The Lack of Growth of Intravenously Inoculated Tumor Cells in Peripheral Wounds*

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SUMMARY

Various doses of Walker 256 and Guerin tumor suspensions were injected intravenously into groups of rats which had been subjected to incisions of skin and muscle less than 1 hour previously. Similar experiments were conducted with Sarcoma 180 and V2 carcinoma of mice and rabbits, respectively. Following intravenous injection of cancer cells no metastases were noted in the scars of 520 incisions made in rats, in 40 incisions made in mice, and in sixteen incisions in rabbits.

In no animal did the intravenous injection of tumor cells result in the growth of a metastasis in an incision. However, when one million V2 carcinoma cells were given intra-arterially, four tumors were found in 24 incisions made in nine rabbits.

Wound contamination with tumor cells at the time of operation for cancer is one of the recognized causes of local recurrence. It has been suggested that deposition in the wound, of cells circulating in the blood stream, may also explain some of the recurrences.

R. R. Smith and associates (8) demonstrated that histologic evidence of vascular invasion in the excised specimen was associated with an increased incidence of malignant cells found in wound washings. They postulated seeding of wounds from severed lymphatic and blood vessels as a cause of local recurrence. Robinson and Hoppe (7) showed that V2 carcinoma of rabbits injected into the descending aorta implanted more frequently in limbs subjected to ischemia or blunt trauma than in normal limbs.

This study evaluates the capacity of cells from several animal tumor systems to implant in surgical incisions from the circulating blood.

MATERIALS AND METHODS

1. Two longitudinal incisions (3 cm. long) and two transverse incisions (2 cm. long) were made in 130 female Sprague-Dawley rats weighing between 150 and 200 gm. The incisions were carried through skin and muscle, but no body cavities were entered.

Walker 256 cell suspensions were prepared by mincing the tumor in Hanks solution and passing it through a stainless steel cytosieve. The suspension was then diluted with sufficient Hanks solution to yield the desired number of cells in 0.5 ml. The suspensions of Guerin uterine tumor were prepared in like fashion. One gm. of tumor tissue in 10 or 20 ml. of Hanks solution was used because it is very difficult, if not impossible, to count Guerin tumor cells.

The incisions were made and closed prior to the injection of the cell suspension into a hind leg vein. This allowed a 10- to 60-minute interval between surgery and cell dissemination. The cells were injected in various doses (see Table 1).

2. Twenty Swiss mice were subjected to one dorsal and one abdominal incision. A suspension of Sarcoma 180 cells was prepared by the above method. The mice received a dose of 500,000 S-180 cells after closure of the incisions.

3. Two 8-cm. longitudinal dorsal incisions and two 4-cm. transverse ventral incisions of the skin and muscle layers were made in seven 3-kg. female rabbits. Four million V2 cells were injected intravenously into a hind leg of four female rabbits weighing 3 kg. One million V2 cells were given into the aorta of the other three animals after two incisions were made on the back and two incisions made on the abdomen.

An intra-aortic injection of one million V2 cells was made in six rabbits after making and closing two incisions on one hind leg on each of the animals. The intra-aortic injections were done via a cannula passed through the ligated left common carotid artery.

4. All animals were observed for the 4- to 6-week period necessary for them to die or become moribund from pulmonary metastases. The skin was then removed from the incised areas, and the skin and muscle were thoroughly palpated and examined for tumors. Any areas suggestive of tumor growth were submitted for histologic examination.

RESULTS

None of the rats subjected to intravenous injection of various doses of Walker 256 or Guerin cells developed metastases in any of the 520 scars (Table 1).

No tumor nodules developed in any of the 40 dorsal and
TABLE 1

IMPLANTS IN WOUNDS AFTER INTRAVENOUS INJECTION OF RAT TUMOR CELLS

<table>
<thead>
<tr>
<th>No. animals</th>
<th>Cell dosage</th>
<th>Tumors/incisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>25,000 W256</td>
<td>0/60</td>
</tr>
<tr>
<td>15</td>
<td>50,000 W256</td>
<td>0/60</td>
</tr>
<tr>
<td>40</td>
<td>100,000 W256</td>
<td>0/160</td>
</tr>
<tr>
<td>20</td>
<td>500,000 W256</td>
<td>0/80</td>
</tr>
<tr>
<td>20</td>
<td>0.5 ml. Guerin 1/10</td>
<td>0/80</td>
</tr>
<tr>
<td>20</td>
<td>0.5 ml. Guerin 1/20</td>
<td>0/80</td>
</tr>
</tbody>
</table>

There has been ample work to show that tumor cells given by the intravenous route are capable of passing through the lungs and appearing in the peripheral blood. Jonasson (3) showed that these cells are viable. Zeidman and Buss (9) also demonstrated transpulmonary passage and viability of intravenous tumor cells. They injected V2 carcinoma and Walker 256 carcinosarcoma into the peripheral veins of rabbits and rats and showed that blood obtained from the aorta would produce tumor growth when given intravenously to a second animal. In spite of this insensitive assay method, one of eleven rats and two of fifteen rabbits developed tumors. Roberts (6) points out that secondary embolization of cancer cells from the lungs can supply circulating cells to the peripheral blood long after the original lodgement of cells in the lungs. Griffiths and Salsbury (2) injected 100,000 Walker 256 carcinoma cells intravenously into rats and recovered nine cells per cc. of blood 45 minutes after injection and eight cells per cc. of blood 85 minutes after injection. In cortisonized animals Moore (5) demonstrated circulating cancer cells in the blood 72 hours after intravenous administration. It appears, therefore, that there should be enough cells circulating in the vascular system for hours after intravenous injection in the animals tested to form metastases in fresh wounds if the area of incision truly represented susceptible tissue.

REFERENCES


vventral scars of the twenty mice subjected to intravenous injection of 500,000 S-180 cells.

No metastases were found in any of the sixteen incisions of four rabbits receiving intravenous V2 cell suspension. Metastatic tumors were found in four incisions of the nine rabbits receiving intra-aortic injections of V2 cells. Two of the tumors were found in dorsal incisions and two were found in incisions of hind legs.

The intravenous tumor injections resulted in a 100 per cent incidence of pulmonary metastases as well as occasional scattered tumors in other organs.

DISCUSSION

The results of this study do not refute previous findings that V2 carcinoma of rabbits tends to become implanted in areas of traumatized tissue when it is injected in high doses into a supplying artery (5). This tendency was shown more vividly by Robinson and Hoppe using blunt trauma rather than the sharp incisions of this study. It has been recognized that the V2 tumor has unique properties such as: a tendency to metastasize to muscle, a strong thromboplastic effect (1), high adhesiveness of cells which is not so markedly present in other tumor systems and which may augment the capacity of the V2 cells to implant from the circulating blood.

The findings with the use of intra-arterial V2 cells are not consistent with the results of experiments with other tumor systems given by intravenous injection. It was felt that intravenous administration of the cells may be more nearly analogous to the clinical situation. Intravenous administration produces a moderate shower of cells which falls off rapidly (4), whereas an intra-arterial injection provides an overwhelming onslaught of cells to the area supplied by the artery.
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