Implantation of VX2 Carcinoma into the Liver of Rabbits: A Comparison of Three Direct-Injection Methods

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ABSTRACT. The efficacy of agarose in preventing VX2 carcinoma cell leakage was evaluated and the results were compared with two traditional methods. Forty-five rabbits were divided into 3 groups: Group 1, VX2 tumor cells were injected directly into the liver and no special procedure after removal of the needle; Group 2, the puncture site was gently compressed, using an alcoholic cotton gauze, for three minutes; Group 3, 0.2 ml of heated liquid agarose was injected to seal the aperture after injection of VX2 cells. The leakage rates were 80%, 53.3% and 6.6% for group 1, group 2 and group 3, respectively. We consider agarose is a useful material in preventing the leakage in the establishment of VX2 liver tumor models.

KEY WORDS: leakage, liver tumor, VX2 carcinoma cell.

The liver is the most common site for metastases from gastrointestinal tumors, malignant melanoma, and primary liver tumors. To establish an animal model with liver tumor is very important for the investigation of diagnostic methods and therapeutic effect. The VX2 carcinoma is an animal tumor which is being used extensively to study different aspects of tumor behavior and is generally accepted for the establishment of a liver tumor. The VX2 carcinoma cells are derived from a virus-induced papilloma of rabbits, first developed by Shope and Hurst [19]. It is a fast-growing adenocarcinoma and has been used in several rabbit models [18]. The tumor may grow in many tissues in the rabbit, including the bones, muscle, and liver [5, 7, 15]. In previous investigations, cell suspension of VX2 tumor has been injected via several routes including directly into the liver parenchyma [9], into the hepatic artery or portal vein [2] and into the gastrointestinal walls [20] to achieve liver tumor (VX2) growth. Direct injection of VX2 cell suspension into the liver parenchyma is simple [1, 2, 9]. However, according to our experience, leakage of tumor cells into the peritoneal cavity via the injection route is not uncommon. Using cotton gauze to press the puncture site after injection is useful in decreasing the leakage rate. According to our experience, however, its efficiency is still not satisfactory.

Agarose is in liquid form after heating and returns to solid form in a short time when cooled down. Based on this characteristic, it may be a good material to fix the tumor cells in the liver. In a study by Kaye et al., agarose was used to prevent leakage of C6 glioma cells in the establishment of intracranial gliomas in mice [10]. Their results were encouraging. A success rate of 90% was achieved. However, to the best of our knowledge, no study regarding agarose applied in the establishment of liver tumor using VX2 carcinoma cells has been reported. In this study, we evaluated the efficacy of agarose in the prevention of tumor cells leakage and compared the results with two traditional methods.

The VX2 carcinoma is an anaplastic squamous cell carcinoma derived from a virus-induced papilloma in the wild rabbit, but appears as a carcinoma in the domestic species. The VX2 carcinoma was maintained through serial transplantation into the hind limb muscle of the New Zealand white rabbit. Following implantation into the hind legs, the tumor enlarged rapidly. Immediately after euthanasia of the rabbit under an intravenous anesthesia of sodium pentobarbital (30 mg/kg), the VX2 tumor was aseptically stripped from the surrounding connective tissues and minced mechanically with scissors in a cold (4°C) physiological buffered saline (PBS). Then, the cell suspension was filtered through an iron mesh with 0.08 mm2 pores to remove macroscopic tissue fragments. The filtrate was centrifuged at 2,000 rpm for 10 min and adjusted to a concentration of 1 × 107 cells/ml. Trypan blue was used to evaluate the viability of the VX2 carcinoma cells.

Thirty grams of agarose suspension was added to 1,000 ml distilled water, which was boiled to dissolve the medium completely. It was then sterilized by autoclaving at 15 lbs pressure(121°C) for 15 min. Before injection, the agarose was maintained in a liquid form under 50°C.

Forty-five New Zealand white rabbits, each weighing about 2.8–3.2 kg, were used for the experiment. The animal care and use procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the Animal Care Committee of National Chung-Hsing University. The rabbit was anesthetized with an intravenous dose (20 mg/kg) of pentobarbital sodium (Nembutal sodium solution, Abbott Laboratories, North Chicago, IL). The hair on the cranial abdomen was

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following injection of the VX2 carcinoma cell suspension, 0.2 ml of liquid agarose (Agarose A9539, Sigma, St. Louis, MO, U.S.A.) was dripped into the puncture site. It is necessary to make sure that the needle is not in a vessel or a bile duct by withdrawing the syringe before injection of the VX2 cells. To prevent leakage and bleeding, a compress on the puncture site using cotton gauze after removal of the needle is the most common method. In our study cases not using compression (group 1), 12 (80%) rabbits showed evidence of leakage. When a compress on the puncture site was performed (group 2), the no statistical difference between the success rates of group 1 and 2 was noted (p=0.19). However, there was a statistical difference in success rate between groups 2 and 3 (p=0.003) and between groups 1 and 3 (p=0.001).

Many types of tumor cells have been used to induce liver tumors in animals and most animal models are developed in small animals such as the mouse and rat [3, 6, 11, 14, 22]. The VX2 carcinoma is a highly malignant anaplastic squamous cell carcinoma and is generally accepted as a transplantable carcinoma that can be implanted in the liver of rabbits [1, 9, 17]. The VX2 carcinoma cell has many characteristics similar to those of human liver cancer such as its vascularization and, therefore, is relevant to clinical regional chemotherapy or radiotherapy [4, 12, 13].

Table 1 shows the result of implantation of VX2 carcinoma cells into the liver of rabbits via three different methods. The VX2 carcinoma cell grew well and rapidly in the rabbits. In group 1, one rabbit showed no evidence of tumor growth. Of the 14 rabbits with tumor growth, only two rabbits had VX2 carcinoma growth confined to the liver (Fig. 1a). Twelve rabbits showed the evidence of leakage and had tumor growth in the peritoneal cavity or surrounding organs (Fig. 1b). The success rate was only 13.3%. In group 2, two rabbits showed no evidence of tumor growth. Of the 13 rabbits with tumor growth, 8 showed evidence of leakage. Five rabbits had VX2 carcinoma confined to the liver and the success rate was 33.3%. In group 3, one rabbit showed no evidence of tumor growth. Of the 14 rabbits with tumor growth, only one showed evidence of leakage. As many as 13 rabbits showed a tumor confined to the liver. The success rate was 86.6%. The leakage rates were 80%, 53.3% and 6.6% for group 1, group 2 and group 3, respectively.

When tumor suspension is injected into the liver parenchyma, the tumor may spread beyond the target area [2, 9]. Two reasons may contribute to this: one is that cells are inoculated, not just to the parenchyma, but also into blood vessels and bile ducts; the other is that tumor cells may leak from the puncture site. It is necessary to make sure that the needle is not in a vessel or a bile duct by withdrawing the syringe before injection of the VX2 cells. To prevent leakage and bleeding, a compress on the puncture site using cotton gauze after removal of the needle is the most common method. In our study cases not using compression (group 1), 12 (80%) rabbits showed evidence of leakage. When a compress on the puncture site was performed (group 2), the

Table 1. Result of implantation of VX2 carcinoma cells into the liver of rabbits via three different methods

<table>
<thead>
<tr>
<th>Method</th>
<th>No.</th>
<th>Tumor growth</th>
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<tbody>
<tr>
<td></td>
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<td>No tumor</td>
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<tr>
<td>Group 1</td>
<td>15</td>
<td>1</td>
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<tr>
<td>Group 2</td>
<td>15</td>
<td>2</td>
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<td>Group 3</td>
<td>15</td>
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a) VX2 tumor cells were injected directly into the liver and no special procedure was performed after removal of the needle.
b) The puncture site was gently compressed, using an alcoholic cotton gauze, for three minutes to prevent bleeding and leakage after removal of the needle.
c) Following the injection of the VX2 carcinoma cell suspension, 0.2 ml of heated liquid agarose was injected to seal the aperture caused by the needle.
number of cases of leakage decreased but there were still 8 (53.3%) cases which showed evidence of leakage. There was no statistical difference between these two groups.

Agarose is a natural colloid extracted from seaweed. The melting point of the agarose we used in this study is about 75°C and the gelling point is about 36°C. After heating the agarose into liquid form, it was kept at a temperature of 50°C before it was used for injection. Following the injection of VX2 carcinoma cell suspension, we injected the melted agarose via the same syringe. In a short time, the agarose coagulated and sealed the aperture, which prevented the leakage of the VX2 suspension. VX2 carcinoma cells are still viable after heating to 67°C for 30 min, but not after heating to 70°C [19]. Therefore, no damage was done to the VX2 carcinoma cells by the melting agarose at a temperature of 50°C. Based on our data, the effect of agarose on preventing leakage was very good. Only one case was found to have evidence of leakage. The leakage rate was only 6.6%. There was a significantly higher leakage rate in group 1 (80%) compared to group 2 (53.3%).

In recent years, a fragment of tumor tissue instead of cell suspension has been implanted into the liver using a surgical technique with laparotomy. Transplantation of a tumor fragment may imply a defined tumor location. There is no risk of initial spread or leakage of the tumor, thus allowing for a more predictable appearance of tumor tissue [8, 16, 21]. However, this surgical technique is more traumatic than using the suspension injection method. Surgical complications in the liver such as hemorrhage, bile leakage, or abscess are more likely to happen. Moreover, it is very difficult to control the same number of viable tumor cells in every tumor fragment but it is easy to achieve using the cell suspension technique. In addition, failure of implantation may occur when necrotic tissue instead of viable tumor cells is implanted into the liver. Therefore, further studies are required to develop a standard experimental model for liver tumor for a precise investigation of diagnostic and therapeutic methods.

In conclusion, agarose is useful in preventing the leakage of VX2 tumor cells from the injected route in the establishment of VX2 liver tumor models.

REFERENCES