**Effects of Valproic Acid in Combination with S-1 on Advanced Pancreatobiliary Tract Cancers: Clinical Study Phases I/II**

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**Abstract.** Background/Aim: Pancreatobiliary tract cancers are amongst the most aggressive human cancers. Histone deacetylase (HDAC) is well-known to be associated with tumorigenesis through epigenetic regulation and its inhibitors (HDACIs) induce differentiation and apoptosis of tumor cells. We conducted a clinical trial of combination therapy using valproic acid (VPA, a HDACI) and S-1, which is an oral fluoropyrimidine derivative consisting of 5-fluorouracil. Patients and Methods: Patients with advanced pancreatobiliary tract cancers were eligible for this clinical trial. Twelve patients, in whom a curative operation was not feasible, were enrolled in the study. Patients received S-1 orally at a daily dose of 80 mg/m² for 28 days, followed by a 14-day recovery period. They also received VPA orally at a total daily dose of 15 mg/kg, twice daily. Results: One patient had partial response (PR); ten patients were recorded with stable disease (SD); and one patient showed progressive disease (PD). Eight patients had clinically significant drug-related adverse events. The most frequent adverse events were platelet depletion and fatigue. Grade 3/4 adverse events, including anemia and platelet depletion, were observed. Significant increases in blood concentrations of VPA were confirmed 2 and 4 weeks after VPA administration. Conclusion: Combination therapy of VPA and S-1 for patients with pancreatobiliary tract cancers had a manageable safety profile and preliminary antitumor activity.

Pancreatic cancer is one of the most aggressive human cancers. The overall five year survival rate among patients with pancreatic cancer is <5% (1). Cholangiocarcinoma is a cancer arising from bile duct epithelium and treatments of choice remain very limited (2, 3). S-1, an oral fluoropyrimidine derivative consisting of the 5-fluorouracil (5-FU) prodrug tegafur, combined with two modulators of 5-FU activity, has been developed (4-6). Several reports have been published regarding the administration of S-1 to patients with solid tumors (7, 8). They showed that S-1 monotherapy was generally well-tolerated and demonstrated promising activity against advanced biliary tract cancer in a phase II study (9). However, since the effects of chemotherapy against pancreas and biliary tract cancers still continue to be limited, new regimens and innovative approaches are currently important subjects for investigation.

Alterations in the epigenetic modulation of gene expression have been implicated in cancer development and progression. Histone acetylation, one of the epigenetic regulations, is a post-translational modulation of the nucleosomal histones that affects chromatin structure and modulates gene expression. Histone deacetylases (HDACs) comprise an ancient family of enzymes that play crucial roles in numerous biological processes (10). It is well-known that HDACs are overexpressed in many tumor types (11, 12). We have reported that the survival rate for patients with histone deacetylase inhibitors (HDACI)-positive pancreatic cancer was significantly lower than that for patients with HDACI-negative cancer, suggesting that HDACI may be a promising therapeutic target in pancreatic cancer (13). It has also been shown that HDACIs induce the differentiation or apoptosis of cancer cells (14, 15). We, thus, examined the combined effects of valproic acid (VPA) and gemcitabine (GEM) and witnessed their inhibitory effects on the proliferation rates of pancreas and biliary tract cancer cell lines (16, 17). In addition, we confirmed that VPA enhanced the anti-tumor effects of 5-FU in pancreas and biliary tract cancer cell lines (18). Therefore, we here conducted a clinical trial to
investigate the effects of a combination therapy with VPA and S-1, in patients with advanced pancreatobiliary tract cancers.

**Patients and Methods**

*Patient eligibility.* Patients with inoperable (locally-advanced or distant metastatic) pancreatobiliary tract cancers, including pancreatic cancer, cholangiocarcinoma and gallbladder cancer were eligible for this clinical trial. Before the initiation of the study, relevant study documentation was submitted to, and approved by, the responsible Ethics Committee: The University of Tokushima Hospital Clinical Research Ethical Review Board, Tokushima, Japan. The guidelines of the World Medical Association Declaration of Helsinki in its revised edition (Edinburgh, Scotland, October 2000) and other applicable regulatory requirements were strictly followed. Written informed consent was obtained from each patient before any study-specific screening procedures were undertaken. This trial has been registered in the University Hospital Medical Information Network Clinical Trial Registry System, Japan (UMIN-ID: 000004525).

*Exclusion criteria.* Patients with severe organ disorders, including liver enzymes >2.5-times the upper level of normal, bilirubin levels >2.0 mg/dl or other active cancers were excluded from the present clinical study.

*Treatment plan.* Patients received S-1 orally at a daily dose of 80 mg/m² for 28 days, followed by a 14-day recovery period. Specifically, during the treatment weeks, patients with a body-surface area of less than 1.25 m² received 80 mg daily (i.e. two doses of two 20-mg capsules, twice daily); those with a body-surface area of 1.25 m² or more but less than 1.5 m² received 100 mg daily (i.e. two doses of two 25 mg capsules, twice daily); and those with a body-surface area of 1.5 m² or more received 120 mg daily (i.e. two doses of three 20 mg capsules, twice daily). S-1 was administered after the morning and evening meals. Chemotherapy was continued until evidence of progression, a request for withdrawal or the development of unacceptable toxicity. Compliance and agent accountability were thoroughly scrutinized and patients were asked to keep a diary tracking the intake of S-1 and other medications.

Patients received VPA orally at a total daily dose of 15 mg/kg, twice daily. VPA was administered to reach serum concentrations between 40 and 120 μg/ml, which is the therapeutic range for the treatment of seizures (19). Baseline safety and toxicity evaluations included a history, physical examination and complete blood counts with differential, metabolic, hepatic and renal function assessment at baseline and on days 3, 10, 17 and 21, and then every three weeks. Other laboratory tests, including pancreatic enzymes and platelet functions, were evaluated as clinically indicated. Disease restaging was done every two cycles (six weeks). Toxicity was graded by the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, and objective tumor response was defined by the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines. Responses were reviewed by an independent study radiologist. All patients at the dose expansion needed to have measurable disease based on the RECIST criteria.

*Pharmacokinetic studies.* Blood samples (5 ml) were collected in heparinized tubes, processed within 30 min after collection and stored at −20°C. VPA concentrations were measured using the Abbott AxSYM VPA assay reagent system (Abbott Laboratories, Abbott Park, IL, USA).

*HDAC expression.* HDACI expression was evaluated by flow cytometric analysis. A 3-ml peripheral blood sample was obtained from each subject and the peripheral blood mononuclear cells (PBMCs) were isolated using 4 ml of Lymphoprep (AXIS-SHIELD, Oslo, Norway), centrifuged at 2,000 rpm for 20 min at room temperature and then resuspended in 4 ml buffer. After the addition of anti-HDACI antibody (PE-conjugated antihuman HDACI antibody; Santa Cruz Biotechnology, Dallas, TX, USA) to the cell pellets, the mixture was centrifuged at 2,000 rpm for 5 min at 4°C, washed twice with FACS buffer, centrifuged at 1,800 rpm for 20 min in the dark and further incubated at 20°C overnight. The cells were then washed twice with FACS buffer, and centrifuged at 1,800 rpm for 5 min at 4°C and washed using a FACSCalibur (BD Biosciences, San Jose, CA, USA). Then, the fluorescence intensities of HDACI were detected.

**Results**

*Patients’ characteristics and efficacy.* Patients’ demographics for the 12 patients enrolled in this study are shown in Table I. Curative operations were not feasible for these 12 patients.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gender</th>
<th>Tumor</th>
<th>Non-resectable factor</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81</td>
<td>Female</td>
<td>Cholangiocarcinoma</td>
<td>Liver metastasis</td>
<td>Death</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>Male</td>
<td>Pancreatic cancer</td>
<td>Portal vein invasion</td>
<td>Death</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>Female</td>
<td>Pancreatic cancer</td>
<td>Post-operative recurrence (Portal vein)</td>
<td>Death</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>Male</td>
<td>Cholangiocarcinoma</td>
<td>Liver metastasis Artery invasion</td>
<td>Death</td>
</tr>
<tr>
<td>5</td>
<td>81</td>
<td>Female</td>
<td>Pancreatic cancer</td>
<td>Portal vein and artery invasion</td>
<td>Alive</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>Male</td>
<td>Pancreatic cancer</td>
<td>Portal vein and artery invasion</td>
<td>Death</td>
</tr>
<tr>
<td>7</td>
<td>78</td>
<td>Female</td>
<td>Cholangiocarcinoma</td>
<td>Post-operative recurrence (Lung metastasis)</td>
<td>Alive</td>
</tr>
<tr>
<td>8</td>
<td>77</td>
<td>Female</td>
<td>Gallbladder cancer</td>
<td>Post-operative recurrence (Lymph node metastasis)</td>
<td>Death</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>Male</td>
<td>Pancreatic cancer</td>
<td>Artery invasion Bone metastasis</td>
<td>Death</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>Male</td>
<td>Pancreatic cancer</td>
<td>Portal vein and artery invasion</td>
<td>Alive</td>
</tr>
<tr>
<td>11</td>
<td>74</td>
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<td>Cholangiocarcinoma</td>
<td>Para aorta lymph node metastasis</td>
<td>Alive</td>
</tr>
<tr>
<td>12</td>
<td>81</td>
<td>Female</td>
<td>Pancreatic cancer</td>
<td>Lung metastasis</td>
<td>Alive</td>
</tr>
</tbody>
</table>
Objective clinical response was assessed according to RECIST. As previously stated, 1 patient had PR, 10 patients had SD, while 1 patient was classified as having PD (Figure 1A).

Safety. All 12 enrolled patients were evaluated for safety (Figure 1B). The current regimen was generally well-tolerated, with 8 patients showing clinically significant agent-related adverse events. The most frequent adverse event was platelet depletion. Regarding Grade 3 or 4 toxicities, there was anemia in one case and platelet depletion in two cases.

Pharmacokinetic and pharmacodynamic evaluations. Blood concentrations of VPA were measured 2 and 4 weeks after VPA administration. Effective blood concentrations of VPA for the treatment of seizures (40 to approximately 120 μg/ml) (19), were achieved within 2 weeks and these blood concentrations of VPA were kept for at least 4 weeks (Figures 2A, B). In addition, a marked decrease in the HDAC expression was confirmed 4 weeks after VPA administration (Figure 3).

Discussion

In the present study, we conducted a clinical trial to examine the safety and efficacy of combination therapy with S-1 and VPA. We first found that our regimen was feasible and safe for patients with advanced pancreatobiliary tract cancers. S-1 is a novel oral fluoropyrimidine derivative consisting of the 5-FU pro-drug tegafur. We demonstrated that VPA...
augmented the anti-tumor effects of 5-FU in pancreas and biliary tract cancer cell lines (18). In our previous study, neither 5-FU (1.0 μM) nor VPA (0.5 mM) suppressed cell viability. However, when combining 5-FU (1.0 μM) with VPA (0.5 mM), the inhibitory effects could be clearly observed and the combination therapy was significantly more effective compared to 5-FU (1.0 μM) alone or VPA (0.5 mM) alone (18).

With regard to toxicities, we observed Grade 3 or 4 anemia and platelet depletion cases. In previous reports, anemia and platelet depletion were common in monotherapy with S-1 (anemia: 60.0%, platelet depletion: 32.5%) (20, 21). It appears that the degree of toxicities in our study was comparable to those with S-1 alone in the previous studies. Three patients (25%) experienced Grade 1 or 2 fatigue in our study, which is also in line with the previous clinical study of VPA administration for advanced solid tumors (22).

S-1 is a widely accepted agent for solid tumors, including advanced pancreatic cancer and cholangiocarcinoma. Several recent studies have demonstrated that monotherapy with S-1 showed non-inferiority to gemcitabine in overall survival with good tolerability representing a convenient oral alternative for locally advanced pancreatic cancer (20). In these reports, the PR was 21.0% and the SD was 42.3%. In our study, the PR rate was only 8.3%. However, the disease control rate (PR and SD) of our combination therapy (S-1 plus VPA) was 91.7%, while their disease control rate of S-1 monotherapy was 63.3% (20). In addition, as far as the biliary tract cancer is concerned, the disease control rate of S-1 monotherapy was shown to be 77.5% (21) while in GEM and S-1 combination therapy, the disease control rate was 70% for the advanced biliary tract cancer (23). The current study indicated that the combination therapy employed showed comparable, or an even better tumor control rate (91.7%) for the advanced pancreatic cancer and cholangiocarcinoma compared to those reported in the previous clinical studies. Moreover, VPA has long been widely used as a therapeutic agent for epilepsy and its toxicity profile, as well as pharmacokinetic properties, have been established. Munster et al. reported that the tumor control rate (PR and SD) was 61% with a combination of VPA and epirubicin in solid tumors such as melanoma, breast cancer and colon cancer (22). Candelaria et al. documented a tumor control rate of 80% with the combination of VPA and antitumor agents (cisplatin, carboplatin, paclitaxel and others) in advanced solid tumors, such as ovarian cancer and breast cancer (24). Therefore, it is conceivable that combination therapy with VPA and other anticancer agents may be promising regimens for life-threatening cancers, such as pancreatic cancer and cholangiocarcinoma.

In this study, patients received VPA at a total daily dose of 15 mg/kg. It has been generally described that the dose of VPA in epilepsy is 15-20 mg/kg/day (25), and that for patients with non-small cell lung cancer 10-20 mg/kg/day (26). The blood concentrations of VPA reached an effective level (40-120 μg/ml) that was kept for at least 4 weeks after VPA administration in this study. However, previous study documented that the maximum-tolerated VPA dose was 140 mg/kg/day (22). Therefore, to obtain a better response-rate or tumor-control rate, a higher dose of VPA might be required as a combination therapy with S-1 for patients with advanced hepatobiliary pancreatic cancers. Further studies using such regimens in larger cohorts of patients will be valuable.

In conclusion, this clinical study on 12 patients with advanced pancreatobiliary tract cancers indicated that combination therapy of VPA and S-1 is generally well-tolerated, exhibiting a significant reduction of HDAC expression, probably associated with acceptable anti-tumor activity because of a high tumor-control rate in this study.

Conflicts of Interest

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