ORIGINAL

Effect of histone deacetylase inhibitor in combination with 5-fluorouracil on pancreas cancer and cholangiocarcinoma cell lines

Shuichi Iwahashi[1,2], Hiroki Ishibashi[1,2] Tohru Utsunomiya[1], Yuji Morine[1], Tovuu Lkhaguva Ochir[1], Jun Hanaoka[1], Hiroki Mori[1], Tetsuya Ikemoto[1], Satoru Imura[1], and Mitsuo Shimada[1]

[1]*Department of Digestive Surgery and Transplantation, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan,* [2]*These authors equally contributed to this study.*

Abstract : Background : 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 examined the therapeutic effects of valproic acid (VPA, a HDACI) with a combination of 5-fluorouracil (5-FU) in vitro. Methods : A human pancreas cancer cell line (SUIT-2) and a cholangiocarcinoma cell line (HuCCT1) were used. Cell viabilities were evaluated by a cell proliferation assay. We determined the anticancer effects of VPA combined with 5-FU in these cell lines. Results : Pancreas cancer (SUIT-2) : No effect of 5-FU (1.0 μM) was observed, but 17% and 30% of proliferation-inhibitory effects were recognized in a dose of 2.5 or 5.0 μM, respectively. Cell viability was only weakly reduced by VPA (0.5 mM). However, in combination of 5-FU (1.0 μM) with VPA (0.5 mM), 19% of inhibitory effect was observed. Cholangiocarcinoma (HuCCT1) : 5-FU (1.0 μM) did not suppress the cell viability, but 5-FU (2.5 μM) suppressed by 23%. VPA (0.5 mM) did not suppress the cell viability, while VPA (1.0 mM) weakly decreased it by 11%. Combination of 5-FU (1.0 μM) and VPA (0.5 mM) markedly reduced the cell viability by 30%. Conclusion : VPA augmented the anti-tumor effects of 5-FU in cancer cell lines. Therefore, a combination therapy of 5-FU plus VPA may be a promising therapeutic option for patients with pancreas cancer and cholangiocarcinoma. J. Med. Invest. 58 : 106-109, February, 2011

Keywords **:** *pancreas cancer, cholangiocarcinoma, HDAC inhibitor, valproic acid, epigenetic regulation*

INTRODUCTION

Pancreas cancer is one of the most aggressive human cancers. The overall 5-year survival rate among patients with pancreatic cancer is < 5% (1). Cholangiocarcinoma is a cancer arising from bile duct epithelium. This cancer is one of the most difficult diseases to treat as pancreas cancer, and no standard chemotherapy has been established (2, 3). Therefore, we have researched about resistance of chemotherapy in pancreatic and biliary tract cancers.

5-fluorouracil (5-FU) is a chemotherapeutic drug which is widely used mainly for the treatment of the digestive system cancer, but the response rate

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Address correspondence and reprint requests to Mitsuo Shimada M.D. Professor and Chairman, Department of Digestive Surgery and Transplantation, Institute of Health Biosciences, the University of Tokushima, Kuramoto-cho, Tokushima, 770-8503, Japan and Fax : +81-88-631-9698.

in pancreatic and biliary tract cancers is very low (4, 5). Therefore, new agents and innovative approach to therapy are the important subjects for research.

Alterations in the epigenetic modulation of gene expression have been implicated in cancer development and progression, and histone acetylation, one of the epigenetic regulations, is a posttranslational modulation of the nucleosomal histones that affects chromatin structure and modulates gene expressions. Histone deacetylases (HDACs) comprise an ancient family of enzymes that play crucial roles in numerous biological processes (6), and HDACs are found to be overexpressed in many tumor types (7, 8). We reported that the survival rate for pancreas cancer patients with HDAC1-positive was significantly lower than that for patients with HDAC1-negative, and HDAC1 was considered to be a promising therapeutic target in pancreas cancer (9). HDAC inhibitors induce the differentiation or apoptosis of cancer cells (10, 11). Therefore, HDAC inhibitors are promising new agents, in this study, we used Valproic acid (VPA). VPA has the antitumor effects of a HDAC inhibitor (12), and VPA has been shown to have anticancer effects in various cancer models (13).

The aim of this study was to investigate the anticancer effects of VPA in combination with 5-FU in pancreas cancer and cholangiocarcinoma cell lines.

MATERIAL AND METHOD

Cell lines and culture conditions

SUIT-2 cell was purchased from the Japanese Collection Research Bioresources Cell Bank (Tokyo, Japan). HuCCT-1 was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. All cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 70 μg/mL penicillin and 100 μg/mL streptomycin (complete medium) and maintained at 37°C in a humidiWed incubator with 5% CO_2 in air. The cells were maintained for no longer than 12 weeks after recovery from frozen stock.

Reagents

Valproic acid was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and kept at 4°C and diluted in PBS as necessary at the time of use. 5-FU was purchased from Kyowa Hakko (Tokyo, Japan) and made fresh in 0.9% NaCl on the day of use.

Cell proliferation assay

All of tumor cells (5×10^3) were seeded into 38-mm^2 wells of flat-bottomed 96-well plates in quadruplicate and allowed to adhere overnight. The spent medium was then removed, and the cultures were refed with new medium (negative control) or medium containing diVerent concentrations of VPA and 5-FU. Incubation was continued for 72 h prior to adding the Cell Counting Kit-8, and after 2 h, the optical density was measured at 450 nm with a microplate reader (Multiskan JX ; Labsystems).

Statistical analyses

Statistical comparisons of mean values were conducted using oneway ANOVA. All the results are presented as mean ± SD. Statistical analysis was performed using Stat View 5.0 J software (SAS Institute, Inc., Cary, NC, USA). A P value of less than 0.05 was considered to be statistically significant.

RESULTS

In pancreas cancer cell line, SUIT-2, no effect of 5-FU was observed in dose of 1.0 μM and 17%, 30% and 33% of proliferation-inhibitory effects were observed in dose of 2.5, 5.0 and 10 μM (Fig. 1A). VPA (0.5 mM) weakly decreased cell viability by 13%, and VPA (1.0 mM) suppressed by 19% (Fig. 1B). In combination of 5-FU and VPA, 19% of inhibitory effect was observed in dose of 5-FU 1.0 μM/VPA 0.5 mM, the combination effect was significant compare

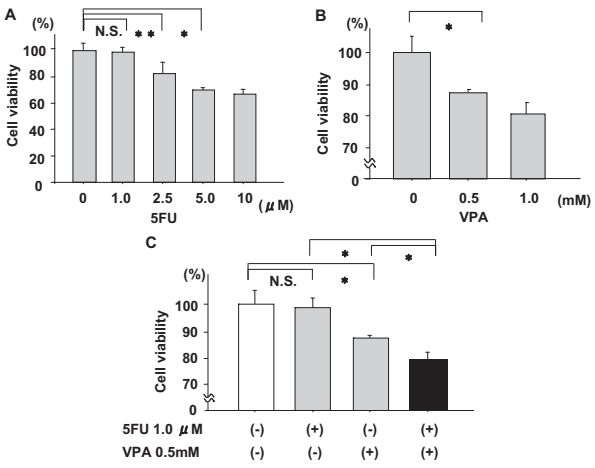

Figure 1 : The effect of 5-FU (A), VPA (B) and combination of 5-FU and VPA (C) in inhibiting cell proliferation of human pancreas cancer cell line, SUIT-2.
** : $p< 0.05$, * : $p< 0.01$.

to 5-FU alone or VPA alone ($P < 0.01$) (Fig. 1C).

In cholangiocarcinoma cell line, 5-FU (1.0 μM) did not suppress the cell viability, 5-FU (2.5 μM) suppressed by 23%, and 34% and 39% of proliferation-inhibitory effects were observed in dose of 5.0 and 10 μM (Fig. 2A). VPA (0.5 mM) did not suppress the cell viability, while VPA (1.0 mM) weakly decreased it by 11% (Fig. 2B). 5-FU (1.0 μM) and VPA (0.5 mM) reduced by 30%, which significantly augmented the anticancer effect of 5-FU alone or VPA alone ($P < 0.01$) (Fig. 2C).

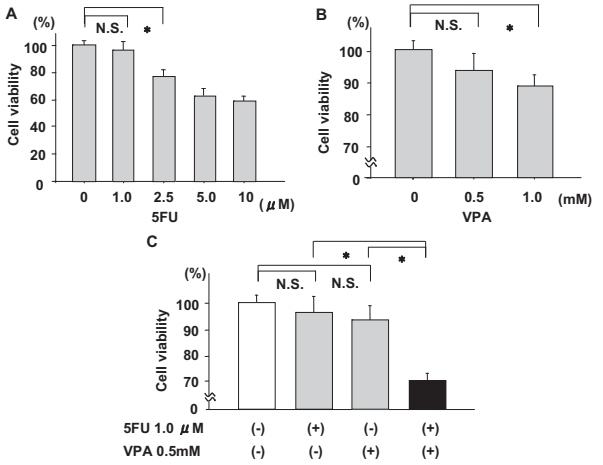

Figure 2 : The effect of 5-FU (A), VPA (B) and combination of 5-FU and VPA (C) in inhibiting cell proliferation of human cholangiocarcinoma cell line, HuCCT1.
* : $p < 0.01$.

DISCUSSION

In the present study, we assessed the effect of HDAC inhibitor (VPA) in combination with 5-FU on pancreatic-biliary carcinoma cell lines. To our knowledge, this is the first report to show that VPA enhances the effect of 5-FU on both pancreas cancer and cholangiocarcinoma cell lines.

HDAC inhibitors are useful in cancer treatment when used in combination with current chemotherapeutic drugs, especially in combination with 5-FU, HDAC inhibitor (MS275) enhance the effect of 5-FU in colorectal cancer cells (14), and other HDAC inhibitor (SAHA) enhance the effect of 5-FU in non-small cell lung cancer (15). The mechanisms of the additional effects on HDAC inhibitors to the cytotoxic agent are the enhancement of apoptosis (14) and the up-regulation of p21(waf1/cip1) expression (15). In this study, the mechanisms may be the augmentation of apoptosis or the enhancement of p21 (waf1/cip1) expression.

However, some HDAC inhibitors are of limited therapeutic use due to toxic side effects at high doses (16). VPA is widely used as a therapeutic drug for epilepsy, its toxicity profile and pharmacokinetic properties are well established. Furthermore, in our study, the dose of VPA was 0.5 mM, because the peak plasma concentration in patients treated for epilepsy ranges between 0.5 and 1.2 mM (17). VPA at a dose of 0.5 mM may not cause any serious side effects in clinical setting.

Recently, S-1, an oral drug consisting of the 5-FU prodrug tegafur, combined with two modulators of 5-FU activity, has been developed (18-20). S-1 contains 5-chloro-2,4-dihydroxypyridine (CDHP), CDHP competitively inhibits the 5-FU degradative enzyme dihydropyrimidine dehydrogenase (DPD), resulting in the retention of a prolonged concentration of 5-FU in blood (18).

VPA has been investigated in clinical studies (21, 22), we plan the clinical trial of the combination therapy, S-1 and VPA. We have expected VPA enhances the anti-tumor effect of S-1 in this trial.

In conclusion, VPA augmented the inhibitory effects of 5-FU on the proliferation rates of both pancreas cancer and cholangiocarcinoma cell lines. Therefore, VPA in combination with 5-FU is suggested to be a promising therapeutic option for pancreatic and biliary tract cancers.

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