INTRODUCTION

The VX2 carcinoma is a widely used model of transplantable neoplasia in the rabbit.1,2 We previously described retrograde intra-arterial administration of a VX2 single cell suspension via the auricular artery to create a model of widespread metastasis in rabbits, for evaluation of novel tumor-avid imaging contrast agents.3

Upon implementation of this model in a pilot study, we found that widespread metastasis was not reliably produced in all rabbits, with some animals developing only unilateral localized head and neck metastasis. We hypothesized that this was due to failure of the tumor cells to reach the aortic arch during injection, and instead reaching only the carotid artery, resulting in dissemination to the ipsilateral head and neck to produce localized disease.

We refined the intra-arterial injection procedure in the following ways to address this problem:

1. Iohexal contrast was injected under fluoroscopy just prior to the VX2 carcinoma cells to verify that the retrograde arterial injection reached the aortic arch.

2. The VX2 cells were labeled with Tc-99mMAA, and nuclear medicine imaging was performed following the injection, to verify widespread dissemination of the neoplastic cells.

FLUOROSCOPY and NUCLEAR MEDICINE IMAGING

Use of Fluoroscopy and Nuclear Medicine Imaging during Intra-Arterial Administration of VX2 Carcinoma Cell Suspension in Rabbits to Verify Widespread Dissemination of Neoplastic Cells

FLUOROSCOPY and NUCLEAR MEDICINE IMAGING

MATERIALS AND METHODS

1. Animals: 2 SPF New Zealand White rabbits, weighing 2.75 - 3.25 kg.

2. VX2 carcinoma model and imaging:
   a. Anesthesia: Rabbits were pre-medicated with 0.15 mg acepromazine IV, and then induced and maintained with isoflurane via face mask (1.2-1.5%).
   b. Catheterization: a 22-gauge catheter was placed in the auricular artery with a 3-way stop cock attached, for administration of contrast and VX2 cell suspension.
   c. Fluoroscopy: 5 mL of iohexal radiographic contrast was injected through the arterial catheter during fluoroscopic imaging to verify delivery to the aortic arch.
   d. VX2 inoculation: once the contrast was seen in the aortic arch, a single-cell suspension of 8 x 10^6 VX2 carcinoma cells/ml was injected through the arterial catheter.
   e. Nuclear medicine imaging: after VX2 cell suspension injection, 1 mL of Tc-99mMAA was injected through the arterial catheter, then nuclear medicine imaging was performed to confirm the ultimate distribution of the VX2 cells.
   f. Monitoring. Following VX2 cell inoculation, a weekly CT scan was performed to monitor for pulmonary metastasis. Once pulmonary metastasis was detected, MRI and PET/CT was performed to assess presence of disseminated metastasis with a novel tumor-avid contrast agent.

MATERIALS AND METHODS, cont.

3. MRI: Coronal head, neck, chest, and partial abdomen pre- and post-contrast image reveals a shoulder mass, low signal. Image post-contrast infusion, vascular phase, reveals faint ring enhancement (a, arrow). Twenty-four hour image reveals rising enhancement in the tumor rim (b, arrow). At forty-eight hours, enhanced signal persists and partly "fills in" the tumor (c, arrow).

4. Magnetic Resonance Imaging (MRI):
   a. A novel contrast agent (3Gd-HPPH) was injected into the auricular vein (80 umol Gd/kg, 10 umol 3Gd-HPPH/kg), and the animals were imaged at 1.5T (GE Sigma HD XT 1.5) in the "vascular phase" and also at 24 a (34.1 MMBq) and coronal lave pre-and post-contrast FOV 28 images were generated; the sequences were repeated for the body with a FOV of 48.

5. Positron Emission Tomography/Computed Tomography (PET/CT):
   a. 0.93 mCi (34.1 MMBq) of Fluorine-18-Fluorodeoxyglucose (FDG) was injected intravenously into each rabbit and at 30 min each underwent PET/CT (GE Discovery ST, FOV 25 cm).

6. Necropsy and histopathology:
   a. After the 48 hour MRI the rabbits were euthanized by IV injection of Pentobarbital Sodium, 390 mg/ml.
   b. Gross necropsy was performed on both rabbits. Grossly visible metastatic lesions were photographed for comparison with imaging findings and then the tumors were collected and submitted for histopathologic evaluation.

RESULTS

1. Fluoroscopy confirmed injection of iohexal contrast and VX2 cells to the aortic arch in both rabbits.

2. Nuclear medicine imaging of Tc-99mMAA labeled VX2 cells confirmed widespread dissemination throughout the thorax and abdomen following injection.

3. MRI with contrast and PET/CT confirmed development of widespread metastasis to the head, thorax, and abdomen in both rabbits.

4. Necropsy and histopathology confirmed widespread metastasis of VX2 carcinoma to the head, thorax, and abdomen in both rabbits, consistent with MRI and PET/CT images.

Conclusion: refinement of the VX2 arterial injection technique via incorporation of fluoroscopy to verify injection to the aortic arch and nuclear medicine imaging to verify dissemination of labeled cells results in consistent widespread metastatic disease in rabbits.

REFERENCES

