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Treatment of locally recurrent Epstein-Barr virus-associated nasopharyngeal carcinoma by anti-viral agent cidofovir.

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ABSTRACT

Nasopharyngeal carcinoma (NPC) is an Epstein-Barr virus (EBV) associated malignant tumor. Recently, anti-tumor effect of cidofovir, an anti-viral drug to an acyclic nucleoside analogue, has been reported to have anti-tumor potential. Thus, two cases of NPC, who have received multi-round therapy, were treated with cidofovir. Cidofovir was topically injected in and around the tumor once per 3 weeks. Tumor growth was suppressed for several months around the injection site in each patient. EBV-encoded RNAs in situ hybrodization revealed the reduction of the tumor cell population, however, EBERs expression was still maintained in the NPC tumor cells. Although anti-tumor mechanism remains unclear, these results suggest that cidofovir is actually an effective and safe agent for the treatment of NPC.

Key words: nasopharyngeal carcinoma, Epstein-Barr virus, cidofovir, chemotherapy, head and neck cancer, EBERs

INTRODUCTION

Chemoradiotherapy has been recognized as a standard treatment of fresh nasopharyngeal carcinoma (NPC). Recurrent NPC is a disease with poor prognosis because re-irradiation and anti-cancer chemotherapy only has poor response rates and raise severe morbidity rates. Moreover salvage surgery plays only a limited role because of its complicated anatomical location. Thus, treatment of locally recurrent NPC has been a matter of issue for several decades (1, 2). NPC is unique with contribution of Epstein-Barr virus (EBV), a member of Herpesvirus, to tumorigenesis and progression (3, 4), which suggests some treatment value of anti-viral drugs against NPC. Cidofovir, an acyclic nucleoside analogue, shows anti-proliferation effect against broad range of viral species including EBV. It has been reported to have anti-tumor potential. Here, we report two cases of NPC, who have received multi-round therapy, treated with topical injection of cidofovir. The protocol in this report was approved by the Institute Review Board of Kanazawa University Hispotal.

CASE REPORT

Case 1: A 30-year-old man was diagnosed to nasopharyngeal carcinoma (NPC) with T4N0M0 disease in 1998. He received cisplatin + 5FU neoadjuvant chemotherapy and radiotherapy at the initial treatment, eventually, achieved complete response. The disease recurred at the surface area of the nasopharynx in 2000, and the tumor was treated with endoscopic laser surgery and with 24Gy of brachytherapy. The treatment

controlled the local disease for a year. MRI revealed tumor regrowth in deeper portion than the previously detected recurrent diseases. The tumor did not respond to docetaxel (80mg/m2) and then, he received nasopharyngectomy via maxillar-swing approach and reconstructed with forearm free flap. Although complete resection was confirmed in surgical specimen, the disease recurred at the primary site in 2003. At that time, the tumor was treated with topical injection of cidofovir (75mg, diluted with normal saline to 1:4) at a time, once per week, 5 times). He received one more series of cidofovir injection. During the treatment, successfully injected site of the tumor did not grow whereas unsuccessful site of the tumor, mainly deeper portion, gradually increased its size (Fig. 1). Eventually, he died of intracranial invasion in 2005.

Case 2. The second case was 63-year-old male with rT2N0M0. At the initial treatment, he received neoadjuvant chemotherapy (Pirarubicine 30mg/m2 and cisplatin 70mg/m2 on day 1, pepleomycin 5mg/m2 on day 2 to 6, 2 course, every 3 weeks) and sequential radiotherapy 52.4Gy in 1995 at a local hospital. Five years after, he received neo adjuvant chemotherapy (5FU 650mg/m2 on day 1 to 5, Nedaplatine 100mg/m2 on day 6) and second-round radiation therapy (34 Gy, 2Gy/day, 5days/week) for the recurrent tumor. Six months after the treatment, the disease recurred. He was treated with topical injection of cidofovir (6.25 mg at a time, once per 3 week, 5 times) in 2002. Similar to the patient 1, tumor size did not increase after completion of cidofovir injection for three months. Tumor

growth stopped, however, not remarkable regression. An endoscopic nasopharyngectomy was undertaken for the purpose of improving nasal congestion and histological evaluation. There were faint tumor cells in submucosal area where tumor invasion was confirmed by previously biopsy. One year later, metastatic disease arose at the rt-parotid gland. Again, topical injection of cidofovir was conducted at the metastatic site. The tumor remained in stable size for a year (Fig. 2). However, multiple organ metastasis developed sequentially, and then, he has been receiving weekly low-dose docetaxel (25mg/m2) for 2 years. The metastatic tumor in the parotid grand began to grow again 3 months after stopping cidofovir injection and 6 months after suspension of cidofovir, facial palsy developed. Although he is at terminal disease stage, he still alive with multiple systemic metastasis.

Histological studies

Effect of cidofovir angiogenesis was studied in the nasopharyngeal carcinoma tissues. For clear identification of EBV positive NPC tumor cells, the tumor tissue samples were examined by EBV-encoded RNAs (EBERs) in situ hybridization. It revealed that the population of EBERs positive tumor cell decreased 3 months after completion of cidoforvir injection. In contrast, proportion of stroma increased where there had been tumor cells. Suggesting anti-tumor effect of cidofovir. There was no EBERs negative tumor cells in the post-cidofovir treatment samples, suggesting that cidofovir had not been working to eradicate EBV. (Fig.

3).

DISCUSSION

Cidofovir belongs to the acyclic nucleoside phosphonate analogues, which is characterized by a stable phosphonate linkage between the acyclic nucleoside and the phosphate moiety. Thus, cidofovir possesses distinct feature to antiviral drugs such as acyclovir and ganciclovir, in that it bypasses the first phosphorylation step by herpesvirus-encoded kinases (5).

NPC is an EBV-associated malignant disease in which EBV is latently infected (3). In addition to the fact that NPC, as well as HPV papillomatosis, is an epithelial tumor caused by an oncogenic DNA virus, recent successful report of cidofovir for EBV-associated disease in athymic nude mice model rendered us to use this drug for the treatment of recurrent human NPC (6, 7). Two drug delivery systems, intravenous and topical injection, have been clinically used for cidofovir administration against human disease. Intravenous injection is recommended for the treatment of CMV retinitis in AIDS patients, and topical injection has been successful for laryngeal papillomatosis (5, 8). Based on three reasons, topical injection was selected for both patients. The first one is the similarity of laryngeal papillomatosis with NPC in that these diseases are epithelial neoplasms. Next one is that intravenous dose was not effective against Kaposi sarcoma (9). The third one is less nephrotoxicity of topical use. The patients reported here had received CDDP-based full dose chemotherapy, major

side effect of which is nephrotoxicity. Nephrotoxicity is also a major side effect of cidofovir.

The deeper portion of tumor such as intracranial invasion site did not seem that cidofovir had been delivered in case 1, however, topical cidofovir injection turned out to be both physically and pathologically effective treatment for topical deliverable portion. Moreover, topically delivered cidofovir controlled parotid gland metastasis for one year. Histological evaluation of the metastatic site was not performed to avoid facial nerve palsy in case 2. Thus, we cannot tell if there were viable tumor cells or not. However, it is obvious that the treatment was clinically quite benefitial for the patients as either progression of the disease in the parotid gland or tumor resection would have been caused facial palsy.

An approach against EBV-associated tumor has been attempted in the way to induce viral lytic replication. Short-chain fatty acids, such as butyrate, induce EBV-Thymidine kinase (TK) expression in latently infected B cells. The combination of arginine butyrate and ganciclovir was reasonably well-tolerated and appears to have significant biologic activity in vivo in EBV-infected lymphoid malignancies which are refractory to other regimens (10). Theoretically, cidofovir does not require an induction of viral lytic replication. Thus, induction of EBV-TK expression by butyrate is not essential for cidofovir treatment.

It is not possible to be conclusive if anti-tumor effect for these NPC tumors is virus dependent or not so far. EBERs are abundantly transcribed in the latently infected EBV positive cells but not in such cells where viral

is replicating. Expression of EBERs in the residual tumor cell seems similar although population of tumor cell itself diminished. Serum EBV-DNA copy-number, as well as intensity of EBERs staining, did not change before and after cidofovir treatment in each case. These results suggest that cidofovir did not act to eradicate latently infected EBV in human NPC. Anti-tumor mechanism of cidofovir for NPC as well as papillomatosis has not been elucidated yet. While the anti-viral effects of cidofovir can be attributed to the interaction of diphosphorylated-cidofovir with the viral DNA polymerase and incorporation of cidofovir into the viral DNA chain, its specific inhibitory activity against the proliferation of HPV-infected cells must imply additional or alternative mechanism of action. The anti-proliferative effect of cidofovir on the growth of HPV-infected cells, akin to its inhibitory effect on the growth of NPC, may be ascribed the induction of apoptosis (11). This, in turn, may be related to the ability of cidofovir to restore the function of the tumor suppressor protein p53 in EBV-infected cells. Interestingly, inhibitory effect of cidofovir against non-viral transformed tumor cells via suppression of angiogenesis has been reported. The dormant attitude of NPC tumor in the two cases may partly be due to this anti-angiogenic effect of cidofovir (12).

The results presented here suggest that cidofovir treatment can be an alternative modality for the patients with recurrent nasopharyngeal carcinoma. The mechanism of the anti-tumor effect should be continuously investigated.

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Figure legends

Figure 1: MRI of the cidofovir injected site in case 1.

(A) Before starting cidofovir injection to the NPC invaded to the left nasal cavity.

(B) Four weeks after completion of a series of cidofovir injection. Nasal obstruction was improved after cidofovir treatment. Maxillary sinus empyema also improved along with the shrinkage of the nasal tumor (white arrowhead).

Arrows in the figures indicate injection site of cidofovir.

Figure 2: MRI of the cidofovir injected site in and 2

(A) Before starting cidofovir injection to the metastasized NPC at the patorid gland.

(B) Three months after completion of a series of cidofovir injection. Tumor size remained stable before and after cidofovir treatment. Arrowheads indicate the metastatic tumor.

Figure 3: Pathological analysis of EBV positive NPC before and after cidofovir treatment in case 1. (In situ hybridization for EBERs).

(A) Before starting cidofovir treatment.

(B) Four week after completion a series of cidofovir treatment.

Tumor cells are identified with dark blue EBERs staining signals (arrow).