fig. 7. This quotient is maximal at dilation, decreases at low and moderate norepinephrine concentrations, and increases again at high dose levels. The renal cortical vasculature is capable of increasing its resistance approximately 7-fold, while the tumor vascular resistance increased 3 to 4 times, becoming apparent only at high norepinephrine dose levels.
DISCUSSION

This study was prompted by contradictory reports on experimental tumor vascular reactivity to vasoactive drugs. In spite of the lack of traditional contractile structures in the tumor vascular wall, most functional studies on experimental tumors have demonstrated that the tumor vascular bed decreases its blood flow upon exposure to, for example, norepinephrine (7, 8, 10–12). It was considered of interest to study a human tumor where radiologists (14, 15) claim that catecholamines mostly affect normal renal vessels.

As in a recently published study on rat mammary tumors (8), an in vitro perfusion technique was used with minor modifications. It allows registration of perfusion at different states of vascular constriction in the same specimen and in a multitude of samples.

The microsphere tracer technique involves certain technical problems. When used in vivo, adequate mixing is often established when injecting the spheres into the left cardiac ventricle. In this work, the microspheres were injected into the perfusate flow at a distance from the kidney, and even distribution was confirmed by a very small variation in multiple specimens from cortical tissue.

In this study a constant flow was used in each experiment. This means that the preparation has to accept the same bulk flow at maximal dilation as at maximal constriction. The perfusion pressure is thus low at maximal dilation and increases upon vascular constriction. If differences in contractile capacity exist within the preparation, a redistribution of perfusate from the one compartment with highest contractility to the one with lower contractility will occur. In fact, the perfusion may be increased proportionally more than the perfusion pressure, and a reduction in vascular resistance will be recorded, as was often found for tumor tissue in the individual experiments of this study. Further increase of the norepinephrine concentration and, hence, of the perfusion pressure will, however, redistribute the perfusate flow from the vessel wall to that of the cortex. However, a reduction of the measured flow in both cortical and tumor tissue at increasing norepinephrine dose levels in several experiments indicates the existence of an undetected perfusate flow, presumably passing arteriovenous passages larger than 15 μm in diameter letting the microspheres through. In this work, the venous effluent was not analyzed for radioactivity, nor was the total amount of spheres trapped in the specimen measured, and direct calculation of the "shunted" flow was not possible. However, assuming that the preparation is a two-compartment system, cortical and tumor tissue, excluding perirenal tissue and medulla, due to their negligible perfusion, equations can be formulated to express the identity of bulk flow and the sum of measured cortical and tumor flow plus undetected, "shunted" flow for each preparation. Solution of these equations showed that the undetected, "shunted" flow at maximal dilation in some individual experiments constitutes a considerable fraction of the total flow and that this fraction increases upon norepinephrine infusion.

At first sight, this behavior may seem puzzling. However, several investigators have found high interstitial tissue fluid pressures in experimental tumors (4, 6, 18, 19), high enough to compromise perfusion. The hemodynamic consequences of such a high tissue pressure were recently studied in an in vitro system similar to that used in this work (7). It was found that tumor vessels are likely to be collapsed under the tissue pressure at low perfusion pressures, while they open up at higher perfusion pressures. Although the tissue pressure has so far not been measured in renal carcinoma, these tumors are hard and tense upon palpation, both in vivo and during perfusion, indicating a high pressure. Upon norepinephrine infusion, the vasculature of the renal cortex constricts, with a subsequent perfusion pressure increase forcing the perfusate into the tumor vasculature, both narrow and wide arteriovenous passages, which opens up. The fine vascular network, now distended to some extent, may not exhibit its capacity to contract until now. Thus the sensitivity of the different vascular beds at low perfusion pressure is, in this system, difficult to evaluate. A difference in pressure-flow relationships between the compartments seems here to overwhelm the pharmacological effects upon the vessels.

A similar in vitro perfusion system was recently used to study the influence of norepinephrine activity upon experimentally induced mammary tumors in the rat (8). In that study, a hypersensitivity, expressed as an early increase in resistance of the tumor vasculature, was found, in contrast to the present findings. The discrepancy between that study and this one may have several explanations. An important factor is that, in the whole rat, the mammary tumors do not compete exclusively with the strongly reacting kidneys for the flow distribution, but with a multitude of organs. Another, equally important, factor is that the type of tumor is very different, the renal carcinoma having much more necrotic areas, together with a denser capillarity and, presumably, also a higher tissue pressure, possibly enhanced by the growth within the renal capsule.

The findings in this study may play a role in link radiologists' concepts of a nonreactive tumor vascular bed (14–16) to most physiological data on tumor blood flow susceptibility to vasoactive agents. We cannot say whether or not there is a hyperreactivity to or within the tumor vascular network, but perfusion through the fine capillary network is clearly reduced in tumor tissue during high norepinephrine exposure.

There is obviously a delicate balance of flow between different vascular beds, which may be assumed to exist in vivo also. Since interference with the tumor blood supply may become part of tumor therapy, further research into its regulation is warranted.

REFERENCES

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