Comparison of the Blood Supply to Diethylnitrosamine-induced Hyperplastic Nodules and Hepatomas and to the Surrounding Liver

Dennis B. Solt, John B. Hay, and Emmanuel Farber

Department of Pathology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

SUMMARY

Intravascular injection of radionuclide-labeled microspheres was used to compare the blood supply to diethylnitrosamine-induced hyperplastic liver nodules and hepatomas with the blood supply to the surrounding, histologically normal liver. Microspheres injected into the heart or portal vein lodged in the organs of control and diethylnitrosamine-treated rats providing a quantitative index of blood supply to the microvascular bed. The blood supply is expressed as percentage of cardiac output (arterial) or cpm (portal) per organ, lobe, g tissue, etc.

The fraction of the cardiac output received by lung, kidneys, spleen, and liver was similar in control and carcinogen-treated animals. The arterial blood supply of 23 nodules and hepatomas was variable [1.17 ± 0.22% (S.E.) cardiac output per g, fixed weight], but it was similar to the arterial supply to the surrounding tissue (1.12 ± 0.21% cardiac output per g, fixed weight). In contrast the portal blood supply to 25 selected lesions was 39 ± 6% that of the surrounding liver tissue. There was no apparent relationship between blood supply and lesion size or histological appearance.

While only 0.13 ± 0.04% of the microspheres injected via the portal system were recovered in the lungs of control rats, approximately 100 times this number bypassed or escaped the liver containing nodules and hepatomas and lodged in the lungs.

Such alterations in blood flow could contribute to biological diversification of hepatic lesions in successive stages of cancer evolution and could facilitate metastasis from the liver.

INTRODUCTION

The concept that cancer may result from a multistep process of cellular evolution rather than from a single immutable event is gaining prominence in oncology (7, 11). Mounting evidence suggests that chemically induced hyperplastic liver nodules may be one example of tissue at an intermediate stage of such a multistep carcinogenic process.

Although chemical hepatocarcinogens can induce hyperplastic liver lesions within days or a few weeks under optimal conditions, several months or even years are required for eventual cancer production (8). Even then, in spite of the formation of hundreds or possibly thousands of hyperplastic lesions, only a comparatively few cancers ever emerge (6). Fewer still are the hepatocellular carcinomas that progress to aggressive metastasizing lesions. What conditions affect the degree and the rate at which precursor cells acquire their ultimate fully malignant potential?

A variety of factors could theoretically intercede to accelerate, retard, or otherwise modulate the course of carcinogenesis following the initial interaction of a carcinogen with the target cell. Of these factors, perhaps none is more apt to exert a crucial modulating influence than blood supply. By regulating influx of exogenous and endogenous promoting agents, hormones, growth factors, toxic substances, environmental carcinogens, and nutrients, alterations in blood flow can reasonably be expected to affect the biological fate of preneoplastic tissue.

This supposition gains some indirect support from the work of Folkman et al. (9) whose findings demonstrate the profound biological effect that neovascularization exerts on frankly malignant tissue. Capillary ingrowth elicited by a tumor product, "tumor angiogenesis factor," appears to be an obligatory step for actuation of malignant properties in the late stages of development of some tumors (10). Similarly, alterations in the blood supply to putative preneoplastic lesions and primary tumors may have significant biological consequences in earlier stages of carcinogenesis.

Radioactive microspheres have been used experimentally to study the circulation in diverse pathological processes with attendant alterations in blood flow (1, 3, 12, 17). When introduced into the vascular system, spheres of the appropriate size will have negligible recirculation capacity and rapidly lodge in the microvasculature. At appropriate doses they have no observable effect on the gross physiology of the animal. Errors and limitations of their use have been reported (5). The application of γ-labeled microspheres for the quantitative measurement of arterial and portal flow to hyperplastic nodules and hepatomas in comparison with the surrounding liver is the subject of this paper.

MATERIALS AND METHODS

Animals and Treatment. Male Fischer 344 rats (150 to 200 mg; Charles River Breeding Laboratories, Wilmington...
distribution in the liver tissue. As a test of this, microspheres were administered, i.e., cardiac output in cpm.

Portal Blood Flow Measurements. Following sodium pentobarbital anesthesia a laparotomy was performed to expose the portal vascular tree. Labeled tracer Sephadex (15 ± 3 μm) (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) was labeled with ¹⁴¹Ce according to the manufacturer’s specifications. The specific activity was 0.5 to 4 cpm/sphere under our counting conditions. The total administered dose was varied. In studies measuring the blood supply to individual liver lesions, the administered dose was approximately 8.2 x 10⁶ to 2.2 x 10⁷ cpm and 4.5 x 10⁶ to 4.0 x 10⁷ cpm for arterial and portal measurements, respectively.

Arterial Blood Flow Measurements. Light anesthesia was induced with 0.25 to 0.30 ml of sodium pentobarbital (60 mg/ml i.p.). The right carotid artery was dissected free of surrounding tissue, and 2 loose 4-0 silk ligatures were placed around the vessel approximately 1 cm apart. A PE-60 polyethylene catheter (Clay-Adams, Parsippany, N. J.) containing a solution of 0.9% NaCl and 5 IU of heparin per ml (NaCl:heparin solution) was pulled out to a narrow diameter to facilitate entry into the vessel. The vessel was pierced, and the catheter was introduced and advanced approximately 3.5 cm into the heart. A small amount of blood was aspirated to ensure patency of the catheter.

Tracer Sephadex suspended in 0.5 ml of NaCl:heparin solution was rapidly injected through the catheter. An additional 1 ml of solution was rinsed through the syringe to wash residual microspheres into the heart. The animal was then sacrificed with an i.c. injection of sodium pentobarbital (60 mg/ml i.p.). The right carotid artery was dissected free of surrounding tissue, and 2 loose 4-0 silk ligatures were placed around the vessel approximately 1 cm apart. A PE-60 polyethylene catheter (Clay-Adams, Parsippany, N. J.) containing a solution of 0.9% NaCl and 5 IU of heparin per ml (NaCl:heparin solution) was pulled out to a narrow diameter to facilitate entry into the vessel. The vessel was pierced, and the catheter was introduced and advanced approximately 3.5 cm into the heart. A small amount of blood was aspirated to ensure patency of the catheter.

As an additional check on the uniformity of microsphere distribution, a liver was fixed after each method of sphere administration, random fragments from each lobe were weighed, placed in vials, and counted in a Intertechnique Model CG30 gamma spectrometer. Syringe counts obtained before and after infusion provided the net amount of radioactivity administered, i.e., cardiac output in cpm.

RESULTS

Distribution of Arterial and Portal Blood Flow among Individual Liver Lobes. The validity of the technique used in this study depended upon adequate mixing of the microspheres in the blood and their widespread and uniform distribution in the liver tissue. As a test of this, microspheres were introduced into either the arterial or portal system of control animals and distribution of radioactivity was measured in relation to the weight of individual liver lobes. Chart 1 shows that both methods of administration resulted in uniform distribution of labeled particles among the lobes.

As an additional check on the uniformity of microsphere distribution, a liver was fixed after each method of sphere administration, random fragments from each lobe were weighed, and the radioactivity was determined. Following arterial administration 17 fragments (257 ± 25 mg) from one liver trapped 2517 ± 263 (S.E., 10% of the mean) cpm/100 mg. For the other liver given spheres via the portal system, 18 random fragments (189 ± 22 mg) had 1810 ± 150 (S.E., 10% of the mean) cpm/100 mg. The low standard errors in each case are an additional indication that sphere distribution is reasonably uniform within a normal liver using this technique.

Organ Distribution of Cardiac Output in Normal and DENA-treated Rats. The percentage of the cardiac output received by each of 4 different rat organs is shown in Chart 2. In control animals the values for liver, kidneys, lung, and spleen were 6.0 ± 1.2, 17.9 ± 2.0, 2.8 ± 0.9, and 0.9 ± 0.1%, respectively. This distribution is very similar to that previously reported using the microsphere technique in the rat (16). In DENA-treated rats the fraction of the cardiac output

* The abbreviations used are: DENA, diethylnitrosamine; i.c., intracardiac.
received by kidneys, lung, and spleen was similar to controls. An apparent elevation in the percentage of cardiac output received by the liver of carcinogen-treated rats (treated 10.0 ± 1.3% cardiac output versus control 6.0 ± 1.2% cardiac output) may not be significant (0.10 > p > 0.05).

Comparison of Arterial and Portal Blood Flow to Lesions and Surrounding Liver. Table 1 summarizes the arterial blood flow measurements from DENA-treated rat livers. The average blood flow (S) to the lesion-free surrounding liver was 1.12 ± 0.21% of the cardiac output per g of fixed liver. Although there was a 4-fold variation in S from 0.42 to 1.65%, the values may not differ significantly (0.10 > p > 0.05) from those obtained from 4 control livers (0.40, 0.53, 0.94, and 0.38%)

The average arterial blood flow (L) to 23 fixed lesions was 1.17 ± 0.22% cardiac output per g. While this average value is similar to that obtained for surrounding liver (1.12 ± 0.21%), the values for individual lesions (not shown) varied within a given liver and ranged from 0.03 to 4.39% cardiac output per g among the 6 animals.

A comparison of arterial blood flow to 23 lesions and to their surrounding liver revealed no consistent relationship between the 2 (Chart 3). L/S × 100% for individual lesions (not shown) in the 6 rat livers varied from 2 to 294% and averaged 112 ± 15%.

In contrast, measurements of the portal blood flow distribution in 5 treated rat livers reveal a striking difference in the portal L/S ratio (Table 2; Chart 3). The lesions received only 39 ± 6% as much portal blood (i.e., spheres, cpm/g, etc.) as the surrounding liver. Only 1 of the 25 lesions appeared to receive more portal blood than its surrounding (L/S = 1.25). For example, L/S x 100% for 8 lesions in Animal P4 ranged from 1 to 125% and averaged 28 ± 15% (L = 115 ± 62 cpm/100 mg; S = 421 cpm/100 mg).

Comparison of Lesion Histology, Size, and Blood Supply. A histopathological study of 48 lesions failed to elucidate any relationship between their histological pattern and their portal or arterial blood supply. The lesions included typical hyperplastic nodules (Fig. 1), unequivocal hepatocellular carcinomas (Fig. 2), and a spectrum of intermediate lesions. No lung metastasis, anaplastic carcinomas, or extensive necrosis were encountered. Each lesion was diagnosed independently by 3 pathologists and graded from 1 (nodule) to 10 (hepatoma), with respect to pleomorphism, mitotic activity, nuclear:cytoplasmic ratio, basophilia, nucleolar prominence, and several other criteria. No convincing association could be drawn with lesion blood supply (L/S) and any of the histological parameters considered.

There was also no apparent correlation between lesion size and portal or arterial blood supply (Chart 3). Thus, there is no suggestion that blood flow per g of tissue increased (or decreased) as the lesions grew.
Liver to Lung Bypass of Portal Microspheres in DENA-treated Rats. Over 99% of the labeled microspheres injected into the portal venous system of 5 control rats appeared to lodge in the liver. Only 0.13 ± 0.04% (range, 0.04 to 0.25%) of the administered cpm (i.e., spheres) passed through the liver to lodge in the lung. In contrast when radionuclide-labeled microspheres were injected into the portal venous system of 10 DENA-treated rats with nodules and hepatomas, 14 ± 4% (range, 5 to 38%) of the total administered cpm were detected in the lung.

DISCUSSION

A few reported quantitative studies comparing the portal or arterial blood flow to liver tumors and the surrounding liver. (3, 4, 17) have dealt primarily with metastatic or transplanted carcinomas. Comparable studies on the blood supply to primary hepatomas and presumptive preneoplastic lesions have been largely neglected, possibly because of the experimental difficulty in developing such lesions in the absence of general distortion in liver architecture and hemodynamics. The carcinogenic regimen used in this study minimizes such distortion while promoting the formation of discrete hyperplastic nodules and hepatomas.

Table 1

<table>
<thead>
<tr>
<th>Blood flow (% cardiac output/g)</th>
<th>Animal</th>
<th>No. of lesions</th>
<th>Lesion (L)</th>
<th>Surrounding (S)</th>
<th>L/S x 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>4</td>
<td>2.17 ± 0.38*</td>
<td>1.65</td>
<td>132 ± 23</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>5</td>
<td>0.37 ± 0.13</td>
<td>1.55</td>
<td>24 ± 11</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>2</td>
<td>0.53 ± 0.11</td>
<td>0.95</td>
<td>56 ± 12</td>
</tr>
<tr>
<td></td>
<td>A4</td>
<td>3</td>
<td>2.54 ± 0.95</td>
<td>1.49</td>
<td>170 ± 64</td>
</tr>
<tr>
<td></td>
<td>A5</td>
<td>6</td>
<td>0.96 ± 0.17</td>
<td>0.67</td>
<td>143 ± 23</td>
</tr>
<tr>
<td></td>
<td>A6</td>
<td>3</td>
<td>0.59 ± 0.03</td>
<td>0.42</td>
<td>140 ± 18</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td>1.17 ± 0.22</td>
<td>1.12 ± 0.21*</td>
<td>112 ± 15</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

Table 2

<p>| Comparison of portal blood flow to 23 DENA-induced hepatic lesions and surrounding liver |
|---------------------------------|--------|---------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Animal</th>
<th>No. of lesions</th>
<th>L/S x 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>A1</td>
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</tr>
<tr>
<td>A6</td>
<td>3</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>1.17 ± 0.22</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

Blood Supply in DENA-induced Liver Nodules and Hepatomas

Blanchard et al. (3) used labeled microspheres to measure the blood flow to V2 carcinoma implants and surrounding liver. After portal infusion the concentration of label in V2 tumors was 23% that of the surrounding liver. This apparent decrease approaches 39 ± 6% measured for portal flow to DENA-induced hyperplastic nodules and primary hepatomas. However, when microspheres were infused into the hepatic artery, there was a variable but consistent elevation of label in V2 implants from 1.2 to 10.2 times that of the surrounding liver. This result differs from the arterial measurements of DENA-induced lesions in which the concentration of labeled microspheres, although variable, averaged 1.1 times that of surrounding liver. These combined results suggest that the more aggressive V2 carcinoma may have acquired a capacity for arterial neoascularization not yet
expressed by the majority of DENA-induced nodules and primary tumors in this series.

There was no obvious correspondence between the histological appearance of individual lesions and their blood supply. While the lesions included typical hyperplastic nodules and classical trabecular carcinomas, no metastasizing or anaplastic tumors were encountered. In addition, the size range of the fixed lesions was rather narrow (90% < 300 mg). On morphological grounds then, the lesions may all be considered to lay toward the benign end of the neoplastic spectrum. Consistent with this interpretation is the apparent inability of the lesions to evoke neovascularization accompanied by a large increase in arterial blood flow.

Of the spheres injected into the portal venous system 14 ± 4% bypassed the liver with hyperplastic nodules and hepatomas and lodged in the lungs. This bypass may have resulted from anastomosis of the portal and systemic venous system within the liver lesions. The presence of large (microscopic) blood-filled channels within some of the lesions studied adds support to this possibility. In addition several of the livers contained cavernous vascular sinuses, often grossly visible on the capsular and cut surface, which suggests another possible explanation for the bypass phenomenon. The presence in DENA-treated rats of hepatic thoroughfares from the portal to the systemic venous system could provide an ideal route for liver to lung metastasis in subsequent stages of tumor development. There was no evidence (e.g., enlarged spleen cirrhosis, ascites, etc.) to suggest the alternate possibility of extrahepatic bypass via collateral vessels, as seen for example with some types of human portal hypertension.

The results of our study and previous studies (2—4, 13—15, 17) indicate that all types of neoplastic lesions in the liver, including hyperplastic nodules, hepatomas, and metastasis in man and animals, can be associated with a relative decrease in portal blood flow when the lesions are compared to the surrounding liver. This alteration in blood flow could have important biological consequences with respect to tumor evolution.

Once formed, “preneoplastic” or early malignant lesions would be partially protected from bacteria or other toxins (e.g., drugs) arriving from the gastrointestinal tract, while the surrounding liver would be left to perform the bulk of detoxification and the usual gamut of other metabolic functions. Gradually, over a period of several months or years, this physiological imbalance, augmented by periodic influx of environmental (or experimentally administered) carcinogens, could inhibit growth of the surrounding liver while exerting a promoting influence on the lesions. Further growth imbalance could possibly result from ingrowth of new capillaries supplying the developing lesions with nutrient-rich blood partially detoxified by the surrounding liver. While obviously theoretical, this scheme is in agreement with our present limited knowledge of the development of liver cancer in man and animals.

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REFERENCES

Blood Supply in DENA-induced Liver Nodules and Hepatomas

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