Distribution to Normal Tissue and Liver Tumors in Rats

Larsolof Hafström, Anders Nobin, Bertil Persson, and Kaj Sundqvist

ABSTRACT

The effects of infusion of adrenaline and noradrenaline were studied in rats with intrahepatic tumors, using \( {\text{\textsuperscript{99m}}\text{Tc}} \)- and \( {\text{\textsuperscript{51}}\text{Cr}} \)-labeled microspheres. The effect on general circulation, cardiac output, and tissue blood flow was pronounced, especially with infusion of noradrenaline. Studies of liver tumor perfusion in relation to surrounding liver parenchyma perfusion showed an increased tumor/liver ratio in noradrenaline-infused rats, thus indicating a preferential blood flow to the tumors induced by this drug. Adrenaline as well as 0.9% NaCl solution infusion had no effect on tumor/liver blood flow ratios. Fluorescence microscopy and monoamine determination could not reveal any noradrenaline containing nerves in the liver tumors. These experiments might suggest that the effect of intraarterial infusion with cytostatic agents might be enhanced by a simultaneously administered vasoconstrictor such as noradrenaline.

INTRODUCTION

The control of tumor growth and palliative treatment of malignant tumors in the liver raise great problems. Several modalities have been tried for these purposes, e.g., dearterialization or intrahepatic or intraportal infusion of cytostatics (4–6, 24–26). Altering the concentration of cytostatics in the tumor vessels by means of injection of degradable microspheres is another manipulation that has been attempted (20). Another possible way to make the treatment of cytostatics more effective could be the administration of vasoactive drugs to redistribute blood to the tumor in order to lead a greater portion of the cytostatic to the tumor and less to unaffected organs, thus improving the cytostatic effect and reducing side effects.

Adrenaline or noradrenaline work mainly by binding to adrenergic receptors, causing alterations in heart rate, blood pressure, and peripheral resistance (14). These agents have been used in pharmaangiography to enhance tumor vascularity (1, 11).

Tumor vessels have been shown to be very immature, lacking smooth muscles and adrenergic nerves in the vessel walls (19). This aspect has also been studied by Mattsson et al. (21), who found no adrenergic innervation in tumor vessels. This means that adrenergic substances would mainly constrict normal vessels and that tumor vessels would probably be unaffected by these drugs. This finding could serve as a background for attempts to deviate or distribute blood flow from normal to pathological tissue during general or regional cytostatic therapy.

MATERIALS AND METHODS

Material

Thirty-seven rats of inbred Wistar and Lister strains with an average weight of 233 ± 8 (S.E.) g were used. Wistar rats were inoculated intrahepatically in the left liver lobe with a standardized adenocarcinoma cell suspension (NG-W). In the same way, Lister rats were inoculated with a hepatoma cell suspension (Hep-H). After 10 to 12 days, the experiments were carried out. At that time, the weights of the tumors in the liver were 0.79 ± 0.12 (S.E.) g (Table 1).

Methods for Determination of Blood Flow

Microspheres and Isotopes. Dextran microspheres (Tracer Sephadex; Pharmacia Fine Chemicals AB, Uppsala, Sweden) with a diameter of 15 μm were labeled with either \( {\text{\textsuperscript{99m}}\text{Tc}} \) or \( {\text{\textsuperscript{51}}\text{Cr}} \) isotopes and dissolved in Ficoll 70 solution (15). The approximate activity per injection was about 25 to 70 μCi for \( {\text{\textsuperscript{99m}}\text{Tc}} \) and for 12 to 14 μCi for \( {\text{\textsuperscript{51}}\text{Cr}} \). Labeling efficiency was about 96 to 99%. Each injection contained about 800,000 to 1 million microspheres. Samples and tissues were counted in a 2-channel well-type scintillation counter. Sample heights in the vials were kept at less than 1.5 cm to minimize geometrical errors. The 140-keV photopeak of \( {\text{\textsuperscript{99m}}\text{Tc}} \) was recorded in the first channel, and the 324-keV photo peak of \( {\text{\textsuperscript{51}}\text{Cr}} \) was recorded in the second channel. Corrections were performed for the Compton contribution of \( {\text{\textsuperscript{51}}\text{Cr}} \) in the \( {\text{\textsuperscript{99m}}\text{Tc}} \) channel.

A computer program was established for the calculations. Cardiac output is expressed in ml • min\(^{-1}\) • 100 g\(^{-1}\) body weight, and tissue organ and tumor blood flow is expressed in ml • min\(^{-1}\) • g\(^{-1}\).

Drugs. The rats were divided in 3 groups. In Series A, 7 rats were infused with 0.9% NaCl solution at a rate of 0.2 ml/min. Series B contained 12 rats infused with adrenaline at a rate of 4 μg per kg body weight per min. The 11 rats in Series C were infused with noradrenaline at a rate of 5 μg per kg body weight per min (Table 1). The dosages were chosen based on a dose-response study in 6 rats. The dosages induced the maximal effect on central hemodynamic parameters without serious side effects.
L. Hafström et al.

**Table 1**

<table>
<thead>
<tr>
<th>Series</th>
<th>Drug</th>
<th>No. Tumors</th>
<th>Tumor wt (g)</th>
</tr>
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<tbody>
<tr>
<td>A1</td>
<td>0.9% NaCl solution</td>
<td>4</td>
<td>NG-W 0.96 ± 0.12*</td>
</tr>
<tr>
<td>A2</td>
<td>0.9% NaCl solution</td>
<td>3</td>
<td>Hep-H 0.41 ± 0.06</td>
</tr>
<tr>
<td>B1</td>
<td>Adrenaline</td>
<td>6</td>
<td>NG-W 1.03 ± 0.34</td>
</tr>
<tr>
<td>B2</td>
<td>Adrenaline</td>
<td>6</td>
<td>Hep-H 1.41 ± 0.54</td>
</tr>
<tr>
<td>C1</td>
<td>Noradrenaline</td>
<td>5</td>
<td>NG-W 1.05 ± 0.33</td>
</tr>
<tr>
<td>C2</td>
<td>Noradrenaline</td>
<td>6</td>
<td>Hep-H 0.23 ± 0.05</td>
</tr>
<tr>
<td>D1</td>
<td>Determinations of monoamine and fluorescence</td>
<td>4</td>
<td>NG-W Not measured</td>
</tr>
<tr>
<td>D2</td>
<td>Determinations of monoamine and fluorescence</td>
<td>3</td>
<td>Hep-H Not measured</td>
</tr>
</tbody>
</table>

\* Mean ± S.E.

**Procedure.** Under pentobarbital anesthesia (30 mg/kg i.p.), catheters with an outer diameter of 0.63 mm were introduced; one catheter was placed in the jugular vein for infusion of drugs, one was placed via the left femoral artery to the lower abdominal aorta for injection of spheres and measurement of heart rate and intra-abdominal pressure and pulse rate were recorded via the catheter in the left ventricle using an Elema-Schönander EMT 34 transducer.

The 99mTc-labeled microspheres were injected into the left ventricle of the heart for 10 sec. The catheter and stopcock were flushed with 0.4 ml of 0.9% NaCl solution. Just prior to this injection, the withdrawal pump was started drawing the reference sample from the aortic catheter at a rate of 0.5 ml/min and was stopped after 40 sec. After this, either adrenaline or noradrenaline or (in the control group) 0.9% NaCl solution infusion was started. Infusion rate was 0.2 ml/min. After circulatory steady state had been established, measured by heart rate and blood pressure, after approximately 3 to 4 min, the second injection of the 99mTc-labeled microspheres was performed, and the second reference sample was drawn.

After 1 min, the rat was sacrificed by an injection of saturated KCl solution. The 2 reference samples, tumor samples, and different tissue samples were taken out, weighed, and counted in the scintillation counter. Cardiac output and tissue blood flow were calculated according to methods previously reported (15). Thus, cardiac output

\[ F = \frac{f_r Q}{q_r} \]

and blood flow of the tissues

\[ f_r = \frac{f_r q_r}{q_r} \]

where \( f_r \) is the known constant withdrawal rate (0.5 ml/min) of the reference sample having the activity \( q_r \) (μCi), \( q_r \) is the measured activity (μCi) of the organ or tissue sample, and \( Q \) is the activity of the administered radioactive indicator.

**Methods for Fluorescence Microscopy and Monoamine Determination.** Seven rats were killed (Series D). Pieces from liver and tumor were also taken for determination of noradrenaline which was performed fluorimetrically according to the method of Bertler et al. (8) and Häggendahl (16).

Results are presented as mean ± S.E. For analysis of significance, Student's t test for paired and unpaired observations was used. Analysis of variance, correlation, and regression analysis was also performed.

**RESULTS**

The circulatory effects of drug infusion in the 3 experimental series are summarized in Chart 1. In the control series infused with 0.9% NaCl solution at a rate of 0.2 ml/min, no significant changes could be noted in blood pressure (128 ± 11 to 125 ± 8 mm Hg), heart rate (304 ± 9 to 360 ± 8), or cardiac output (39 ± 3 to 40 ± 4 ml·min⁻¹·100 g⁻¹). No significant differences between Lister and Wistar rats were noted in Series A.

When adrenaline (series B) was injected, there was an average increase in blood pressure from 98 ± 8 to 131 ± 6 mm Hg. This was of the same magnitude when Groups B1 and B2 were analyzed separately. Heart rate was unchanged (333 ± 6 to 346 ± 7). Cardiac output was not significantly changed in the Wistar rats [26 ± 3 to 29 ± 5 ml·min⁻¹·100 g⁻¹ (Series B)]. The Lister rats (Series B2) showed a significant decrease in cardiac output by the adrenaline infusion from 38 ± 6 to 23 ± 3 ml·min⁻¹·100 g⁻¹ (Chart 1).

Noradrenaline infusion (Series C) induced a significant increase in blood pressure in the Wistar series from 104 ± 8 to 134 ± 6 mm Hg. Heart rate was not affected, 362 ± 3 to 360 ± 5. Cardiac output was significantly lowered from 31 ± 2 to 24 ± 3 ml·min⁻¹·100 g⁻¹. No differences between Lister and Wistar rats were noted in these parameters (Chart 1).

No changes occurred in tissue and organ blood flow after 0.9% NaCl solution infusion in the control group. There were no significant differences between Lister and Wistar rats (Chart 2).
arterial flow to the liver and the lungs. In Series B2, a significant decrease in blood flow was found in the spleen, intestine, and the perfusion of the heart when Series C1 and C2 were analyzed, but an increase in arterial liver blood flow (Chart 2).

Increased blood flow to the myocardium but a decreased solutIon (Series A), adrenaline (Series B), and noradrenaline (Series C). Bars, c1; @, noradrenaline C2. S.E. • p < 0.05; ' ' , p < 0.01 (Student’s t test for paired observations). 0, separately. When the 2 groups were analyzed together, an increase was noted. A decrease in blood flow was registered in both Wistar and Lister rats in kidneys, liver, spleen, and lungs but an increase in arterial liver blood flow (Chart 2).

In the Wistar rats (Series B.), adrenaline infusion caused an increased blood flow to the myocardium but a decreased arterial flow to the liver and the lungs. In Series B2, a significant decrease in blood flow was found in the spleen, intestine, and lungs but an increase in arterial liver blood flow (Chart 2).

Noradrenaline infusion caused no significant difference of the perfusion of the heart when Series C1 and C2 were analyzed separately. When the 2 groups were analyzed together, an increase was noted. A decrease in blood flow was registered in both Wistar and Lister rats in kidneys, liver, spleen, and lungs (Chart 2).

The correlation coefficient between blood flow in the left and right kidneys was not significantly different from 1.0 in the 3 series. After infusion of 0.9% NaCl solution, adrenaline, and noradrenaline, the correlation remained unaltered.

The ratio between blood flow in liver tumors related to liver parenchyma flow increased significantly in noradrenaline-infused rats (Series C1 and C2) but was unchanged in the control series (Series A1 and A2) and in the adrenaline-infused rats (Series B1 and B2) (Table 2), when using Student’s t test for paired observations.

The fluorescence microscopic investigation revealed a dense network of noradrenaline-containing nerves in connection with the blood vessels in the normal liver tissue (Fig. 1). No parenchymal nerve fibers in the rat liver could be observed. In the tumors, a larger number of blood vessels were observed, but neither the vessels nor the tumor tissue were innervated with noradrenaline-containing nerve fibers (Fig. 2). The chemical results confirmed the morphological observations. Thus, the content of noradrenaline in normal rat liver was 0.15 ± 0.003 μg noradrenaline per g liver tissue (mean ± S.E. of 7 determinations), while the noradrenaline content was not detectable in tumor tissue.

DISCUSSION

The results of different methods for treatment of liver cancers leave much to be desired. Several methods for this have been tried without success but remain still in clinical experiments (4, 5, 22). Cytostatic drug administration is one of these. The best clinical results of this technique produce objective improvement in approximately 40 to 60% of patients (6, 24, 26). Any technique that will improve this figure with a reasonable magnitude of side effects is well worth trying. One such technique could be to enhance the blood flow through the tumor in relation to normal tissue by a vasoactive drug and thereby reduce the toxic effects. This has been suggested by Ackerman (2) and Wickersham et al. (28).

The catecholamines, adrenaline and noradrenaline, have profound effects on the cardiovascular apparatus and on blood circulation. The effects are based on the action of these drugs on the α- and the β-adrenergic receptors in the vessel walls. If these receptors and/or muscle cells are lacking in small arterioles and capillaries, the constrictor effect would be minimal if noradrenaline and adrenaline are administered. Krylova (19) has found that during tumor growth there is no development of smooth muscles in the neoplastic vessel walls and that there is an absence of nervous elements.

In this study, radioactively labeled microspheres were used for blood flow measurements. This technique has been evaluated by the present authors (15) and has also been widely used by others (7, 10, 18, 23). With a double isotope technique, blood flow can be studied in the same tissue before and after infusion of the vasoactive drug because of the different γ energies of the 2 isotopes. With this technique, each animal is used as its own control.

In our study on anesthetized rats, adrenaline and noradrenaline produced an increase in the blood pressure consistent with the known effect on the heart. The effect on heart rate showed a slight but not significant rise in the adrenaline-infused rats, an effect that could be masked by the anesthetic agent used (14). Cardiac output showed a significant decrease in both Lister and Wistar rats infused with noradrenaline. The Lister rats showed a significant decrease in cardiac output after adrenaline infusion, but the Wistar rats were unaffected. This decrease in cardiac output in the Lister rats is surprising inasmuch as the expected effects from adrenaline are an increased heart labor with an increase of cardiac output, possibly caused by an increased peripheral resistance or a depressant effect of the anesthetic agent. The difference in car-

<table>
<thead>
<tr>
<th>Tumor/liver blood flow ratio before and after infusion of drugs</th>
<th>Tumor blood flow/liver blood flow</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>A1</td>
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<tr>
<td></td>
<td>A2</td>
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<td>Adrenaline</td>
<td>B1</td>
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<td></td>
<td>B2</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>C1</td>
</tr>
<tr>
<td></td>
<td>C2</td>
</tr>
</tbody>
</table>

*a Mean ± S.E.

*b p < 0.05. Student’s t test for paired observations.
The blood flow to the kidneys showed, as in previous studies (15, 26), a good correlation between the 2 kidneys before and after infusion of the drug or 0.9% NaCl solution, indicating a homogeneous mixing of the spheres. This is a prerequisite for the utilization of the microsphere method (10). Noradrenaline produced a pronounced reduction of the renal blood flow averaging 40%. The adrenaline effect on the kidney blood flow was not as marked in spite of the known effects in humans, in whom adrenaline produces a pronounced constrictor action on the kidneys. This difference might be due to species differences.

Noradrenaline induced a decrease in arterial liver blood flow as well as in the blood flow of the spleen. In Lister rats, adrenaline caused a decrease in liver, spleen, and intestinal blood flow; but in Wistar rats, there was an increase of liver blood flow and an unchanged flow in the spleen and intestine. These differences between the 2 strains may correspond to the variations in response to adrenaline in cardiac output and peripheral resistance.

The ratio between liver tumor and liver parenchyma blood flow was grossly inversely related to tumor size, findings which agree well with our earlier experience (27).

After infusion of 0.9% NaCl solution or adrenaline, no changes in the tumor/liver blood flow ratio was noted. Noradrenaline infusion produced an increase in tumor/liver ratio significant both in Wistar and Lister rats. An explanation for this might be that tumor vessels do not react to the constricting action of noradrenaline, possibly due to the lack of smooth muscles and/or adrenergic receptors adjacent to the vessels.

This hypothesis was tested by fluorescence microscopy, and it was found that noradrenaline-containing nerves could not be seen adjacent to the vessels of the tumors, whereas in the surrounding liver parenchyma the vessels were normally innervated. Moreover, quantitative estimations of noradrenaline content were close to zero. Others have also found an almost complete absence of catecholamine-containing nerves adjacent to tumor vessels (19, 21).

The findings do not agree with those of Ackerman et al. (3), who found that both constrictors and dilators administered intraarterially increased the relative arterial perfusion of experimental tumors in the rat. The method used in their experiments does not reflect the actual flow of the liver but only the relative distribution between portal and arterial blood flow. They also performed injections of the tracers (radiological human serum albumin and 86-μm microspheres labeled with 99mTc) into the hepatic artery after clamping the aorta below the origin of the celiac trunk or by injection into a mesenteric vein to study the portal flow.

Wickershaw et al. (28) noticed that topical administration of adrenaline and noradrenaline caused a significant difference between normal and tumor tissue with no response in tumor vessels indicating a lack of smooth muscles and/or receptors. In a study by Iwaki et al. (17) performed on rabbits, the administration of epinephrine preceding mitomycin C infusion in the hepatic artery significantly increased the ratio of mitomycin C concentration between liver tumor tissue and normal liver tissue. This indicated a redistribution of blood caused by the vasoconstrictor effect on the normal tissue exerted by epinephrine.

This effect on blood flow distribution has also been shown in our study, but noradrenaline seemed to be the most active agent in the rat.

This study points to the possibility of potentiating the effects of intraarterial infusion of antineoplastic agents by concomitant administration of noradrenaline in the treatment of hepatic malignant tumors.

REFERENCES

15. Hafström, L., Persson, B., and Sundqvist, K. Measurements of cardiac output between the 2 strains is difficult to explain, and further studies must be carried out.
Adrenergic Drugs and Tumor Blood Flow


Fig. 1. Fluorescence photomicrograph of normal liver parenchyma. Fluorescence specific for noradrenaline can be seen adjacent to the vessel wall. \( \times 180 \).

Fig. 2. Fluorescence photomicrograph of an adenocarcinoma (NG-W) induced in the liver; dark areas, blood vessels. No fluorescence specific for noradrenaline can be seen adjacent to the vessels. \( \times 220 \).
Effects of Catecholamines on Cardiovascular Response and Blood Flow Distribution to Normal Tissue and Liver Tumors in Rats


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