Relationship between renal capsular artery feeding and size of VX-2 carcinoma implant in the rabbit kidney

G. Gadeholt-Göthlin · J. H. Göthlin

Abstract When regional intraarterial infusion is applied in the treatment of malignant tumors it is essential to reach the tumor via all its major feeder vessels. In this study VX-2 carcinoma was implanted into the lower pole of the left kidney in 24 rabbits to investigate whether the renal capsular artery takes part in tumor feeding. The rabbits were divided into four groups that were followed for 8, 10, 12 or 14 days after tumor implantation. At that time the renal artery was ligated close to the kidney and subsequently silicone rubber or barium sulfate/gelatin suspension was injected into the capsular artery. The tissue was cleared, and the tumor carefully removed and examined microscopically for traces of silicone rubber. When barium sulfate had been injected, the kidney was examined radiographically in order to detect possible presence of contrast medium in the tumor. This study revealed no vascular supply to the implanted VX-2 carcinoma from the capsular artery when the tumor was confined intracapsularly, i.e., up to 12 days after tumor implantation in untreated rabbits.

Key words Tumor blood supply · Renal capsular artery · Kidney neoplasm · VX-2 carcinoma · Angiography

Materials and methods

Twenty-four male and female French Burgundy/Chinchilla hybrid rabbits, average weight 3.0 ± 0.5 kg, were used. The rabbits were housed individually in stainless steel cages with free access to standard laboratory pellets and water.

The VX-2 carcinoma is a highly malignant anaplastic squamous cell carcinoma characterized by rapid and predictable metastases to lymph nodes and lungs. Its growth characteristics and histology are well described [1, 6, 8, 9]. The VX-2 carcinoma was passed serially by intramuscular injection of approximately 1 million cells into the hind limb of a rabbit every 12-14 days. The donor rabbit was anesthetized and its skin shaved and cleansed with 70% alcohol. Using sterile technique, the skin and subcutaneous tissues were deflected and the tumor (1-2 cm in diameter) was dissected free from surrounding muscles and fasciae. Small pieces of tumor were removed from the peripheral, less necrotic area, and finely chopped using a MacIlwain tissue chopper (Mickle Laboratory Engineering, UK). The tumor tissue was suspended in Hanks’ balanced salt solution (Flow Laboratories, UK) and gently homogenized with a Downe’s homogenizer before the tissue was forced through a cytosieve into a sterile petri dish. The cell viability was checked with trypan blue stain (Sigma, USA), and the cell concentration adjusted to approximately 10^8 cells/ml in Hanks’ solution.

For surgical procedures the rabbits were anesthetized with fentanyl 0.2 mg-fluanisone 10 mg/ml (Hypnorn, Janssen Pharma-
Table 1 Tumor size and extent at autopsy 8, 10, 12, and 14 days after renal implantation of VX-2 carcinoma in rabbits

<table>
<thead>
<tr>
<th>Days after tumor implantation</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor diameter (range in mm)</td>
<td>1-3</td>
<td>3-6</td>
<td>6-12</td>
<td>7-15</td>
<td></td>
</tr>
<tr>
<td>Intrarenally confined tumor</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Renal capsule invasion</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Perirenal invasion</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Fig. 1 Experimental setup for study of the capsular artery. a-d, ligatures around the aorta, e-f, ligatures around the distal part of the left and right renal arteries.

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The results are summarized in Tables 1 and 2. All 24 rabbits developed a kidney tumor after implantation. At autopsy the maximum tumor diameter ranged between 1 and 3 mm, 3 and 6 mm, 6 and 12 mm and 7 and 15 mm after, 8, 10, 12 and 14 days, respectively (Table 1).

In 3 rabbits there were technical problems when applying the glue (solidified glue sticking to the needle as it was withdrawn after implantation). When this occurred a new drop of glue was applied to the puncture site immediately. One of these rabbits had capsular infiltration but no feeders from the capsular artery on day 12 and two rabbits had tumor invasion of the perirenal tissue on day 14. Of the 21 rabbits in which there were no problems with the radiographic equipment, barium angiography was substituted for the silicone rubber technique in the remaining 15 rabbits. Microfil was injected via the catheter to fill the entire superior capsular artery and was allowed to cure at room temperature overnight. Thereafter, the left kidney was removed and in to approximately 5-mm-thick slices, and the tumor’s three longest perpendicular diameters were measured. The tumor was marked with a pin before the specimens were dehydrated using ethyl alcohol and cleared in methyl salicylate. The white tumor was marked with a pin before the specimens were dehydrated using ethyl alcohol and cleared in methyl salicylate. The white, Microfil was easily seen both macro- and microscopically. Finally, the tumor-containing specimens were cut into 1-mm-thick slices and examined microscopically for silicone compound in the tumor.

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