The Combination of 5-Fu/DDP and Suicide Gene in the Treatment of Tongue Squamous Carcinoma

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Abstract **Objective** To investigate the therapeutic efficiency of combination of herpes simplex virus thymidine kinase (HSV-TK)/ganciclovir(GCV) system and 5-fluorouracil (5-FU) and/or cisplatin (DDP) in the treatment of tongue squamous carcinoma. **Methods** Tca8113 cells were inoculated subcutanously in the right flank of BALB/c nude mice. Mice were randomly assigned to 8 groups and treated with: ①Control, ②HSV-TK/GCV, ③5-FU, ④5-FU+HSV-TK/GCV, ⑤DDP, ⑥DDP+HSV-TK/GCV, ⑦DDP+5-FU, ⑧DDP+5-FU+HSV-TK/GCV. Observe the tumor growth and calculate inhibition rate and tumor doubling time; Observe tumor histopathological changes. The anticancer effect between 5-FU/DDP and HSV-TK/GCV was analyzed by factorial experiment. **Results** Compared with control groups, tumor grouth in treated groups (group ②-⑧) had significant in hibition (P<0.001) and the tumor doubling time prolonged. There had cooperative anticancer effect between 5-FU and HSV-TK/GCV, DDP and HSV-TK/GCV, DDP+5-FU and HSV-TK/GCV by factorial experiment (P<0.001). There had cyst-formation and substantial tumor necrotic area in each treated group under light microscope. **Conclusion** The combination of chemotherapy (5-FU± DDP) and HSV-TK/GCV has the cooperative anticancer effect on tongue squamous carcinoma.

Key Words Herpes simplex virus thymidine kinase gene; Gene therapy; chemotherapy; Tongue neoplasms

Tongue squamous cell carcinoma (TSCC) comprises the majority of the estimated head and neck cancer. The current standard therapies include deforming radical surgical procedure, radiotherapy and chemotherapy. Although conventional cancer therapies produce a high rate of cure for patients with early-stage disease, many cancers recur and the majority of patients with advanced cancer eventually succumb to the disease. So we must look for more effective treatment for TSCC. In our previous researches herpes simplex virus thymidine kinase (HSV-TK)/ganciclovir (GCV)suicide gene therapy system had demonstrated good therapeutic effect for tongue squamous cell carcinoma in vitro and in vivo[1,2]. But the clinical curative effect of gene therapy was still unsatisfactorily[3], the main reason was related to poor target to special tissue and low efficiency of transfer into cells during the gene therapy. To data, suicide gene therapy can't replaced the present conventional cancer therapies, but researches had also revealed that suicide gene therapy can be used to improve the sensitivity of chemotherapy[4,5].

5-fluorouracil (5-FU) combined with cisplatin (DDP) were standard chemotherapy for TSCC, but the effectiveness of 5-FU and DDP is limited by the systemic toxicity associated with treatment. In order to enhance the therapeutic effect of tongue carcinoma we combined HSV-TK/GCV suicide gene therapy and 5-fluorouracil and/or cisplatin chemotherapy, and to investigate the possibility of the combination.

METHODS AND MATERIALS

Materials

Female BALB/c nude mice (6~8 weeks, weight 20~30g, SPF) were purchased from Animal Center of Sun Yat-sen University. The AdCMVHSV-TK vector (purchased from Prof. Xu Dehua of our u-

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Foundation item: this work was supported by the grant from the foundation of the Bureau of Health of Guangdong (A2002189)

niversity) was propagated in 293 cells, purified by cesium chloride gradient ultracentifugation and titrated by TCID50[1]. The titration was 2×10^{10} PFU (plaque forming units)/ml. Human tongue carcinoma cell line (Tca8113 cell line, kindly presented by Prof. He Ronggeng) were grown in RPMI 1640´s medium (GIBCO) with 10% fetal calf serum (GIBCO), 100 units/ml penicillin G, 100μg/ml streptomycin. GCV was purchased from Sigma Inc. 5-Fu was purchased from Nantong pharmacy factory of Jiangsu province, china and DDP from Qilu pharmacy factory of Shandong province, china.

Methods

Establish and treat the Xenograft model of tongue squamous carcinoma in nude nice

2×10^6 Tca8113 cells (tongue squamous carcinoma) were inoculated subcutanously in the right flank of nude mice. Mice were randomly assigned to 8 groups (n=6) after xenografts became palpable and were about 4 mm in diameter (about the seventh day): ① control (blank) group, ② HSV-TK/GCV treated group, ③5-FU treated group, ④5-FU+HSV-TK/GCV treated group, ⑤DDP treated group, ⑥DDP+HSV-TK/GCV treated group, ⑦DDP+5-FU treated group, ⑧DDP+5-FU+HSV-TK/GCV treated group.

AdCMVHSV-TK was intratumorally injected (2×10^9 PFU/0.1ml) at day 8; GCV (25 mg/kg in PBS)、DDP(2 mg/kg) or 5-FU(20 mg/kg)was intraperitoneally injected once daily from day 9 to day 14, respectively. The doses of DDP and 5-Fu were consulted from Zhang et al [6]. In control group, the same volume of PBS was injected intratumorally or intreperitoneally, respectively. The doses were adjusted with the weight of mice which was weighed every 3 days.

Observe the tumor growth status and the tumor histopathological changes

Tumor size from seventh day to thirty-fifth day was measured by caliper (length and width) for every 3 days. Calculate the tumor volume ($V=1/2$ length $\times$ width^2), depict tumor growth curve ($y=A e^{kt}$) and calculate the tumor doubling time ($T=(\ln2)/K$, k: growth speed). The tumor inhibition rate of each group at thirty-fifth day was calculated. Inhibition rate =(1-the volume change of experiment group/the volume change of control group) $\times$ 100%. All mice were sacrificed at day 35 and tumor tissue was harvested and placed in 10% buffered formalin. Routine hematoxylin-eosin staining was performed after paraffin embedding and sectioning of tissues, and the tumor tissues were examined for morphologic alterations.

Statistical analysis

The anticancer effect between HSV-TK/GCV suicide gene therapy and 5-FU chemotherapy, HSV-TK/GCV and DDP chemotherapy, HSV-TK /GCV and 5-FU+ DDP chemotherapy was analyzed by factorial experiment, respectively. Results are presented as mean±standard deviation of the mean. Significance was defined at $P<0.05$ level.

RESULTS

Inhibition effect of 5-FU ±DDP combined with HSV-TK/GCV on tumor growth

Tumor growth curves were showed in Fig.1~Fig. 3. Compared with control group, mice treated with 5-FU, DDP, DDP+5-FU, HSV-TK/GCV, 5-FU+ HSV-TK/GCV, DDP+HSV-TK/GCV, DDP+5-FU+ HSV-TK/GCV had longer tumor doubling time and the tumor growth was significant inhibited. The tumor doubling time and inhibition rate were showed in table 1.

The tumor volume at day 35 between HSV-TK/GCV suicide gene therapy and 5-FU chemotherapy, HSV-TK/GCV and DDP chemotherapy, HSV-TK/GCV and 5-FU+ DDP chemotherapy was analyzed by factorial experiment, respectively. The results showed in table 2 demonstrated that there had significant inhibition for the growth of xenograft in each treatment group($P<0.001$)and cooperation effect was exist between chemotherapy and suicide gene therapy ($P<0.001$).

Histopathological changes of xenograft

All the tongue squamous carcinoma cells inoculated in nude mice became xenograft in this study. After treated with DDP or 5-FU mice had slightly diarrhea and became thin and all this symptom disappear after treatment, no any serious side effect appear. no any side effect appear in HSV-TK/GCV treated group; no any adding side effects appear in each combined treated group. Histopathological analysis revealed there were many huge tumor cells and mitotic figures in control groups under light microscope. In each treated group there had cyst-formation and substantial tumor necrotic area (Fig. 4).

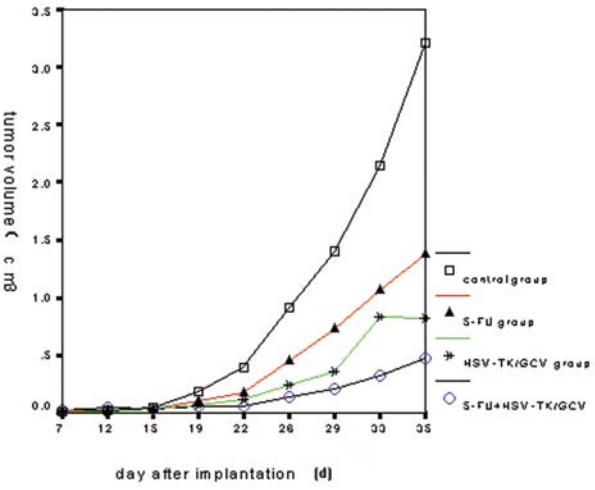


Fig.1 Tumor growth curves of the xenografts after treated with 5-FU combined with HSV-TK/GCV

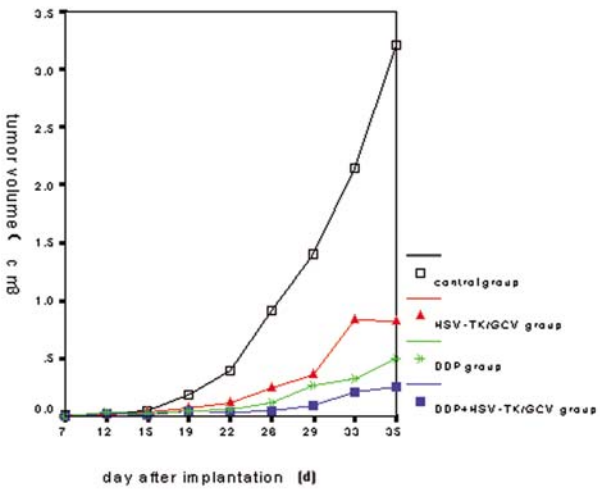


Fig.2 Tumor growth curves of the xenografts after treated with DDP combined with HSV-TK/GCV

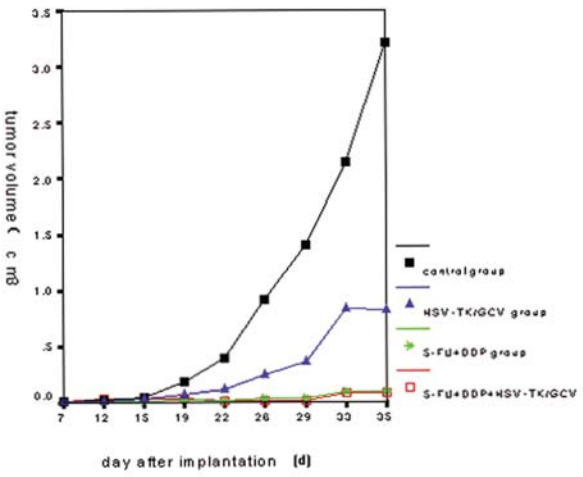
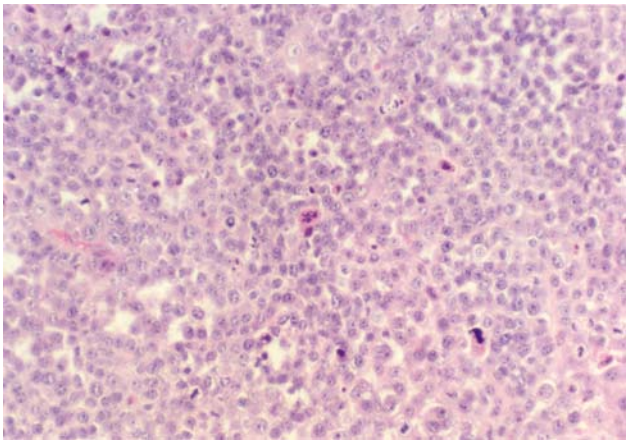
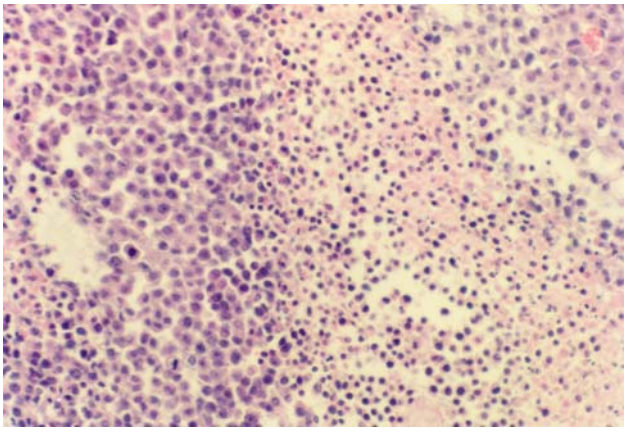


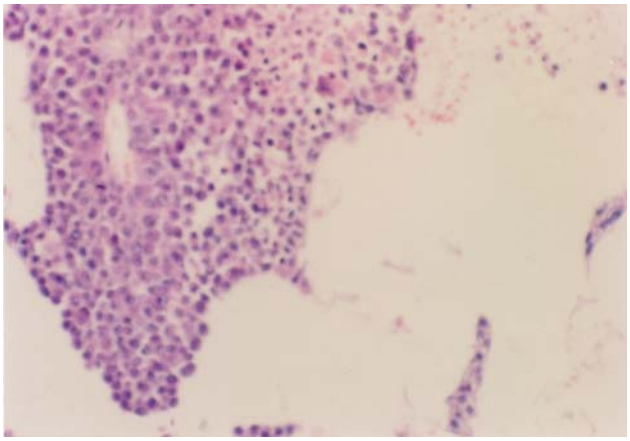
Fig.3 Tumor growth curves of the xenografts after treated with 5-FU+DDP combined with HSV-TK/GCV



A (control group): tumor cells arranged tightness, many huge tumor cells and mitotic figures were found.



B (5-Fu treated group): there had substantial tumor necrotic area



C (5-FU+AdCMVHSV-TK/GCV): there had cyst-formation and substantial tumor necrotic area

Fig.4 Histopathological changes of xenografts of tongue squamous cell carcinoma (H&E, × 200).

Table 1 The tumor doubling time and inhibition rate of each group

	control	5-FU	DDP	5-FU+ DDP	HSV-TK/ GCV	5-FU+ HSV-–TK/GCV	DDP+ HSV-TK/GCV	5-FU+DDP+ HSV-TK/GCV
tumor doubling time	3.2d	3.9d	4.6d	7.0d	4.0d	6.7d	5.7d	24.1d
inhibition rate		56.9%	84.4%	97.1%	74.2%	85.3%	92.0%	97.6%

Table 2 The anticancer effect between 5-FU/DDP chemotherapy and HSV-TK/GCV suicide gene therapy was analyzed by factorial experiment

source	DF	SS	MS	F volume	P volume
5-FU and HSV–TK/GCV					
corrected model	3	26.671			
5-FU(A_1)	1	7.157	7.157	23.599	0.001
HSV-TK/GCV(B_1)	1	16.266	16.266	53.635	0.001
(A_1B_1)	1	3.248	3.248	10.710	0.004
error	20	6.065	0.303		
DDP and HSV-TK/GCV					
corrected model	3	33.353	16.149	92.116	0.001
DDP(A_2)	1	16.149	10.344	59.002	0.001
HSV-TK/GCV(B_2)	1	10.344	6.860	39.129	0.001
(A_2B_2)	1	6.860	0.175		
error	20	3.506			
DDP+5-FU and HSV-TK/GCV					
corrected model	3	39.469			0.001
DDP+5-FU(A_3)	1	22.443	22.443	303.310	0.001
HSV-TK/GCV(B_3)	1	8.613	8.613	116.402	0.001
(A_3B_3)	1	8.413	8.413	113.697	
error	20	1.48	0.074		

*SS: sum of squares, MS: mean squares

DISCUSSION

Suicide genes typically code for nonmammalian enzymes that convert nontoxic prodrugs into highly toxic metabolites. Therefore, systemic application of the nontoxic prodrug results in the production of the toxic drug at the tumor site[7]. The herpes simplex virus TK gene is a prototype “suicide gene” because it encodes a viral enzyme that is foreign to mammalian cells, which will phosphorylate nucleoside analogs such as acyclovir and ganciclovir to their monophosphate metabolites. These monophosphates are subsequently phosphorylated by cellular kinases to the di-and triphosphates. After integration of the GCV metabolites into DNA, chain termination occurs, followed by cell death. The anticancer effect of HSV-TK/GCV suicide gene therapy was mainly through direct killing effect to the HSV-TK-transferred cells and killing the non transferred cells through bystander effect[8].

In our previous study we found that HSV-TK/GCV system had strong killing effect on TSCC in vitro and in vivo. The growth of tongue carcinoma xenograft was significantly inhibited after treated with adenovirus-mediated HSV-TK (2×10^9 PFU)/ GCV (25 mg/kg, twice every day, 6 d) system[2]. The inhibition rate was 90.69%. In this study the dose of GCV was decreased to once a day and the growth of xenograft was also significantly inhibited and the inhibition rate was 74.2%.

The scheme of DDP+5-FU combined chemotherapy is one of the best chemotherapy for tongue squamous carcinoma. 5-FU exerts its toxic effect by interfering with DNA and protein synthesis due to substitution of uracil by 5-FU in RNA and inhibition of thymidilate synthetase by 5-fluorodeoxyuridine monophosphate, resulting in impaired DNA

biosynthesis[9]. Research had revealed that these inhibitor of thymidilate synthetase can enhance the efficacy of HSV-TK/GCV by increasing the phosphorylated GCV, enhancing the ability of phosphorylated GCV to incorporate into DNA and inhibiting the reparation of DNA [10]. In this study the growth of xenograft was obviously inhibited after treated with single or combined 5-FU and HSV-TK/GCV, the tumor doubling time prolonged. There had cooperative anticancer effect between 5-FU and HSV-TK/GCV by factorial experiment. That´s 5-FU can enhance the efficacy of HSV-TK/GCV. At the same time, this study also revealed that DDP or 5-FU combined DDP can enhance the efficacy of HSV-TK/GCV in the treatment of tongue squamous carcinoma, but the mechanism need to more study. In this study histopathological analysis also revealed that cyst-formation and substantial tumor necrotic area were found in each treated group. So there had obviously cytotoxicity to tongue squamous carcinoma cells in the single or combined treatment of 5-FU, DDP and HSV-TK/GCV.

From above, we can conclude that the combined therapy of HSV–TK/GCV suicide gene therapy and 5-FU 1 DDP chemotherapy can enhance the curative effect of tongue squamous carcinoma.

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