Differential Pulmonary Microcirculatory Response to Tumor Cell Emboli as Observed in Vivo*

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SUMMARY

Malignant tumors frequently exhibit characteristic patterns of metastatic spread. In the rabbit, the VX2 carcinoma metastasizes to the lung, whereas the Brown-Pearce carcinoma usually spreads to organs distal to this structure. A system of in vivo microscopy has been established in which the response of the microcirculation of the lung has been observed during embolization of tumor cells from these neoplasms. The VX2 tumor characteristically produces microcirculatory arrest or slowing, but the Brown-Pearce does not show this effect. Microcirculatory response to specific cancer cells may be one of the determinants of metastatic pattern.

Malignant tumors frequently exhibit characteristic patterns of metastatic spread. An illustrative example of this tendency is seen in the metastatic patterns produced by two tumors of the rabbit—the VX2 and the Brown-Pearce carcinomas. The VX2 carcinoma when growing intramuscularly in the thigh of the rabbit metastasizes to the lung and the regional lymph nodes but rarely to other viscera. Also following an intravenous injection of these tumor cells lung tumors are frequent, but tumors elsewhere occur in less than 5 per cent of cases. The Brown-Pearce tumor, when growing intramuscularly, often produces metastases to organs distal to the lung, and after intravenous injection metastases in other organs occur in 90 per cent of cases (1).

These differences in metastatic patterns indicate that a selectivity of organ sites for metastatic growth exists. The causes for such selection are still uncertain, but two theories have been developed to explain this phenomenon. One of these postulates that the site of metastasis is the result of the mechanics of circulation in which physical delivery of tumor cells to an organ plays the prime role in determining metastatic distribution. An example of this is seen in the frequent hepatic metastases from carcinomas of organs drained by the portal system. An alternate explanation is that of the “fertile soil” theory which postulates that a specific organ provides a uniquely suitable environment for the growth of the neoplastic cell. The nature of this suitability is undefined. A large amount of experimental and clinical data has been amassed to support both of these theories.

Attempts to determine the causes for this metastatic specificity have been made in the past by the study of fixed tissue preparations of necropsy material. Pathologic sections suffer from the inherent disadvantage of being unable to reflect the physiologic response of the host which is associated with tumor dissemination and growth. To study these physiologic reactions, stimulated by the commendable work of Wood (4), a laboratory model with in vivo microscopy has been designed (3). This technic is utilizable for observing the dynamic processes in the pathogenesis of metastasis and has been useful in detecting differences in the response of the rabbit lung to emboli from these two carcinomas.

METHODOLOGY

An experimental model has been established for in vivo study of the passage of cancer cells in the microcirculation of the lung. Domestic, albino, male rabbits were anesthetized with pentobarbital sodium, 25 mg/kg. A tracheotomy was performed, and oxygen was administered at a rate of 2 liters/min to produce respiratory arrest. A sternum-splitting incision was made, the thymus gland was excised, and the pericardial sac opened. A polyethylene catheter was passed through a purse-string suture into the infundibulum of the right
ventricle and was directed into the left pulmonary artery. A left posterolateral thoracotomy incision was then made. The position of the catheter in the left pulmonary artery was checked by an injection of methylene blue, which momentarily discolored the lung.

Instrumentation for microscopy utilized an operating unit of heavy sheet aluminum supporting a vertical steel tube on which were mounted two horizontal arms to which were attached a compound microscope and a Bolex Rex 16-mm. picture camera driven by an electric motor. These arms permitted vertical, horizontal, and rotary adjustment of the microscope and camera by means of sleeves and set screws. The edge of the lung was transilluminated by light from a shielded Tru Flector Tungsten spring filament light bulb delivered through a methyl methacrylate rod. The surface of the lung was irrigated with mammalian Ringer's solution at a constant temperature of 37°C. Direct observation of the pulmonary microcirculation with cinephotomicrographic recording on Ektachrome ERB film at speeds of 16–64 frames per second was performed.

A cell suspension of VX2 rabbit carcinoma was prepared in a cytosieve, and this suspension further filtered through a metal sieve with a pore size averaging 65 μ. The cell suspension was vitalized with methylene blue, and microscopically undamaged single cells with occasional clumps were obtained. This cell suspension was then washed with saline, centrifuged, and the supernatant fluid removed. A cell suspension was then injected into the pulmonary circulation via the catheter in the pulmonary artery.

The Brown-Pearce tumor was prepared and injected in a similar fashion. A suspension of rabbit muscle cells was also prepared in a similar manner and injected.

A 10 per cent homogenate of VX2 tumor was prepared at 0°C to preserve enzymatic activity of this tumor. The homogenate was centrifuged in a vacuum at 40,000 r.p.m. for 30 minutes to produce a particle-free solution of soluble enzymes of the cytoplasm.

RESULTS

With this system direct observation was possible of blood flow through arterioles, capillaries, and venules. The passage of discrete red cells through capillaries was easily seen. The vitaly stained tumor cells were readily apparent when passing through the field of observation.

Criteria were selected to designate the result of injections of neoplastic cells. The results were classified in the following manner: (a) Microcirculatory arrest: This designation was used if the injection of tumor cells produced a complete cessation of pulsatile progressive blood flow in the capillaries, arterioles, and venules under observation within a period of 15 seconds. (b) Microcirculatory slowing: This designated a definite and unequivocal slowing of the circulation occurring within 15 seconds of injection which did not eliminate all progressive motion of the circulation. (c) No effect: This indicated that only an equivocal slowing of the circulation developed or that no change at all was evident.

In those instances in which circulatory arrest was noted, the arrest immediately followed the injection of the tumor cell suspension and was often of only temporary duration with subsequent reestablishment of flow. However, if the dosage of embolic cells was massive, permanent arrest of the microcirculation, usually resulting in eventual death of the animal, was noted.

Arrest of the circulation occurred with the arterioles, venules, and capillaries engorged with cells. It did not appear that simple mechanical blocking of the larger arterioles was responsible for this effect, because the size of the cells in the suspension was smaller by far than the diameter of these arterioles. Also when mechanical obstruction of the lobar artery to the lung was produced by advancing the pulmonary artery catheter, cessation of flow was produced with an ischemic lung. This differed from the appearance of the engorged lung after neoplastic embolization.

With inocula which did not produce immediate circulatory arrest, reversal of blood flow in arterioles and venules was frequently seen.

A total of 41 observations was made of the effect of VX2 tumor (Table 1). Nineteen initial observations were performed with inocula prepared by suspending similar amounts of tumor in equal volumes of saline. These suspensions were not counted for cell concentration. With this technic nineteen observations of the effect of the injection of VX2 tumor were made. Fifteen of these nineteen showed a prompt arrest of the microcirculation, and in four no effect was noted. In the instances of circulatory arrest the VX2 cells could often be seen trapped in arterioles and capillaries.

Brown-Pearce tumor cells prepared in a similar manner were injected 22 times. In nineteen instances there was no discernible effect on the circulation, and the cells almost always disappeared rapidly from the microscopic field. On three occasions arrest was noted when massive numbers of cells were injected.

With this difference in result noted, quantification of the response was established. A standard-
ized preparation of tumor with a concentration of 500,000 cells/cc, counted in a counting chamber, was prepared. In 22 observations with the VX2 tumor on injection of this suspension prompt arrest of the circulation was noted on ten occasions, slowing of the circulation in four, and no effect was noted in eight. Brown-Pearce tumor in a similar dosage produced no arrest or slowing of the circulation in seventeen observations.

The suspension of rabbit muscle cells produced no slowing or arrest of the circulation in the course of twenty injections.

The 10 per cent homogenate of VX2 tumor did not produce slowing or arrest in ten injections.

The total results indicate that with the VX2 tumor in 41 observations arrest was noted in 25, Brown-Pearce tumor cells to twenty rabbits and simultaneously collected blood from the aorta. This aortic blood was injected into other rabbits and produced growth of Brown-Pearce tumor in ten, indicating that immediate transpulmonary passage of the Brown-Pearce tumor cell had occurred in the original animals. With the VX2 tumor similarly injected, collected aortic blood produced tumor growth in only two of fifteen rabbits given injections. These studies indicated that immediate transpulmonary passage of cancer cells is possible and that this passage may differ with specific tumors. The size of the cancer cell could not be incriminated as the cause for this difference, since the Brown-Pearce cell which passes the microcirculation of the lung much more readily is 20 per

TABLE 1

RESULTS OF EFFECTS OF VX2 AND BROWN-PEARCE TUMOR CELL EMBOLI, VX2 HOMOGENATE, AND MUSCLE CELL SUSPENSION ON THE PULMONARY MICROcirculation

<table>
<thead>
<tr>
<th>Tumor and dosage</th>
<th>No. observations</th>
<th>Arrest</th>
<th>Slowing</th>
<th>No effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>VX2</td>
<td>19</td>
<td>15</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>VX2, 500,000 cells/cc</td>
<td>22</td>
<td>10</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Total VX2:</td>
<td>41</td>
<td>25</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Brown-Pearce</td>
<td>22</td>
<td>3</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Brown-Pearce, 500,000 cells/cc</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Total Brown-Pearce:</td>
<td>39</td>
<td>3</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>VX2 homogenate (cell-free)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Muscle cell suspension</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

slowing in four, and no effect in twelve. With the Brown-Pearce tumor in 39 observations no arrest was observed in 36.

The results of these differences in all the observations of the effect of the VX2 and the Brown-Pearce tumor cells are statistically significant when subjected to the $\chi^2$ system of analysis. The value of $\chi^2$ is 33.08 for 2 degrees of freedom (smaller probability than 0.05).

DISCUSSION

In attempting to determine whether metastases in organs distal to the lung without gross evidence of pulmonary involvement were the result of secondary embolization from microscopic pulmonary metastases or whether they develop from cancer cells which pass immediately through the circulation of the lung, Zeidman (6) studied the behavior of VX2 and Brown-Pearce tumors. This investigator gave injections intravenously of a suspension of cent larger than the VX2 tumor cell. The average mean size of the Brown-Pearce cell is 12.6 μ, and the VX2 cell measures 10.7 μ.

Zeidman (5) has recently utilized in vivo technics to study differences in these tumor cells. Using an in vivo preparation to study the rabbit mesentery he noted that the VX2 tumor cell apparently does not mold itself readily to conform to capillary size. As a result this cell could not easily squeeze through the capillary network. The Brown-Pearce cell, however, possesses a compressible cell wall capable of adapting itself to small capillaries.

Lawrence et al. (2) established the presence of thromboplastic activity in the VX2 tumor by injecting 3–5 cc. of a 10 per cent suspension of cells disrupted by a Waring Blender. It is not believed that the effects noted in our study are due to thromboplastic action, since a cell-free VX2 homogenate did not produce the result of circulatory arrest.
On the basis of the data collected in this laboratory, which are in accord with Zeidman’s observations, it is postulated that there is a difference in the response of the pulmonary microcirculation to tumor cells which correlates with the metastatic pattern which these tumors produce when metastasizing spontaneously. Characteristically, the VX2 tumor cell injection was followed by a prompt arrest of the microcirculation. This arrest was more marked at the higher dosage level, and the discrepancy between the effects of the two tumors was greater at this level. Similarly, the maintenance of normal pulmonary microcirculatory flow with the Brown-Pearce tumor injection and the rapid disappearance of these cells from the field of observation correlates with the pattern of metastases distal to the lungs with this tumor.

It is hypothesized that the physical property of this cell or its membrane may evoke the difference in response. The arrest of the circulation may play a significant role in delineating the sites of metastatic involvement.

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