Combination therapy of gemcitabine or oral S-1 with the anti-VEGF monoclonal antibody bevacizumab for pancreatic neuroendocrine carcinoma

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Abstract. We previously reported that the administration of bevacizumab for pancreatic neuroendocrine tumors inhibited angiogenesis in the host, resulting in tumor growth inhibition. In light of these results, we compared the effect of bevacizumab/gemcitabine/S-1 combination therapy vs. bevacizumab monotherapy. The QGP-1 pancreatic neuroendocrine carcinoma cell line and the BxPC-3 ductal cell carcinoma cell line were transplanted into the subcutaneous tissue of mice, and the mice were treated for 3 weeks with bevacizumab [50 mg/kg intraperitoneally (i.p.) twice weekly], gemcitabine (240 mg/kg i.p. once weekly) and S-1 (10 mg/kg orally five times weekly). The antitumor effect and side effects were evaluated by measuring the tumor volume and weight and by changes in body weight, respectively. The tumor volume became smaller (from the maximum volume) in the group treated with bevacizumab, gemcitabine and S-1 (BGS) and the group treated with bevacizumab and gemcitabine (BG). A significant difference was noted in the tumor weight between the BG group and the group treated with bevacizumab alone. A relatively significant decrease in the body weight was observed in the BGS and BG groups. We conclude that gemcitabine is appropriate as a drug used in combination with bevacizumab for pancreatic neuroendocrine tumors.

Introduction

Both functional and non-functional pancreatic neuroendocrine tumors (PNETs), including pancreatic neuroendocrine carcinomas (PNECs), are hypervascular tumors and they are known to express angiogenic molecules (1,2). For these reasons, anti-angiogenic therapy is expected to be effective against PNEC (3). Bevacizumab (Avastin®; Genentech Inc., San Francisco, CA, USA) is a recombinant human IgG1 monoclonal antibody against vascular endothelial growth factor (VEGF) (4). We previously reported that bevacizumab inhibited the induction of host angiogenesis, resulting in significant tumor growth inhibition, but not in tumor cell proliferation using QGP-1 which is a PNEC cell line, and expected a further potent cytotoxic effect by various combinations with anticancer drugs (5). On the basis of the suggestion above, we compared an additional effect between the combination of gemcitabine hydrochloride (Gemzar®, Eli Lilly and Company, Indianapolis, IN, USA) (6) or oral S-1 (TS-1®, Taiho Pharmaceutical Co. Ltd., Tokyo, Japan) (7) with bevacizumab and bevacizumab alone.

Materials and methods

The QGP-1 PNEC cell line (8) was purchased from the Japanese Collection of Research Bioresources (Osaka, Japan), and the BxPC-3 human pancreatic ductal carcinoma (DCC) cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured at 37˚C in RPMI-1640 (Gibco, Life Technologies Japan Ltd., Tokyo, Japan) supplemented with 10% fetal calf serum (FCS; Sigma, St. Louis, MO, USA) in a humidified atmosphere containing 5% CO_2.

Athymic female Balb/c-nu/nu nude mice (4-6 weeks old) with a body weight (BW) of 20-22 g, obtained from Clea Japan Inc. (Tokyo, Japan), were kept at the Animal Care and Use Facilities at Tokyo Medical University under specific pathogen-free condition. The cell suspension of each cell line with an adjusted cell suspension of $2x10^7$ cells/ml in RPMI-1640 (Gibco) was mixed with Matrigel matrix (BD Biosciences, San Jose, CA, USA) on ice at a 1:4 ratio. The mixture was implanted subcutaneously in the back of mice. At predetermined time points during a 1-week period after the cancer transplantation, 25 mice were randomly divided into five groups and treated with bevacizumab and gemcitabine or S-1 for 3 weeks. Bevacizumab (4 mg/kg) or human IgG (Sigma) was administered intraperitoneally (i.p.) twice a week (9). Gemcitabine (240 mg/kg) was administered i.p. once a week (10). Hydroxypromethyl cellulose [0.2 ml of

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0.5% (w/v); Shin-Etsu Chemical Co., Ltd., Tokyo, Japan], including dissolved powder-form S-1 (10 mg/kg) were orally administered five times a week (11,12). The treatment groups were as follows: BGS group, mice received bevacizumab, gemcitabine and S-1; BG group, mice received bevacizumab and gemcitabine; BS group, mice received bevacizumab and S-1; B group, mice received bevacizumab alone; and IgG group, mice received human IgG as non-treatment. Tumor volume was calculated by the multiplication of π x longitudinal axis x minor axis x minor axis; measurement was carried out using digital calipers, once a week. The weight of the mice was measured once a week. On the last day of the third week after start of the therapies (on 28 day after cancer cell transplantation), each tumor was removed and weighed. All experiments were approved by the Animal Care and Ethics Committee of Tokyo Medical University.

Statistical analysis. Statistical analyses were performed using Stat View (Abacus Concepts Inc., Berkely, CA, USA). The volume of the tumor was compared using the Mann-Whitney U test. A two-side p-value of <0.05 was considered to denote statistical significance.

Results

BxPC-3 cell tumors grew to approximately double that of the QGP-1 cell tumors. The mean tumor volume (mm^3) 1 week after QGP-1 transplantation, and 1, 2 and 3 weeks after each treatment was as follows: for the BGS group: 300.6, 389.8, 567.6 and 442.2, respectively; for the BG group: 486.8, 531.8, 546.3 and 358.2, respectively; for the BS group: 381.4, 436.1, 638.9 and 725.6, respectively; for the B group: 462.0, 549.7, 970.4 and 949.9, respectively; and for the IgG group: 414.2, 607.0, 935.4 and 1,504.2, respectively (Fig. 1A). The BGS and the BG groups receiving gemcitabine showed marked tumor growth inhibition from the 2nd week or later. By contrast, the BS and the B groups not receiving gemcitabine showed tumor growth inhibition in comparison to the IgG group; however, the tumor increased after the 2nd week or later. The tumor volume of all treatment groups apart from group B at the 3rd week was significantly smaller than that of the IgG group ($p<0.05$). There was no significant difference among the treatment groups. The mean tumor weight in the BGS, BG, BS, B and IgG groups at the time of tumor dissection was 382.9, 515.5, 114.7, 532.8 and 1,653.6 mg, respectively. There was a significant difference between all treatment groups and the IgG group ($p<0.05$), and between the BS and the B group ($p=0.03$) (Fig. 1B).

The mean tumor volume (mm^3) of the BxPC-3 cell tumors was as follows: for the BGS group: 546.1, 527.5, 473.0 and 496.9, respectively; for the BG group: 567.4, 639.7, 528.8 and 475.8, respectively; for the BS group: 437.7, 665.5, 447.1 and 347.7, respectively; for the B group: 526.6, 493.6, 341.8 and 523.6, respectively; and for the IgG group: 743.7, 1,243.1, 2,350.8 and 2,991.2, respectively (Fig. 2A). The BGS, BG and BS groups showed slight tumor inhibition from the 2nd week or later; however, only the B group exhibited tumor growth. The tumor volume of all treatment groups at the 3rd week was significantly smaller than that of the IgG group ($p<0.05$), but not among the treatment groups. The mean tumor weight in the BGS, BG, BS, B and IgG groups was 339.2, 325.7, 217.2, 322.8 and 1,782.7 mg, respectively. There was a significant difference between all treatment groups and the IgG group ($p<0.05$), but not among the treatment groups (Fig. 2B).

Macroscopic findings of the QGP-1 cell tumors showed comparatively solid and little central necrosis and no marked differences among the treatment groups (Fig. 3A-C). By contrast, the macroscopic findings of the BxPC-3 cell tumors indicated intratumoral bleeding and necrosis in all groups (Fig. 3D-F). Numerous subcutaneous blood vessels were

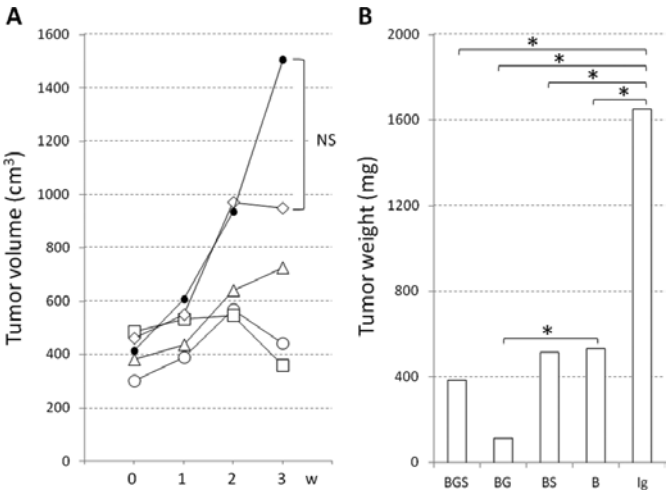

Figure 1. (A) Change in tumor volume with serial time course of QGP-1. x-axis: the time period (weeks). 0 (w) is the start day of therapy (1 week after transplantation). y-axis: tumor volume (cm^3). Tumor volume of mice administered: •, human IgG (Ig); ◇, bevacizumab alone (B); △, bevacizumab and S-1 (BS); ○, bevacizumab and gemcitabine (BG); and □, bevacizumab, gemcitabine and S-1 (BGS). Significant difference between the IgG group in all treatment groups except the B group was noted. (B) Final tumor weight after treatment. x-axis: treatment groups. y-axis: tumor weight (mg). *Significant difference between the untreated group and all treatment groups, and between B and BG group is shown.

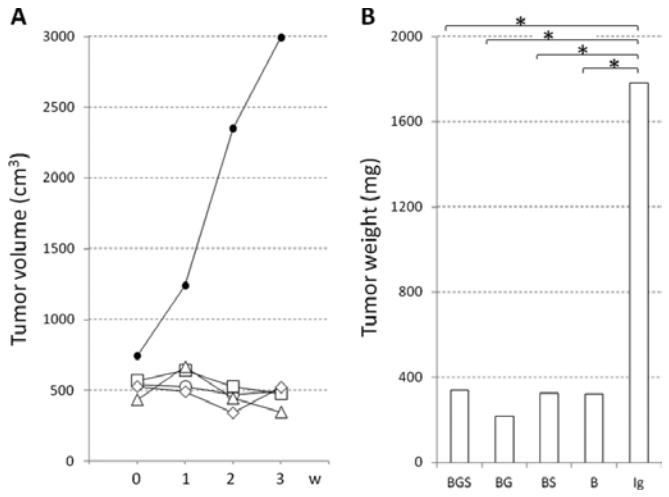

Figure 2. (A) Change in tumor volume with serial time course of BxPC-3. x-axis: the time period (weeks). 0 (w) is the start day of therapy (1 week after transplantation). y-axis: tumor volume (cm^3). •, Ig group; ◇, B group; △, BS group; ○, BG group; □, BGS group. Significant difference was noted between the IgG group and all treatment groups. (B) Final tumor weight after treatment. x-axis: treatment groups. y-axis: tumor weight (mg). *Significant difference was noted between the IgG group and all treatment groups.

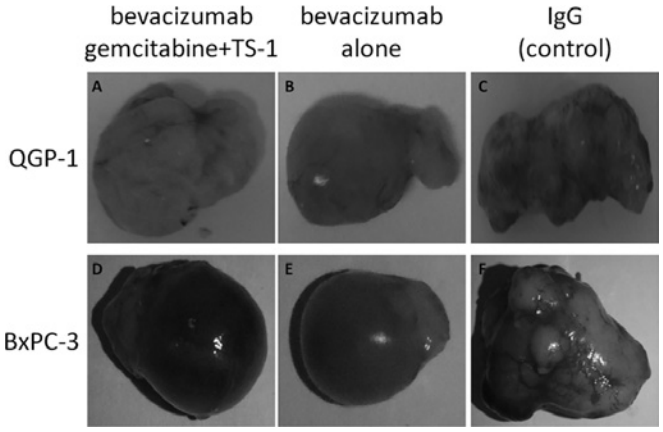

Figure 3. Macroscopic features of subcutaneous (A-C) QGP-1 cell and (D-F) BxPC-3 cell tumors. (A and D) Tumors treated with bevacizumab, gemcitabine and TS-1. (B and E) Tumors treated with bevacizumab alone. (C and F) Tumors treated with IgG as a control. As for QGP-1 cell tumors, the surface was yellowish white, and the inside of the tumor was solid. By contrast, for BxPC-3 cell tumors, the surface exhibited a reddish tinge, and the treated tumors (D and E) showed internal hemorrhage and necrosis.

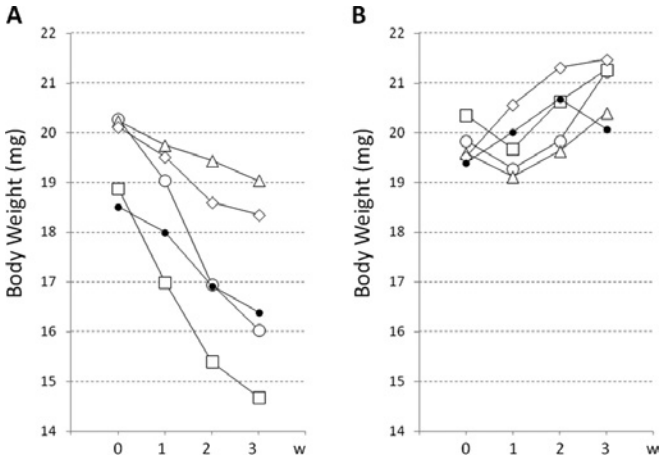

Figure 4. Body weight of mice bearing (A) QGP-1 and (B) BxPC-3 cell tumors. x-axis: time course (weeks). y-axis: body weight (mg). As for the weight change in the QGP-1 cell-transplanted mice, all groups, especially the BGS and BG groups, showed weight loss. By contrast, the body weight of mice bearing the BxPC-3 cell tumors was increased. •, Ig group; ◇, B group; △, BS group; ○, BG group; □, BGS group.

overlying the tumors in the IgG group bearing the QGP-1 and BxPC-3 cell tumors, while few blood vessels were observed in the bevacizumab-treated group.

As for the weight change in the QGP-1 cell-transplanted mice, all groups showed weight loss. In the BGS and BG groups, the drug caused weight loss which was in particular stronger than that in the IgG group. By contrast, weight loss was not evident, but weight instead rather increased in the BxPC-3 cell-transplanted mice. We determined that the above results reflected solely the characteristics of the cell lines.

Discussion

Inhibition of angiogenesis has become a target of cancer therapy, and the anti-VEGF antibody/bevacizumab is representative. Bevacizumab specifically binds to VEGF in the bloodstream and inhibits the binding of VEGF to VEGF receptors in vascular endothelial cells, thereby inhibiting angiogenesis. The interstitial pressure around a tumor is usually increased, inhibiting the delivery of anticancer drugs to tumor tissue. Bevacizumab normalizes tumor blood vessels, reduces the interstitial pressure and thereby improves the delivery of anticancer drugs to tumor tissue (4). PNECs are also hypervascular tumors and are known to express angiogenic molecules (1-3). For these reasons, anti-angiogenic therapy is expected to be effective against PNEC. In a randomized phase II trial of bevacizumab vs. interferon-α for the treatment of patients (n=44) with unresectable carcinoid tumors treated with octreotide, a somatostatin analogue, the added effect of combining bevacizumab with the somatostatin analogue, was reported (13). The therapeutic response rates were 18 vs. 0%, and the 8-week progression-free survival rates were 95 vs. 68%. We previously reported that bevacizumab inhibited the induction of host angiogenesis, resulting in significant tumor growth inhibition (5).

In the selection of therapeutic agents, we focused on the site of origin and growth of PNEC. PNECs are considered to arise from Langerhans cells, endocrine acinar cells and multipotent stem cells in the pancreatic ducts. By contrast, it has been reported that pancreatic ductal cell carcinoma may arise from pancreatic endocrine cells (14). In addition, Langerhans cells or pancreatic endocrine cells are reportedly involved in the growth of pancreatic ductal cell carcinoma (14). In light of these observations, we selected gemcitabine and S-1, which are therapeutic agents for pancreatic ductal carcinoma, as candidate therapeutic agents for PNEC, and confirmed a more beneficial effect of gemcitabine/bevacizumab combination therapy over bevacizumab monotherapy. Concerning the combination treatment of gemcitabine and bevacizumab, a randomized controlled trial of gemcitabine + placebo vs. gemcitabine + bevacizumab for the treatment of advanced unresectable pancreatic cancer was conducted. However, no significant differences were observed between the gemcitabine + placebo and gemcitabine + bevacizumab groups in the therapeutic response rates, median progression-free survival times and median survival times. Thus, gemcitabine + bevacizumab therapy did not prolong the survival time compared to gemcitabine therapy (15). On the contrary, a case report of the utility of the combination therapy including bevacizumab and gemcitabine for the progression of pancreatic cancer was reported (16). Another candidate therapeutic agent, S-1, was first developed in Japan (7,17). Currently, gemcitabine and S-1 are the only drugs that contribute to improving the prognosis of pancreatic cancer. Either gemcitabine or S-1 is commonly used as a first-line treatment, but they are sometimes used in combination with each other (18). Combination therapy with S-1, irinotecan and bevacizumab has been reported to be useful in the treatment of colorectal cancer with metastasis (19). In this study, we expected to obtain better results using a combination therapy with bevacizumab, gemcitabine and S-1, and confirmed a more beneficial effect of bevacizumab/gemcitabine combination therapy over bevacizumab monotherapy. However, the triple therapy was not superior to bevacizumab/gemcitabine combination therapy in the QGP-1 cell-transplanted mice.

The effect of the mammalian target of rapamycin (mTOR) inhibitor everolimus (Afinitor®) in patients with

advanced pancreatic neuroendocrine tumors has recently been reported (20). In this clinical trial, treatment with the mTOR inhibitor extended the median survival time from 4.6 (in a placebo group) to 11 months (in the treated group). It was also found that the mTOR inhibitor exerted an angiogenesis-inhibitory effect through VEGF (21). Future research will be conducted to investigate how to combine drugs for the treatment of pancreatic neuroendocrine tumors.

In conclusion, we compared the effect of bevacizumab/gemcitabine/S-1 combination therapy vs. bevacizumab monotherapy on pancreatic neuroendocrine tumor cell lines. Bevacizumab/gemcitabine combination therapy showed a strong antitumor effect (a decrease from the maximum tumor volume) from 2 weeks after treatment initiation. By contrast, bevacizumab/S-1 combination therapy resulted in a slowdown of tumor growth, but not in a decrease from the maximum tumor volume. Thus, we conclude that gemcitabine is appropriate for use in combination with bevacizumab for pancreatic neuroendocrine tumors.

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