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Adenovirus Vector-mediated Gene Therapy Using Iodized Oil Esters for Hepatocellular Carcinoma in Rats

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Abstract. *Background: When gene therapy is performed for malignant tumors, gene transfer efficiency and selectivity are extremely important. The usefulness of gene therapy by intra-arterial injection of an adenovirus vector with iodized oil esters (IOEs) for hepatocellular carcinoma (HCC) was studied. Materials and Methods: HCC was induced in rats with diethyl nitrosamine and phenobarbital, after which either adenovirus vector expressing the herpes simplex virus thymidine kinase (AxCAHSVtk) and IOEs or AxCAHSVtk alone was injected through the hepatic artery. On postoperative days 2, 4 and 6, gancyclovir was injected into the peritoneum; blood sampling was performed on day 7. Results: Aspartate aminotransferase and alanine aminotransferase levels in the AxCAHSVtk with IOEs group were lower than in the AxCAHSVtk alone group ($p=0.0274$, $p=0.0323$). However, the survival rate was not significantly different between groups ($p=0.7122$). Conclusion: Intra-arterial injection of an adenovirus vector with IOEs can result in cancer-selective but not effective gene therapy for HCC.*

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world, for which current treatments other than liver transplantation for non-advanced HCC are regarded as palliative. Therefore, new modalities of treatment should be developed to further improve the outcome of HCC. To date, effective gene therapy has not been reported for HCC because the efficiency and selectivity of the gene transfer to the tumor tissue are too low. Unlike normal hepatocytes that receive dual blood supply from the hepatic artery and the portal vein, HCC receives its blood supply exclusively from the hepatic artery. Based on such a peculiar feature of HCC, the efficiency and tumor selectivity of anticancer agents can be increased by injection of such agents with embolic material through the hepatic artery, a technique known as transarterial chemoembolization (TACE) (1). When embolic material is also injected through the feeding artery, the anticancer agents remain within the tumor vessels and exert a stronger and more selective antitumor effect. Therefore, injection of an embolic agent with an adenovirus vector may be useful for achieving efficient and selective gene transfer to HCCs. In fact, we have already reported that gene transfer efficiency and selectivity are increased by intra-arterial injection of an adenovirus vector with iodized oil ester (2) and degradable starch microspheres (DSM) (3), an embolic agent.

We hypothesized that gene therapy by injection of an adenovirus vector with IOEs into the feeding artery might improve the duration of survival without severe liver dysfunction in HCC patients because of the efficiency and cancer-selectivity of this gene delivery system.

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Materials and Methods

Recombinant adenovirus vector. The replication-defective recombinant adenovirus vector, AxCAHSV*tk*, expressing HSV*tk* gene, was obtained from the Riken Cell Bank (RDB1429, Tsukuba, Japan). The recombinant adenovirus vector was generated from adenovirus type 5, and the E1 and E3 regions were deleted to prevent virus replication. The HSV*tk* gene was driven by the cytomegalovirus-enhancer-chicken β-actin hybrid promoter (CAG promoter) (4-6) and a rabbit beta-globin poly (A) signal located downstream from the gene. The vector was purified by two rounds of CsCl centrifugation (7) and stored at –80˚C until use. The vector was used at a concentration of $2.0\text{x}10^8$-$2.0\text{x}10^{10}$ plaque formation units (pfu) /ml.

Rat HCC model. Male Wistar rats (6 weeks old and 180 g) received 10 mg/kg per day of diethyl nitrosamine (DENA) (Sigma Chemical Co., St. Louis, MO, USA) and 50 mg/kg per day of phenbarbital

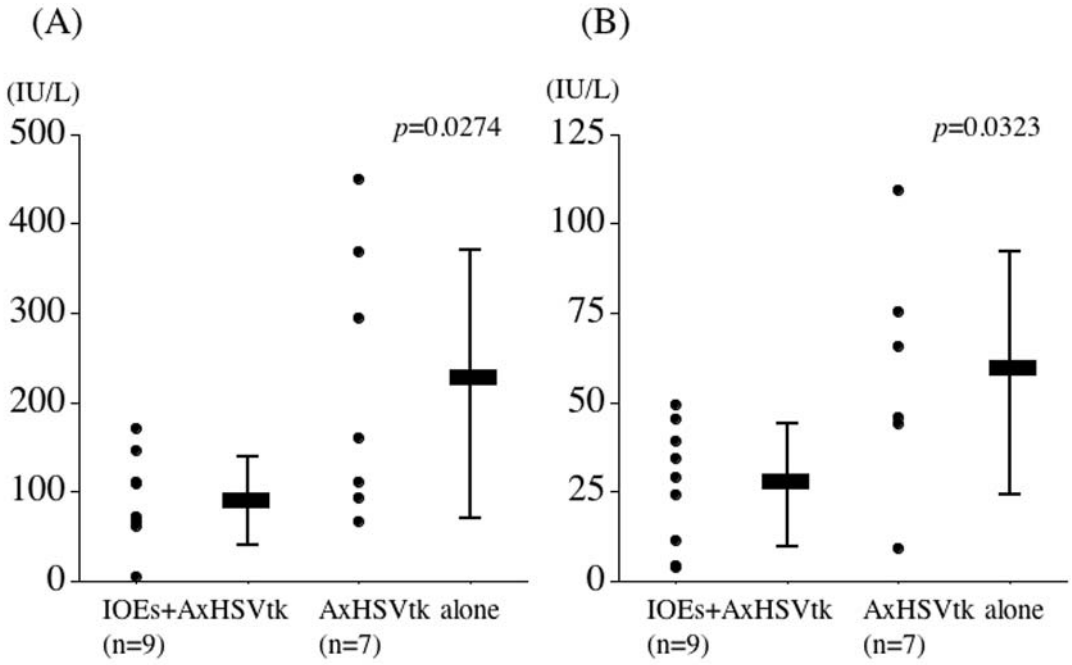

Figure 1. *(A) Serum AST and (B) ALT levels one week after gene therapy with AxCAHSVtk with IOEs plus gancyclovir (GCV) or AxCAHSVtk alone plus GCV. The levels of both enzymes were significantly lower in the former group.*

(Wako Pure Chemical Industries, Ltd., Osaka, Japan) in drinking water for 16 weeks. It has been previously reported that HCC induced in rats by DENA and phenobarbital is hypervascular and is supplied by a tumor-feeding artery (8). The protocol for this animal study was approved by the Laboratory Animal Facility of Jikei University School of Medicine and was performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Gene therapy for HCC. The animals were anesthetized with pentobarbital (Nembutal®; Abbott Laboratories, North Chicago, IL, USA). After clamping the gastroduodenal artery, either $5.0x10^7$ pfu/100 μl of AxCAHSV*tk* plus 150 μl of IOEs (n=19), or $5.0x10^7$ pfu/250 μl of AxCAHSV*tk* alone (n=11) was injected through the hepatic artery into the liver using a 30-gauge needle and a 1-ml syringe. After injection, the hepatic artery was compressed to stop any bleeding. On postoperative day 2, 4 and 6, the animals received 50 μg/g of gancyclovir (GCV) (DENOSINE®; F. Hoffmann-La Roche, Basel, Switzerland) into the peritoneum. On postoperative day 7, blood sampling was performed for liver function evaluation; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured. The animals were followed-up without any further treatment till their death. The effects and side-effects of gene therapy by transarterial injection of AxCAHSV*tk* with IOEs and GCV were evaluated using for indexes of duration of survival and liver dysfunction.

Statistical analysis. Non-paired Student's *t*-test and Log-rank test were used for statistical analyses. All *p*-values were considered statistically significant when the associated probability was less than 0.05.

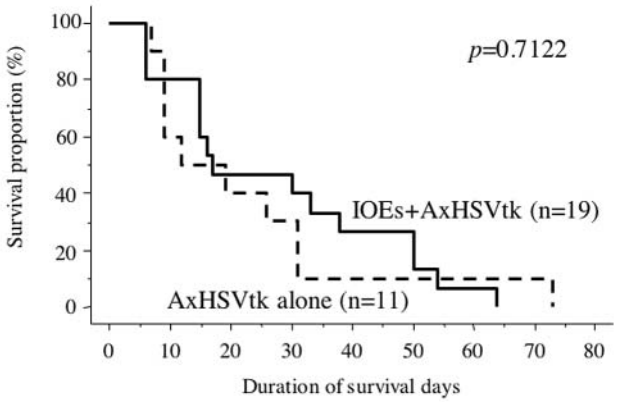

Figure 2. *Survival of rats treated with AxCAHSVtk with IOEs plus GCV or AxCAHSVtk alone plus GCV. There were no significant differences between the survival rates of the groups.*

Results

Assessment of liver dysfunction caused by gene therapy for HCC. The serum AST in the AxCAHSV*tk* with IOEs group (n=9) was 90.0±16.6 IU/l (mean±standard error (SE)), whereas in the AxCAHSV*tk* alone group (n=7) it was 220.5±56.7 IU/l (mean±SE) (Figure 1A). Serum ALT in the AxCAHSV*tk* with IOEs group (n=9) was 26.8±5.7 IU/l (mean±SE), whereas in the AxCAHSV*tk* alone group

(n=7) it was 58.3±13.8 IU/l (mean±SE) (Figure 1B). Serum AST and ALT levels one week after gene therapy in the AxCAHSV*tk* with IOEs group were significantly lower than in the AxCAHSV*tk* alone group (AST; $p=0.0274$, ALT; $p=0.0323$). These results suggested that arterial injection of AxCAHSV*tk* with IOEs plus GCV could treat HCC with less liver functional impairment than with AxCAHSV*tk* alone plus GCV.

Assessment of survival rate under gene therapy for HCC. The survival of rats after gene therapy with arterial injection of AxCAHSV*tk* with IOEs (n=19) was 22.1±4.7 days (mean±SE); on the other hand, that in the AxCAHSV*tk* alone group (n=11) was 20.7±6.1 days (mean±SE). There were no significant differences in the survival of the two groups ($p=0.7122$) (Figure 2).

Discussion

In gene therapy for malignant tumors, efficient gene transfer to the tumor and minimization of transfer to normal tissues are essential. We have already reported the feasibility of the use of IOEs and DSM as embolic materials for satisfactory gene transfer to liver cancer (2, 3). It seems likely that the prolonged residence of the vector within the tumor vessels with the embolic agent increases contact between the vector and tumor cells, resulting in enhanced gene expression within the tumor and limited gene transfer to the surrounding normal tissues. In the case of HCC, almost all HCC patients have viral hepatitis or liver cirrhosis as a complication and their liver function has deteriorated, so that prevention of gene transfer to the surrounding non-tumor tissue is absolutely necessary.

In this study, liver dysfunction one week after gene therapy in the AxCAHSV*tk* with IOEs group was lower than in the AxCAHSV*tk* alone group, however, the survival rate was not significantly different. These results suggested that arterial injection of AxCAHSV*tk* with IOEs plus GCV could provide cancer-selective gene transfer to HCC and cause less liver dysfunction in surrounding normal tissue, but not improve the prognosis.

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References

1 Konno T, Maeda H, Iwai K, Tashiro S, Maki S, Morinaga T, Mochinaga M, Hiraoka T and Yokoyama I: Effect of arterial administration of high molecular weight anticancer agent SMANCS with lipid lymphographic agent on hepatoma. Eur J Cancer Clin Oncol *19*: 1053-1065, 1983.
2 Shiba H, Okamoto T, Futagawa Y, Ohashi T and Eto Y: Efficient and cancer-selective gene transfer to hepatocellular carcinoma in a rat using adenovirus vector with iodized oil esters. Cancer Gene Ther *8*: 713-718, 2001.
3 Shiba H, Okamoto T, Futagawa Y, Misawa T, Yanaga K, Ohashi T and Eto Y: Adenovirus vector-mediated gene transfer using degradable starch microspheres for hepatocellular carcinoma in rats. J Surg Res *133*: 193-196, 2006.
4 Kanegae Y, Lee G, Sato Y, Tanaka M, Sakaki T Sugano S and Saito I: Efficient gene activation in mammalian cells by using recombinant adenovirus expressing site-specific Cre recombinase. Nucleic Acids Res *23*: 3816-3821, 1995.
5 Tani K, Lin T, Hibino H, Takahashi K, Nakazaki Y, Takahashi S, Nagayama H, Ozawa K, Saitoh I, Mulligan R and Asano S: Transduction of LacZ gene into leukemia cells using viral vectors of retrovirus and adenovirus. Leukemia *9(suppl 1)*: 64-65, 1995.
6 Niwa H, Yamamura K and Miyazaki Y: Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene *108*: 193-200, 1991.
7 Kanegae Y, Makimura M and Saito I: A simple and efficient method for purification of infectious recombinant adenovirus. Jpn J Med Sci Biol *47*: 157-166, 1994.
8 Grandin C, Van Beers BE, Demeure R, Goudemant JF, Mottet I and Pringot J: Comparison of gadolinium-DTPA and polylysine-gadolinium-DTPA-enhanced magnetic resonance imaging of hepatocarcinoma in the rat. Invest Radiol *30*: 572-581, 1995.

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