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

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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

When regional intraarterial infusion is applied in the treatment of malignant tumors, a sufficient drug concentration at the tumor site is a prerequisite for successful treatment, and it is essential to reach the tumor via all its major feeding vessels. Therefore, it is important to know all the main tumor feeders to make a comparison of various treatments possible, especially in an investigation comparing results after intraarterial and intravenous treatment.

In order to study the various aspects of local arterial treatment of renal malignany, a rabbit model with implanted kidney VX-2 carcinoma has been established. Each rabbit kidney is supplied from one renal artery. However, in rabbits as in humans [5], a capsular artery supplying the renal capsule and the perirenal tissue is given off as a separate branch from the renal artery immediately after the origin of this vessel. Whether the capsular artery takes part in feeding an implanted renal tumor may be of importance, since the amount of drug to enter that vessel during renal artery infusion may vary and possibly influence the treatment result. The aim of this investigation was to study whether the capsular artery participates in feeding an intrarenally implanted VX-2 carcinoma.

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 (Hypnorm, Janssen Pharma-

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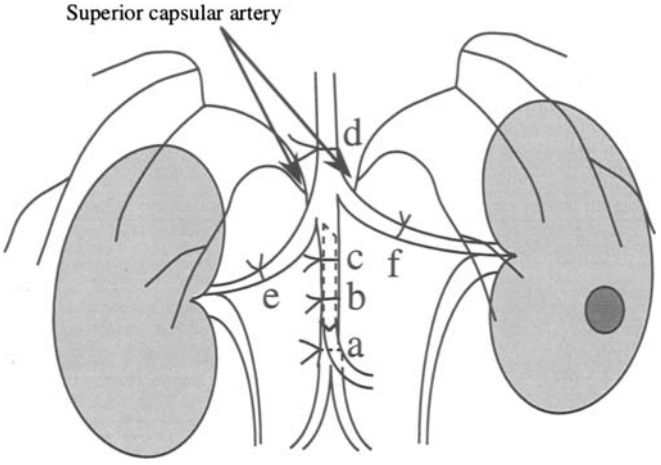

Fig. 1 Experimental setup for study of the capsular artery. *a–d,* ligatures around the aorta; *e, f,* ligatures around the distal part of the left and right renal arteries

Table 1 Tumor size and extent at autopsy 8, 10, 12, and 14 days after renal implantation of VX-2 carcinoma in rabbits

	No. of rabbits				
Days after tumor implantation	8	10	12	14	Total
Tumor diameter (range in mm)	1–3	3–6	6–12	7–15	
Intrarenally confined tumor	6	5	6	3	20
Renal capsule invasion	0	0	1	1	2
Perirenal invasion	0	0	0	2	2

ceutica, Belgium) 0.3 ml/kg i.m. and midazolam (Dormicum, Roche, Switzerland) 2 mg/kg i.p. Local anesthesia mepivacaine hydrochloride 5 mg-epinephrine 5 µg/ml (Carbocaine adrenaline, Astra, Sweden) was given at the incision site. Additional anesthesia was given when required. The rabbit was placed in the right lateral decubitus position with a tissue roll underneath to facilitate access to the left kidney. Using sterile technique, the left kidney was exteriorized through a transverse oblique left flank incision, and stabilized outside the wound. Using a 25-gauge needle approximately 0.015 ml tumor cell suspension was injected at a depth of approximately 2–3 mm into the cortex laterally between the midportion and lower pole of the left kidney (day 0). To seal the needle track and prevent retrograde seeding of tumor cells, a drop of cyanoacrylate adhesive (Sicomet, Henkel, Germany) [4] was placed on the renal capsule at the injection site as the syringe was withdrawn. The kidney was carefully replaced, and the wound was closed in three layers. Metal clips were used to close the skin. Postoperative buprenorphinum (Temgesic, Reckitt & Colman, UK) 0.1 ml/kg was given s.c. for analgesia.

Rabbits were assigned at random to be studied 8, 10, 12, or 14 days after tumor implantation. The capsular artery was not examined in 3 rabbits, due to death from anesthesia (1 rabbit) and technical problems with the radiological equipment (2 rabbits). In these cases only autopsy was performed to measure the tumor and examine the relationship between the tumor and the renal capsule.

The capsular artery was studied in 21 rabbits 8, 10, 12, or 14 days after tumor implantation in 4, 5, 6, and 6 rabbits, respectively. Through a midline abdominal incision and blunt dissection the infrarenal abdominal aorta was uncovered. The experimental setup is shown in Fig. 1. Silk ligatures were placed loosely around the aorta approximately 1 cm above the bifurcation (a) and midway between the bifurcation and the renal arteries (b, c). Additional ligatures were placed around the aorta just above the renal arteries (d), and the distal part of the left as well as the right renal artery (e, f). Ligature a was tightened, thus occluding the distal aorta, whereas ligatures b–f were left loosely applied. Through an incision in the aorta between ligature a and b, a polyethylene catheter (PE 200) was inserted into the aorta and advanced proximally. It was, however, ensured that the catheter tip remained below the origin of the renal arteries. The catheter was secured by tightening ligature c.

In rapid succession perfusion was started with heparinized Ringer-Locke's solution at 30 ml/min. The renal veins were incised bilaterally and ligature d was tightened to prevent continued blood flow to the kidneys. The kidneys were flushed with 150–200 ml Ringer-Locke's solution until the infusate from the renal veins was colorless. Ligatures e and f were then securely tightened, and the rabbit was put to death with intracardial KCl. In order to visualize the capsular artery, either barium suspension of silicone rubber (Microfil, Canton Bio-medical Products, USA) was injected manually into the catheter.

In the first 6 rabbits barium angiography was performed using a suspension of 10% barium sulfate (Micropaque, Nicholas Laboratories, UK) in water containing 5% gelatin in solution. Using fluoroscopic control, enough barium suspension was injected to visualize the entire capsular artery before anteroposterior and oblique radiographs were obtained. The left kidney was then freed from perirenal tissue and removed. Radiographs with 2.5 times magnification of the tumor-containing kidney in situ, and after removal, were obtained.

Owing to technical 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 cut 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 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.

Results

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 application of the tissue adhesive, 2 rabbits had extrarenal tumor extension, (1 of capsular infiltration on day 12 and 1 of perirenal fat infiltration on day 14).

Of the 21 rabbits that had the capsular artery examined, the injection of Microfil or Micropaque was successful in 18 (Table 2). Tumor feeding from the capsular artery was found in 1 rabbit 14 days after tumor implantation and this rabbit had extracapsular tumor growth. In the remaining 17 rabbits there was no tumor feeding from the

Table 2 Results of capsular artery examination 8, 10, 12, and 14 days after renal implantation of VX-2 carcinoma in rabbits

	No. of rabbits				
Days after tumor implantation	8	10	12	14	Total
No. of rabbits	6	5	7	6	24
Capsular arteriography not attempted	2	0	1	0	3
Capsular arteriography attempted	4	5	6	6	21
Total No. of Microfil/Micropaque angiographies	4/0	5/0	4/2	2/4	15/6
Evaluable Microfil/Micropaque angiographies	4/0	4/0	4/2	2/2	14/4
Non evaluable Microfil/Micropaque anigographies	0/0	1/0	0/0	0/2	1/2
Capsular artery feeding extrarenal tumor					
By Microfil or Micropaque method	0	0	0	1 of 4	
By macroscopic evaluation	0	0	0	2 of 2	
Total	0 of 4	0 of 5	0 of 6	3 of 6	

capsular artery. Poor contrast medium filling of the capsular artery due to a gas bubble occurred in 1 rabbit (day 14), and in two others (days 10, 14) there was a leak of contrast medium through the left renal artery ligature into the intrarenal vessels, rendering an angiographic evaluation of the vascular supply impossible. In the 6 rabbits where no angiography was carried out macroscopic evaluation was performed (Table 2). This demonstrated renal tumors with extracapsular extension into the perirenal fat, and a direct inspection of the capsular artery at autopsy demonstrated feeders from this vessel to the extracapsular parts of the tumor and in 2 rabbits on day 14, whereas the tumor blood supply could not be assessed in 3 rabbits with intrarenal tumor in 1 with tumor infiltrating the renal capsule.

Discussion

The rabbit kidney receives its main blood supply from one renal artery. A superior capsular artery is given off from the left renal artery shortly after its origin from the aorta [7]. It runs over the cranial pole of the kidney and inferiorly along the lateral renal margin with branches extending into the perirenal fat equivalent to the superior capsular artery in man, and supplies parts of the renal capsule and perirenal fat [5]. In man sometimes a middle capsular artery arising from the renal artery or its dorsal branch and an inferior capsular artery arising from the gonadal arteries can be identified. If they take part in tumor supply they are often enlarged and more easily identified [5]. In the rabbits we were only able to identify the equivalent of the superior capsular artery, as the renal artery had been ligated too proximal to visualize the middle capsular artery and the aorta ligature was too proximal to permit visualization of the gonadal vessel.

For comparison of renal intraarterial drug treatment with and without temporary renal artery occlusion only the superior capsular artery may cause differences between the groups in this model with intrarenally confined tumor. In order to occlude the renal artery in occlusion-infusion treatment the catheter will in most instances have to be advanced into the distal part of the artery, thus excluding tumor portions supplied from the superior capsular artery from treatment. For intraarterial infusion without occlusion it is, however, important to avoid wedging of the catheter in the renal artery. Thus, the catheter tip must be placed proximally in the renal artery, resulting in a combined renal and superior capsular artery infusion [3]. Independent of administration method the amount of drug reaching a possible middle capsular artery will probably be the same, whereas an inferior capsular artery will not be infused in either case with selective renal artery therapy.

As frequently observed in man, a large tumor would be expected to receive parts of its nutrition from capsular arteries. However, since capsular arteries supply the renal capsule, it seems logical that an intracapsular tumor would be fed from these arteries only when invading or growing very close to the capsule. Boijsen [2], using selective renal angiography, found that capsular arteries supplied a renal tumor in 10 of 11 patients when the tumor invaded the perirenal tissue. When the renal capsule was intact, the capsular vessels supplied the tumor in 16 or 28 cases, but the intrarenal tumor localization was not described.

In this series with tumor implanted in the lower kidney pole, capsular artery involvement in tumor feeding could not be detected within 12 days after tumor implantation. In 3 rabbits examined 2 days later there was, however, a vascular supply from the capsular artery. The kidney tumor had grown laterally and posteriorly to invade the renal capsule (1 rabbit) and perirenal tissue and the psoas muscle (2 rabbits). In addition to the capsular artery, branches of intercostal or lumbar arteries probably also took part in the vascular supply of the tumor.

Although Micropaque/gelatin suspension and Microfil was injected until the superior capsular artery was filled as judged by fluoroscopy or direct inspection, one cannot be sure that minute vessels, too small to be detected radiographically or with simple microscopy, did not take part in the tumor supply. However, even if such small vessels do take part, the amount of blood should not be significant enough to influence intraarterial therapy in this model.

The present animal model has been used to test various administration modes of doxorubicin, and as long as the tumor does not invade the renal capsule (within 12 days after implantation) all its blood supply should come from the renal artery.

Two conclusions may be drawn from the result: (1) when the VX-2 carcinoma is confined intrarenally there is no supply from the capsular artery and (2), when the carcinoma spreads outside the renal capsule, capsular artery supply is seen in three out of six rabbits 14 days after tumor implantation.

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